

Genetic control and dynamics of the cellular immune response to the human T-cell leukaemia virus, HTLV-I

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About 1% of people infected with the human T-cell leukaemia virus, type 1 (HTLV-I) develop a disabling chronic inflammatory disease of the central nervous system known as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Patients with HAM/TSP have a vigorous immune response to HTLV-I, and it has been widely suggested that this immune response, particularly the HTLV-I-specific cytotoxic T-lymphocyte (CTL) response, causes the tissue damage that is seen in HAM/TSP. In this paper we summarize recent evidence that a strong CTL response to HTLV-I does in fact protect against HAM/TSP by reducing the proviral load of HTLV-I. We conclude that HTLV-I is persistently replicating at a high level, despite the relative constancy of its genome sequence. These results imply that antiretroviral drugs could reduce the risk of HAM/TSP by reducing the viral load, and that an effective anti-HTLV-I vaccine should elicit a strong CTL response to the virus. The dynamic nature of the infection also has implications for the epidemiology and the evolution of HTLV-I.

Keywords: cytotoxic T lymphocyte (CTL); human retrovirus; HLA; immunogenetics; central nervous system

1. INTRODUCTION

Human T-cell leukaemia/lymphoma virus type I (HTLV-I) was the first pathogenic human retrovirus discovered (Poesz *et al.* 1980; Hinuma *et al.* 1981; Yoshida *et al.* 1982). It was found because it causes a T-cell malignancy, but it has since become clear that the same virus also causes a range of chronic inflammatory conditions, of which the most commonly recognized is HTLV-I associated myelopathy, also known as tropical spastic paraparesis (HAM/TSP) (Montgomery *et al.* 1964; Gessain *et al.* 1985; Osame *et al.* 1986).

HTLV-I is related to the human immunodeficiency virus HIV-1. However, while HIV-1 causes disease in almost all infected people, HTLV-I causes disease in only a small fraction: about 1–2% develop adult T-cell leukaemia (ATL; Uchiyama *et al.* 1977), and about the same proportion develop a chronic inflammatory disease such as HAM/TSP.

The two main questions that arise are therefore (i) why do some HTLV-I-infected people develop disease, while the great majority remain well? and (ii) what is the pathogenesis of the different diseases? Since there is no

satisfactory animal model of HTLV-I disease, conclusions on the pathogenesis of these diseases can only be inferred. We have therefore concentrated on the first question, looking for factors associated with disease or health among the HTLV-I-infected population. We consider only the chronic inflammatory diseases; the pathogenesis of the leukaemia appears to be quite different.

From a study of immune factors, virus factors, host genetics and mathematical modelling, we reach a clear conclusion: one of the chief determinants of disease is the efficiency of the individual's cytotoxic T-lymphocyte (CTL) response to HTLV-I, which in turn is strongly influenced by that individual's genetic make-up. An efficient CTL response to HTLV-I protects against HAM/TSP and reduces provirus load. Although a vigorous CTL response may contribute to inflammation, the net effect of a strong CTL response is clearly beneficial. This conclusion strongly supports the view that vaccines against HTLV-I and other persistent viruses such as HIV-1/2, hepatitis B or hepatitis C should elicit a vigorous CTL response to the virus.

In this paper we describe how this conclusion is reached. We emphasize first that a mathematical consideration of the equilibrium dynamics of virus replication and the host immune system is an important ingredient of a complete understanding of persistent virus infections. Second, a knowledge of the within-host dynamics of

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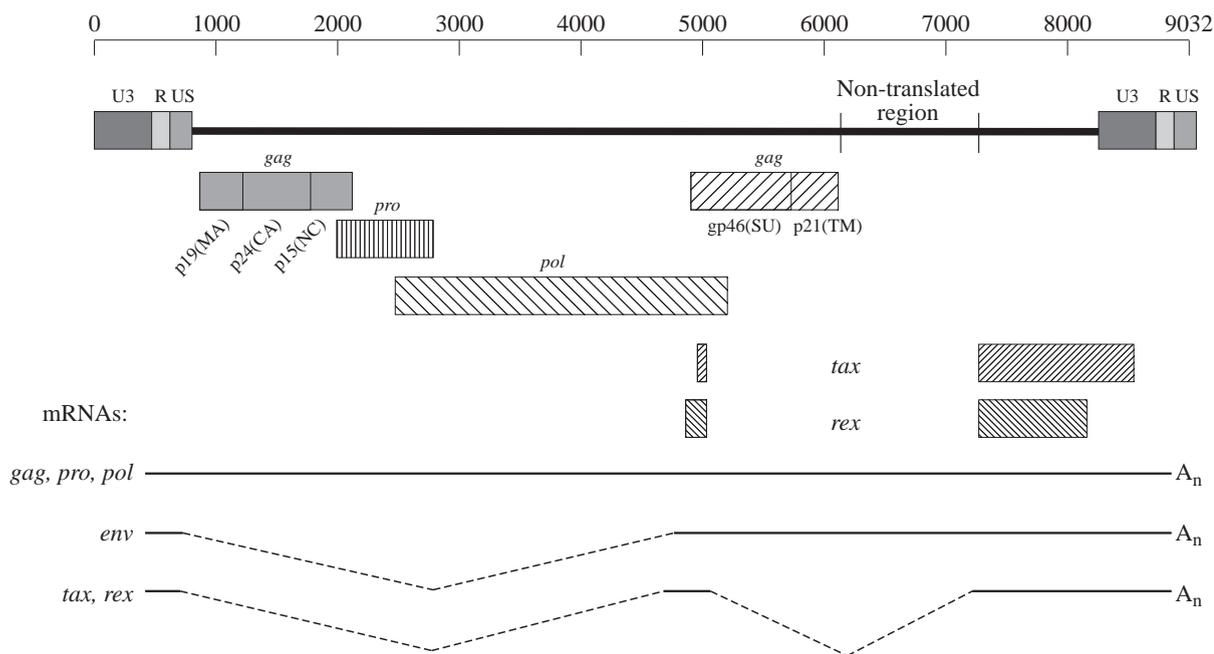


Figure 1. Structure and organization of the HTLV genome (Seiki *et al.* 1983). The HTLV genome is shown at the top of the figure. Positions of known genes are indicated. Sizes and positions of the proteins encoded by the provirus are shown beneath the provirus. The structure of the three messenger RNA species produced are shown at the bottom of the figure. Modified from fig. 1, p. 1850 in Cann & Chen (1996), by permission of the publishers.

persistent virus infections is needed because the dynamics directly influence both the epidemiology of the virus and the risk of virus-induced disease.

2. HTLV-I

HTLV-I contains the typical retrovirus *gag*, *pol* and *env* genes (figure 1), and codes for at least two other regulatory proteins: Tax, the transcriptional transactivator of the virus, and Rex, which regulates viral mRNA splicing (Cann & Chen 1996). The Tax protein is expressed early in the viral replication cycle. It is of central importance in the virus dynamics because, as well as transactivating viral transcription, it drives host cell proliferation by transactivating a large number of host genes, notably *IL-2* and *IL-2 receptor*. Finally, Tax is the dominant target antigen recognized by HTLV-I-specific cytotoxic T lymphocytes in most responding individuals (Jacobson *et al.* 1990; Kannagi *et al.* 1992; Parker *et al.* 1992, 1994). Thus the Tax protein is at the centre of both efficient HTLV-I replication and the host attack on the virus.

HTLV-I can infect most human cell types *in vitro*, but the only cell type that permits significant viral replication is the CD4⁺ T cell, which also appears to be the only cell that carries a significant load of HTLV-I *in vivo*. Most infected cells also carry the CD45RO antigen (Richardson *et al.* 1990). However, the cellular receptor for the virus has not been identified (Sommerfelt *et al.* 1988; Hildreth *et al.* 1997). HTLV-I produces little or no free plasma virus *in vivo*: infection appears to spread by cell-cell contact.

3. EPIDEMIOLOGY

Between 10 and 20 million people worldwide are infected with HTLV-I (de Thé & Bomford 1993). The

virus is chiefly found (Mueller & Blattner 1996) in tropical areas, with large infected populations in South America, Central Africa, the Caribbean islands, and southern Japan; it is also found in southern India, The Seychelles, and southern Africa. In Europe and North America it is uncommon, and exists mainly in individuals born in the endemic areas mentioned; it is also present in some communities of intravenous drug abusers. In endemically infected populations, HTLV-I is maintained principally by transmission from mother to child in the breast milk (Kajiyama *et al.* 1986; Ueda *et al.* 1993). Sexual spread is also important: transmission from men to women is significantly more frequent than from women to men (Mueller & Blattner 1996). The probability of sexual transmission is strongly influenced by the provirus load, that is, the proportion of infected T cells in the host (Mueller & Blattner 1996). The third important mode of transmission is by infected blood, either by transfusion or by the sharing of needles among drug abusers. Transfusion of cell-free blood products appears to carry a negligible risk of HTLV-I transmission, because of the paucity of free infectious virus in the plasma.

4. HAM/TSP AND OTHER CHRONIC INFLAMMATORY DISEASES ASSOCIATED WITH HTLV-I INFECTION

A patient with HAM/TSP develops a progressive spastic weakness of the legs, often accompanied by low back pain and urinary frequency or urgency (Gessain & Gout 1992; Nakagawa *et al.* 1995). The disease typically progresses for several months, leaving the patient bed-bound, chair-bound, or walking with sticks. In some cases, the progression seems to continue, but the relative importance of disuse atrophy and persistent disease activity is still not decided. Women are more commonly affected by HAM/TSP: the ratio of affected males to

females is 1:2. The peak age at onset of HAM/TSP is in the 40s, but the disease can present at almost any age; it is rare in children (Nakagawa *et al.* 1995).

The main neuropathological features of HAM/TSP (Iwasaki 1993; Umehara *et al.* 1993) are local demyelination, with an associated mononuclear cell infiltrate. The mononuclear cells are present both in perivascular cuffs and more diffusely in the neighbouring parenchyma of the central nervous system (CNS). The areas worst affected are the lateral columns of the lower cervical and upper thoracic spinal cord. In fresh lesions, CD4+ T cells predominate in the mononuclear cells; in long-standing lesions there are fewer cells, and CD8+ cells predominate. After some years of disease, the volume of the spinal cord is reduced in the affected areas, and the overlying leptomeninges are thickened.

Clinical investigations, including CT and MRI scans, often show abnormalities elsewhere in the CNS, but associated symptoms are uncommon (Nakagawa *et al.* 1995).

HTLV-I is also associated with chronic or subacute inflammatory diseases in the eye (Sagawa *et al.* 1995), lung (Higashiyama *et al.* 1994) and skeletal muscles (Morgan *et al.* 1989; Higuchi *et al.* 1996). There are also suggestions of similar conditions in the skin (LaGrenade *et al.* 1990), joints (Nishioka *et al.* 1989) and liver, but these associations are less certain.

5. THE PROVIRAL LOAD OF HTLV-I IS HIGH AND DETERMINES THE RISK OF DISEASE

The proviral load in a typical HAM/TSP patient is remarkably high: around 10% of peripheral blood mononuclear cells (PBMC) carry the provirus (Yoshida *et al.* 1989; Gessain *et al.* 1990; Kira *et al.* 1991; Nagai *et al.* 1998). The proviral load in asymptomatic carriers is significantly lower, usually 0.1–1% of PBMC. However, this is still high compared with other persistent viruses: HIV-1 usually infects only 0.01–0.5% of PBMC, and Epstein Barr virus (EBV) is typically present in about one in 10^5 peripheral blood B cells in someone who has recovered from acute EBV infection.

Since CD4+ CD45RO+ cells account for some 20–30% of PBMC, a provirus load of 10% PBMC corresponds to infection of 30–50% of the susceptible host cells. It follows that the rate at which susceptible cells can be infected becomes limiting at high proviral loads, since infection occurs by cell–cell contact. This is probably an important factor in the within-host dynamics of the infection (Wodarz *et al.* 1999a; see below).

We have recently measured the proviral load in a cohort of about 200 HAM/TSP patients and 200 asymptomatic carriers in southern Japan (figure 2) (Nagai *et al.* 1998). In agreement with previous work, we found that HAM/TSP patients had a median proviral load approximately 16-fold greater than asymptomatic carriers. In addition, we found no correlation between proviral load and either age at onset, duration of symptoms, or age at sampling. These results indicate that the HTLV-I proviral load is stable, perhaps indefinitely, in most infected individuals, although a slight fluctuation may occur (Kubota *et al.* 1993).

The large cohort also allowed us to calculate the importance of proviral load in predisposing to HAM/TSP. The surprising result is shown in figure 3; until the

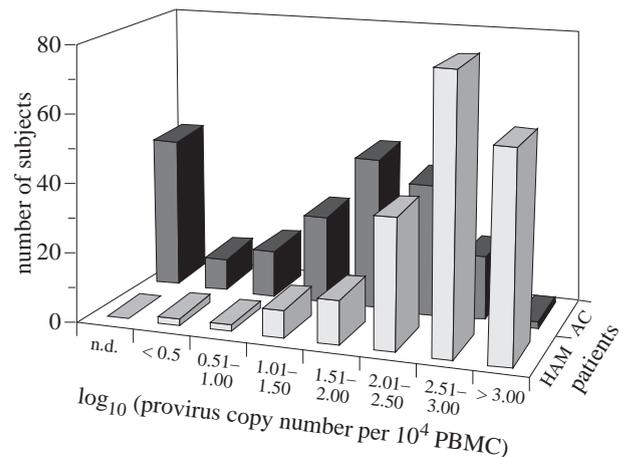


Figure 2. Distribution of HTLV-I proviral load in 202 HAM/TSP patients (HAM) and 200 asymptomatic carriers (AC) in Kagoshima (Nagai *et al.* 1998). The proviral load was measured by a competitive PCR technique, with a lower limit of detection of one provirus per 10^4 PBMC (Nagai *et al.* 1998). The median proviral load in HAM/TSP patients was 16 times greater than in asymptomatic carriers. n.d., not detected.

proviral load reaches 1% of PBMC, it has little impact on the risk of disease. But above this apparent threshold, the risk of HAM/TSP rises exponentially as the log(proviral load) rises. The reason for the existence of this threshold is not known: a suggestion will be made below.

6. DO HOST OR VIRAL FACTORS DETERMINE THE EQUILIBRIUM PROVIRAL LOAD?

(a) Viral factors

An early possibility was that HAM/TSP was caused by a distinct strain of HTLV-I. There is a clear precedent for this in murine leukaemia viruses, where certain mutations in the virus' receptor-binding protein (Env) can confer neurotropism on the virus (Szurek *et al.* 1988; Paquette *et al.* 1989). But analysis of a great deal of HTLV-I sequence data has shown no convincing association between any sequence variant of HTLV-I and disease (see Bangham (1993) for review). However, only certain regions of the HTLV-I genome have been examined: it remains possible that polymorphism elsewhere in the viral genome significantly influences the risk of inflammatory disease.

We have also investigated sequence variation in the *tax* gene of HTLV-I, because Tax is the dominant target antigen recognized by HTLV-I-specific CTL. The consensus sequence of the gene differed little between patients, but there was significant within-isolate sequence heterogeneity (Niewiesk *et al.* 1994). To test whether the CTL exerted significant selection on the *tax* gene, we calculated the ratio (Nei & Gojobori 1986) of non-synonymous (coding) nucleotide changes to synonymous (silent) changes amongst HTLV-I clones in each infected person. There was an excess of non-synonymous mutations in *tax* in asymptomatic carriers, but not in HAM/TSP patients (Niewiesk *et al.* 1994). Since the most plausible selection force acting on the *tax* gene is the anti-HTLV-I-CTL, this result implied that the CTL response to the Tax protein is more effective in asymptomatic carriers than in HAM/TSP

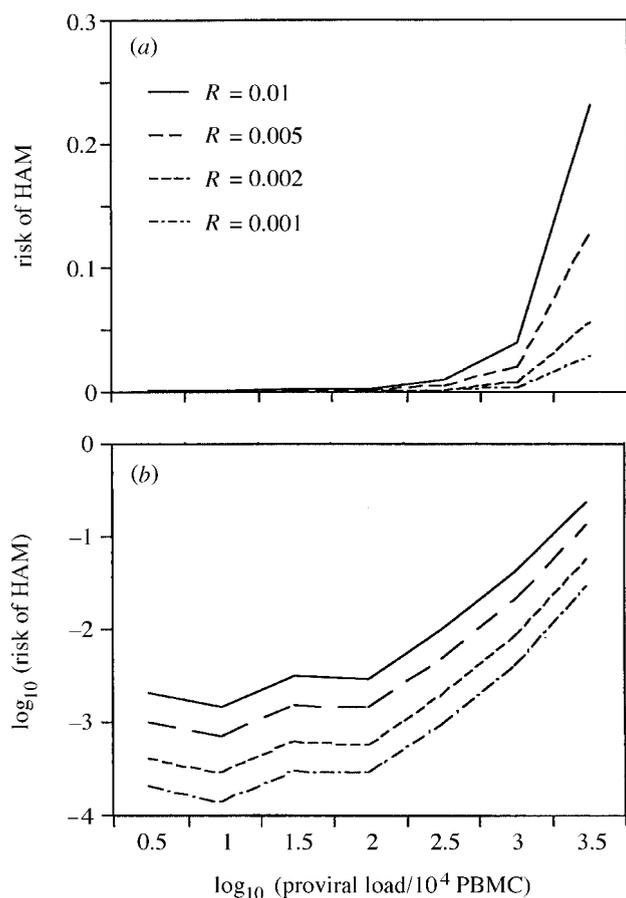


Figure 3. The risk of HAM/TSP depends on the proviral load. The vertical axis shows (a) the risk of HAM/TSP or (b) \log_{10} (risk of HAM/TSP) at a given \log_{10} (HTLV-I proviral load). The risk remains very low until the proviral load reaches 1% PBMC; above this apparent threshold, the risk of disease rises rapidly with increasing load. To calculate the risk of HAM/TSP at a given proviral load we use Bayes' theorem of conditional probabilities. Using the standard notation for conditional probability, where $p(\text{HAM}|L)$ denotes the probability of HAM/TSP in an HTLV-I infected person with a provirus load of L ,

$$p(\text{HAM}|L) = \frac{p(\text{HAM} \times p(L|\text{HAM}))}{p(\text{HAM}) \times p(L|\text{HAM}) + p(\text{AC}) \times p(L|\text{AC})}$$

AC denotes asymptomatic carriers of HTLV-I; R denotes the prevalence of HAM/TSP in the infected population. We estimate $p(L|\text{HAM})$ and $p(L|\text{AC})$ from the distribution of proviral load in the HAM/TSP and healthy carrier cohorts in the case-control study in Kagoshima (Jeffery *et al.* 1999; see text). Since R is not known with certainty, four curves are shown with values of R from 0.001 to 0.01.

patients. This raised the important possibility that an individual's CTL responsiveness to Tax determines their equilibrium proviral load, and so their risk of HTLV-I-associated inflammatory disease.

(b) *Host immune response*

HAM/TSP patients have consistently been found to have very high titres of antibody to HTLV-I (see Gessain & Gout (1992) for review); the titre correlates with provirus load (Mueller & Blattner 1996; Nagai *et al.* 1998). However, there has been no satisfactory test of the protective or pathogenic effects of anti-HTLV-I antibody.

We have studied the CTL response to HTLV-I (Parker *et al.* 1992, 1994; Niewiesk *et al.* 1995; Daenke *et al.* 1996; Jeffery *et al.* 1999), because anti-viral CTL are the chief effectors of the immune system that are responsible for eradicating viruses from the body (McMichael 1995), and because abundant CD8+ cells have been observed in spinal cord lesions in HAM/TSP (Umehara *et al.* 1993). CTL to HTLV-I were first demonstrated in patients with ATL in remission (Kannagi *et al.* 1983). In 1990, Jacobson *et al.* found that anti-HTLV-I CTL were abundant and chronically activated in the peripheral blood in HAM/TSP patients. These observations led to the suggestion that a vigorous host CTL response might cause the inflammatory disease. However, there are a number of problems with this suggestion.

- (i) There are few if any host cells in the inflammatory lesions that are infected with HTLV-I, other than the invading CD4+ T cells (Kira *et al.* 1991, 1992; Ohara *et al.* 1992; Hara *et al.* 1994; Lehky *et al.* 1995; Moritoyo *et al.* 1996). It is therefore unlikely that HAM/TSP is caused by a direct host immune attack against HTLV-I-infected cells which are normally resident in the CNS. A similar conclusion has been reached in polymyositis associated with HTLV-I infection (Higuchi *et al.* 1996).
- (ii) A substantial proportion of asymptomatic carriers also have abundant, chronically activated anti-Tax CTL (Parker *et al.* 1992, 1994; Daenke *et al.* 1996; Jeffery *et al.* 1999).
- (iii) There is no evidence that antibodies or CTL directed against HTLV-I cross-react on host antigens, although this possibility is hard to exclude.
- (iv) The results of *tax* gene sequence analysis (Niewiesk *et al.* 1994; Niewiesk & Bangham 1996; see above) suggested that the CTL response to Tax was, if anything, more efficient in asymptomatic carriers than in HAM/TSP patients.

The possibility remains that activated CTL might damage uninfected bystander cells in the HTLV-I-associated inflammatory diseases (Biddison *et al.* 1997). However, recent genetic evidence (Jeffery *et al.* 1999; see below) strongly implies that the net effect of anti-HTLV-I CTL is beneficial, by reducing the provirus load. It is therefore more likely that activated CD4+ T cells cause bystander cell damage in these conditions.

Because there is a vigorous, chronically activated CTL response chiefly directed against a single HTLV-I antigen, Tax, it was natural to ask whether sequence variation in the *tax* gene allowed the virus to escape CTL surveillance and so to persist. We found that each of nine naturally occurring single amino-acid substitutions in Tax led to failure of recognition by CTL of an HLA-A2-restricted epitope (Niewiesk *et al.* 1995). Moreover, these putative CTL escape mutations were found significantly more often in subjects with HLA-A2, consistent with the idea that such mutations were positively selected by the HLA-A2-restricted CTL. However, the same mutations in most cases also severely impaired the transactivation ability of the Tax protein. We concluded that the Tax mutations were indeed positively selected by CTL pressure, but that they never reached fixation in the viral

population because of the impaired Tax function (Niewiesk *et al.* 1995).

7. ARE ANTI-HTLV-I CTL NECESSARY OR SUFFICIENT TO CAUSE HAM/TSP?

In an acute virus infection, a small amount of tissue damage is usually a price that is worth paying for eradication of the virus. But in a persistent viral infection, such as HIV-1, hepatitis B or HTLV-I, the balance of good and harm exerted by CTL is less certain. Indeed, Zinkernagel has proposed that anti-HIV-1 CTL contribute significantly to the depletion of CD4+ T cells associated with progression to AIDS (Zinkernagel & Hengartner 1994).

To answer the question whether CTL are necessary or sufficient to cause HAM/TSP, we measured the frequency of HTLV-I-specific CTL in HAM/TSP patients and asymptomatic carriers in three ways: (i) the proportion of patients in whom activated HTLV-I-specific CTL could be detected in freshly isolated lymphocytes (Daenke *et al.* 1996); (ii) by limiting dilution analysis of peripheral blood CD8+ T cells (*ibid.*); and (iii) using a tetrameric complex (Altman *et al.* 1996) of HLA-A2- β 2 microglobulin-Tax peptide as a specific ligand for CTL directed against an immunodominant HLA-A2-restricted epitope (Jeffery *et al.* 1999). All three techniques produced the same result: there was a tendency to a higher frequency of HTLV-I-specific CTL in HAM/TSP patients than in asymptomatic carriers, but the difference was not statistically significant. Some HTLV-I-infected people appear to remain asymptomatic for many years with a high frequency of HTLV-I-specific CTL in the peripheral blood. We concluded that, even if CTL are necessary for HAM/TSP pathogenesis, a high frequency of HTLV-I-specific CTL in the peripheral blood is not sufficient to cause HAM/TSP.

8. VARIATION IN HOST IMMUNE RESPONSIVENESS CAN EXPLAIN THE OBSERVATIONS

The data so far presented a paradox: if CTL do indeed select variants of HTLV-I more efficiently in asymptomatic carriers than in HAM/TSP patients, why is the frequency of virus-specific CTL not higher in asymptomatic carriers than in HAM/TSP patients? To suggest a resolution of this paradox, we proposed (Bangham *et al.* 1996; Nowak & Bangham 1996) that individuals might differ in the efficiency of their anti-HTLV-I CTL response. On this hypothesis, a 'high CTL responder' will rapidly produce specific and effective CTL in response to a given concentration of Tax; the replication of HTLV-I will be efficiently controlled, and an equilibrium will be reached with abundant CTL and a low proviral load. This individual will therefore remain unaffected by HTLV-I-associated inflammatory disease. However, a low CTL responder to Tax will be unable to control HTLV-I replication efficiently: the equilibrium virus load (and so the risk of inflammatory disease such as HAM/TSP) will be high, and the resulting high Tax antigen load provides a strong stimulus to both CTL and CD4+ T cells. As a consequence, the equilibrium abundance of HTLV-I-specific CTL shows little or no difference between HAM/TSP patients and asymptomatic carriers.

A mathematical analysis of the dynamics of virus replication and the CTL response showed (Nowak & Bangham 1996) that this explanation was logical, robust, and sufficient to explain the observations. The mathematical model accounted for the observation that anti-HTLV-I CTL were almost equally abundant in HAM/TSP patients and asymptomatic carriers. Furthermore, the model produced a number of experimentally testable predictions. One particular prediction was that there would be a positive correlation between the proviral load and the frequency of anti-HTLV-I CTL, even if the CTL were responsible for reducing the proviral load. This prediction has now been verified by experiment (Wodarz *et al.* 1999c).

We conclude that the efficiency of the host's CTL response to the virus may determine the outcome of HTLV-I infection (see below).

9. IF HTLV-I REPLICATES PERSISTENTLY, WHY DOES IT VARY LITTLE IN SEQUENCE?

Another paradox was presented by the constancy of the HTLV-I sequence. Retroviruses are propagated from cell to cell by two routes. Integrated proviruses are replicated with the host cell genome and are therefore transmitted to daughter cells. This replication involves the cellular DNA polymerase, and the nucleotide misincorporation rate is therefore very low (less than 10^{-10} substitutions per site per replication cycle). This may be called the 'mitotic' route of retroviral spread. Infectious retrovirus, however, is first transcribed from the DNA provirus by the relatively error-prone cellular RNA pol II, and then reverse transcribed by the equally error-prone viral reverse transcriptase. This second route may be called the 'infectious' route of retroviral spread. Each of these two RNA-dependent polymerases has a nucleotide misincorporation rate of about 10^{-5} substitutions per site per replication cycle. Therefore, the retrovirus can accumulate mutations much faster as the ratio of infectious to mitotic replication increases (Gojobori & Yokoyama 1985).

HIV-1, which replicates persistently at a high level by infectious spread, rapidly accumulates mutations. Because HTLV-I varies much less in sequence, it was presumed that HTLV-I replicates very little. However, a number of observations strongly implied that HTLV-I did indeed replicate persistently (reviewed in Wodarz *et al.* 1999a): the presence of Tax-Rex mRNA in freshly isolated PBMC; the persistently activated CTL response to Tax; persistent anti-HTLV-I IgM; the measurable within-isolate sequence diversity of HTLV-I; and the evidence of positive selection of Tax in asymptomatic carriers. How could these observations be reconciled?

Again, we used mathematical modelling to test possible explanations. The result was clear: when the proviral load becomes high, the supply of uninfected, susceptible cells rapidly becomes limiting, and so most new provirus-carrying cells that are produced *in vivo* arise by mitotic spread. However, infectious spread significantly increases the proviral load, and indeed may be required to maintain the very high proviral load that is observed. In fact, the model demonstrated the non-intuitive result that the greater the rate of infectious spread of the virus between cells *in vivo*, the greater the proportion, at equilibrium, of

all provirus transmission events that occur by mitotic spread. Since mitotic spread is accompanied by a much lower mutation rate, it is clear that the constancy of HTLV-I proviral sequence does not imply a lack of HTLV-I virion replication.

Is there any evidence for frequent HTLV-I replication or gene expression in peripheral blood cells? Recently we have used a highly sensitive antibody detection method, with tyramide signal amplification, to detect Tax protein expression in PBMC. The results (Moritoyo *et al.* 1999) indicate that Tax expression is detectable in up to 24% of provirus-carrying PBMC at any one time. This figure implies an intense exposure to selection by CTL, and is consistent with the observations on tax sequence diversity and with the theory summarized above.

The same mathematical analysis (Wodarz *et al.* 1999a) also offers a satisfying explanation for the observed selection effect on the *tax* gene (see above). In HAM/TSP patients, almost all of the provirus is transmitted mitotically, and is therefore unexposed to immune (CTL) selection. But in asymptomatic carriers, a higher proportion of the proviral load has arisen from infectious spread: the opportunity for CTL-mediated selection is therefore greater in asymptomatic carriers. This may contribute to the higher ratio of non-synonymous:synonymous nucleotide substitutions that we observed in the *tax* gene in asymptomatic carriers (Niewiesk *et al.* 1994; Niewiesk & Bangham 1996; see above). However, the recently observed protective effect of class I MHC alleles (see below) suggests that the efficiency of selection also correlates with the strength of the CTL response itself.

The constraint on the mutation rate of HTLV-I that is caused by the very high proviral load may also limit the rate of evolution of the virus in the population. However, in an asymptomatic carrier with a lower proviral load of HTLV-I, why does the virus not accumulate more mutations? Recent analysis (Wodarz *et al.* 1999b) suggests the following explanation. An individual with a strong anti-HTLV-I CTL response restricts HTLV-I replication and Tax expression to a low level. This in turn limits the amount of proliferation of bystander lymphocytes that is induced by HTLV-I-infected cells (Wucherpfennig *et al.* 1992). Since HTLV-I, like most retroviruses, can only efficiently infect activated or proliferating cells, the rate at which new lymphocytes can be infected is again limited.

To summarize, the rate of infectious spread of HTLV-I *in vivo* may be constrained by the lack of susceptible host cells. High immune responders have few activated cells because the viral load is low, while in low immune responders the majority of activated T cells are already infected. As a result, the virus evolves slowly, both in individuals and in the population.

10. IMMUNOGENETICS OF HTLV-I INFECTION

The mathematical modelling summarized above showed that a simple explanation of the experimental observations on the proviral load and the immune response in HTLV-I infection would be that HAM/TSP patients mount a less efficient CTL response to the Tax protein than do asymptomatic carriers. The variation between individuals in the efficiency of the immune response to a given pathogen is largely attributable to

polymorphism in genes that control the immune response (Allison 1964; Zinkernagel & Doherty 1974; Sorensen *et al.* 1988; Hill *et al.* 1991). We are therefore carrying out a candidate gene survey in Kagoshima, Japan, where HTLV-I is present in nearly 10% of adults. In this survey we pose two questions: are there variants of genes that control the efficiency of the immune response that are associated with either (i) the risk of HAM/TSP, or (ii) a high (or low) proviral load of HTLV-I? In a case-control study, we compare the genotypes of HAM/TSP patients resident in the Kagoshima prefecture with those of asymptomatic HTLV-I carriers ascertained by serological screening in the Kagoshima Red Cross Blood Transfusion Centre. The list of candidate gene polymorphisms is long. However, the major histocompatibility complex (MHC; HLA in the human) is the most important group of candidate genes, because of their central role in the immune response (McMichael 1995).

The results of class I HLA genotyping of 216 HAM/TSP patients and 197 asymptomatic carriers (Jeffery *et al.* 1998) indicated a remarkable deficiency of HLA-A*02 alleles among the HAM/TSP patients. The difference in the frequency of these alleles remained statistically significant after correction for the large number of comparisons that are made in such a study. That this result is not due to chance is strongly confirmed by the observation that asymptomatic carriers with HLA-A*02 have a proviral load about 3.5 times lower than those that lack HLA-A*02 ($p = 0.004$).

The most plausible explanation of these results is that HLA-A*02-restricted CTL are particularly efficient at limiting the rate of replication of HTLV-I, and so limiting the proviral load and reducing the risk of HAM/TSP. This result is therefore consistent with the hypothesis (above) that genetically determined differences in immune (particularly CTL) responsiveness to HTLV-I influence the equilibrium proviral load and the risk of inflammatory disease. That is, asymptomatic carriers are indeed 'high immune (CTL) responders' to Tax, and HAM/TSP patients are low CTL responders to Tax. This conclusion is consistent with the observation (above) that the *tax* gene is subjected to more intense selection in asymptomatic HTLV-I carriers than in patients with HAM/TSP.

Although it has been known for nearly 25 years that class I MHC alleles control the specificity of the CTL response (Zinkernagel & Doherty 1974), there has been no clear example of protection by single class I alleles against a virus disease in any outbred population. In most virus infections, individuals differ in the dominant antigen recognized by their CTL. However, in HTLV-I infection Tax is immunodominant in the great majority of responding individuals, and HLA-A*02 alleles are present in nearly 50% of the population. We believe that this is why it has been possible to show such a protective effect of individual class I HLA alleles in HTLV-I infection.

11. CONCLUSIONS

On infection with HTLV-I, the host genetic make-up strongly influences the outcome. If the individual has a protective class I HLA genotype such as HLA-A*02, an efficient class I-restricted CTL response to Tax restricts

the provirus load and reduces both the risk of development of HAM/TSP and the risk of transmission of the virus. The same CTL select escape mutants of HTLV-I, but the mutants do not predominate in the viral population because the function of the mutant Tax protein is impaired.

The exceptionally high proviral load that is seen in HTLV-I infection, especially in HAM/TSP patients, is maintained by persistent virus production as well as mitotic division of provirus-carrying cells. The high load reduces the proportion of the virus that is exposed to immune selection pressure, and therefore favours constancy of the HTLV-I genome sequence, both in one host and (therefore) in the population. This could explain the relatively slow rate at which HTLV-I evolves in the population (Ina & Gojobori 1990), despite the persistent replication of the virus in the host.

These conclusions support the view that an anti-retroviral vaccine should elicit a strong CTL response to the virus.

We suggest that HTLV-I differs from HIV-1 in three respects that critically affect the within-host dynamics of the infection. First, there is a single highly immunodominant (though not exclusive) CTL target antigen in HTLV-I, Tax, while in HIV-1 the immunodominant target antigen recognized by CTL differs between individuals. Second, HTLV-I is not itself cytolytic, so the CTL play a more important role than the virus *per se* in limiting the lifespan of uninfected T cells. The converse may be true in HIV-1 infection (Wei *et al.* 1995). Third, the dominant CTL antigen, Tax, is intolerant of amino-acid changes, while certain HIV-1 antigens recognized by CTL can tolerate more sequence variation without loss of protein function (Zhang *et al.* 1994). HTLV-I is therefore unable to use CTL escape as a significant means of persistence *in vivo*. The advantage conferred on HTLV-I replication by Tax appears to outweigh the disadvantage that Tax elicits a strong CTL response.

HTLV-I induces proliferation of both the host cell (Itayama *et al.* 1988) and bystander lymphocytes (Wucherpfennig *et al.* 1992). It is likely (Wodarz *et al.* 1999b) that both processes contribute to maintaining the basic reproductive ratio of the virus (R_0 , the average number of cells that is infected by each HTLV-I-positive lymphocyte at the start of the infection) above unity, and so allow persistence of the infection. Transactivation of the *IL-2* and *IL-2R* genes by Tax might provide essential cytokine support for these proliferating cells, without which they would die by apoptosis.

The protective effect of class I HLA does not alone determine the outcome of HTLV-I infection, and it is likely that other polymorphic genes play a part. For example, a polymorphism that increases lymphocyte adhesion, migration or cytokine secretion might increase the risk of development of HAM/TSP at a given proviral load. We are therefore continuing a search for other polymorphisms that influence the outcome of HTLV-I infection in the endemically infected population in southern Japan.

The results summarized here do not answer the question of the pathogenesis of the inflammatory diseases associated with HTLV-I. By exclusion of less likely possibilities, we have suggested (Ijichi *et al.* 1993; Daenke & Bangham 1994; Bangham *et al.* 1996) that the most likely mechanism is bystander damage to infected cells,

caused by the activated T cells that are abundantly found in HTLV-I infection. Although HLA class I-restricted CTL reduce the chance of developing HAM/TSP, once the disease has developed, they may contribute to the bystander damage, for example by secreting toxic cytokines or enzymes. However, it is perhaps more likely that CD4+ cells play an important part in causing bystander damage, since these cells predominate in the early, most active lesions in HAM/TSP. If this is the case, certain class II HLA genes, which control the rate of proliferation of CD4+ cells, may be associated with predisposition to HAM/TSP. There is indeed some evidence that a haplotype containing HLA-DR1 and DQ5 is associated with a higher risk of HAM/TSP (Usuku *et al.* 1990; Kitze *et al.* 1998; Jeffery *et al.* 1999).

The reason for an apparent threshold in the provirus load, above which the risk of HAM/TSP rises rapidly, is unexplained. However, if CD4+ cells play an important part in a pathogenesis, it is possible to suggest a simple explanation. Since activated CD4+ cells enter the central nervous system more frequently than resting cells (Wekerle *et al.* 1986), irrespective of their antigen specificity, the rate of transit of CD4+ T cells through the parenchyma of the CNS—and indeed through other organs—may be abnormally high in all HTLV-I-infected people. During transit through the CNS, there is a finite chance that an HTLV-I-specific CD4+ cell will encounter an HTLV-I-expressing CD4+ cell, giving rise to the possibility of a self-sustaining inflammatory focus. Since the frequency of activated CD4+ cells, HTLV-I-infected CD4+ cells and HTLV-I-specific CD4+ cells are each proportional to the proviral load (to a first approximation), the chance of establishment of a self-perpetuating inflammatory lesion will rise as the cube of the proviral load. This may explain the observed form of the curve of HAM/TSP risk versus provirus load (figure 3).

The ability to estimate the rate of HIV-1 replication (Wei *et al.* 1995; Ho *et al.* 1995) has shown the extraordinary dynamism of the infection. Similar data are now needed in HTLV-I and other persistent viral infections, and, to complement the data on the virus, experimental data are needed in each case on the rate of turnover of lymphocytes and the rate of infection of host cells.

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