Table 1: Clinical and pathological characteristics of HAM/TSP.

Clinical characteristics		References
Onset	Insidious, slowly progressive	[11]
Major clinical symptoms	Spastic paraparesis Sphincter dysfunction Mild sensory disturbance in the lower extremities	[11]
Complications	Uveitis Arthritis T-lymphocyte alveolitis Polymyositis Sjögren syndrome	[14]
Mean age at onset	43.8 years	[11]
Male-to-female ratio	1 : 2.3 (male : female)	[11]
Laboratory data	Positive anti-HTLV-1 antibody in both serum and CSF Moderate pleocytosis and raised protein content in CSF	[11]
Pathological characteristics		**References**
Spinal cord	Loss of myelin and axons in the lateral, anterior, and posterior columns-predominantly at the thoracic level Perivascular and parenchymal lymphocytic infiltration with the presence of foamy macrophages, proliferation of astrocytes, and fibrillary gliosis-predominantly at the thoracic level	[27]
Brain	Perivascular and parenchymal lymphocytic infiltration with the presence of foamy macrophages, proliferation of astrocytes, and fibrillary gliosis Perivascular inflammatory infiltration and fibrosis only in the cases with active-chronic lesions in the spinal cord. The composition of cell subsets was similar both in the spinal cord and in the brain	[28]
Peripheral nerve	Varying degrees of demyelination, remyelination, axonal degeneration, regeneration, and perineurial fibrosis	[29, 30]

cells *ex vivo* [42], granzymes and perforin are more highly expressed in individuals with a low PVL [43], and the lytic efficiency of the $CD8^+$ T cell response, that is, the fraction of autologous HTLV-1-expressing cells eliminated per $CD8^+$ T cell per day, was inversely correlated with both PVL and the rate of spontaneous proviral expression [44]. These findings indicate that the CTL against HTLV-1 reduces PVL and risk of HAM/TSP. Moreover, using a combination of computational and experimental approaches, MacNamara et al. recently reported that a CTL response against HBZ restricted by protective HLA alleles such as HLA-A*02 or Cw*08, but not a response to the immunodominant protein Tax, determines the outcome of HTLV-1 infection [45].

Analysis of non-HLA host genetic factors by candidate gene approaches revealed that non-HLA gene polymorphisms also affect the risk of developing HAM/TSP (Table 2). For example, the TNF-α promoter-863 A allele [47] and the longer CA repeat alleles of matrix metalloproteinase (MMP)-9 promoter [48] predisposed to HAM/TSP, whereas IL-10-592 A [49], stromal-derived factor (SDF)-1 +801A, and IL-15 +191 C alleles [47] conferred protection against HAM/TSP. The polymorphisms in the MMP-9 and IL-10 promoters were each associated with differences in the HTLV-1 Tax-mediated transcriptional activity of the respective gene [48, 49]. However, the contributions of these non-HLA genes to the pathogenesis of HAM/TSP are largely unknown, and these data have not yet been reproduced in different populations. Further candidate gene studies together with genome-wide association studies in different ethnic populations in larger sample size may provide evidence for the association of non-HLA genes with HAM/TSP pathogenesis.

3.2. HTLV-1 Genotype and Genomic Integration Site. Although most studies of HTLV-1 genotype have reported no association between variants of HTLV-1 and the risk of HAM/TSP, Furukawa et al. reported the association between HTLV-1 *tax* gene variation and the risk of HAM/TSP [46]. The *tax* subgroup A, which belongs to cosmopolitan subtype A, was more frequently observed in HAM/TSP patients, and this association was independent of the protective effect of the HLA allele HLA-A*02. HLA-A*02 appeared to give protection against only one of the two prevalent sequence variants of HTLV-1, *tax* subgroup B which belongs to cosmopolitan subtype B, but not against *tax* subgroup A in the Japanese population [46]. Interestingly, HLA-A*02 appeared not to give protection against infection with cosmopolitan subtype A in a population in Iran [40]. Moreover, the Iranian HTLV-1 strain has a Rex protein that is 20 amino acids longer than that of the Japanese strain that belongs to cosmopolitan subtype B. Experiments are now underway to compare the functions of these Rex proteins.

Table 2: Host genetic and viral factors associated with the risk of HAM/TSP.

Factor	Condition	Effect	Reference(s)
Viral factors	HTLV-1 *tax* subgroup A	Susceptible	[46]
	Proviral load	Susceptible	[36]
Host factors			
HLA	A*02	Protective	[37, 41]
	Cw*08	Protective	[41]
	B*5401	Susceptible	[41]
	DRB1*0101	Susceptible	[37]
Non-HLA	TNF-α promoter −863 A allele	Susceptible	[47]
	longer CA repeat alleles of MMP-9 promoter	Susceptible	[48]
	IL-10 promoter −592 A allele	Protective	[49]
	SDF-1 promoter +801 A allele	Protective	[47]
	IL-15 +191 C allele	Protective	[47]

Recently, to test whether the genomic integration site determines the abundance and the pathogenic potential of an HTLV-1-positive T-cell clone, Gillet et al. reported the results of high-throughput mapping and quantification of HTLV-1 proviral integration in the host genome [50]. They mapped >91,000 unique insertion sites (UISs) of the provirus from 61 HTLV-1-infected individuals in primary PBMCs and showed that a typical HTLV-1-infected host carries between 500 and 5000 UISs in 10 μg of PBMC genomic DNA. They calculated an oligoclonality index (OCI) to quantify the clonality of HTLV-1-infected cells *in vivo* and found that the OCI did not distinguish between ACs and patients with HAM/TSP and that there was no correlation between OCI and HTLV-1PVL in either ACs or HAM/TSP patients. These results indicate that the higher PVL observed in patients with HAM/TSP was attributable to a larger number of UISs but not, as previously thought, from a difference in clonality. They also obtained evidence that the abundance of established HTLV-1 clones is determined by genomic features of the host DNA flanking the provirus. Namely, HTLV-1 clonal expansion *in vivo* is favored by a proviral integration site near a region of host chromatin undergoing active transcription, or same-sense transcriptional orientation of the provirus. Negative selection of infected clones, probably by CTLs during chronic infection, favors establishment of proviruses integrated in transcriptionally silenced DNA, and this selection is more efficient in ACs than in HAM/TSP, indicating the selection of HTLV-1-infected T-cell clones with low pathogenic potential.

4. Immune Response to HTLV-1

4.1. Innate Immune Response

4.1.1. Natural Killer (NK) Cells. Previous reports indicated that patients with HAM/TSP had both a lower frequency and a lower activity of NK cells (especially the $CD3^+CD16^+$ subset) than ACs although the results were not normalized with respect to PVL [51]. Since an important mechanism of induction of NK cell-mediated killing is recognition by the NK cell of a complex of the nonpolymorphic MHC molecule HLA-E bound to a peptide derived from the signal sequence of some other MHC class I molecules, a synthetic tetramer of HLA-E with the HLA-G signal sequence peptide was used to identify NK cells in HAM/TSP patients [52]. The results showed a significantly lower frequency of HLA-E tetramer-binding cells in HAM/TSP patients than ACs, and as in the earlier studies [51], this reduction in frequency was particularly notable in the $CD3^+$ cells, whereas there was no significant difference in the frequency of HLA-E tetramer-binding $CD3^-$ cells between patients with HAM/TSP and ACs [52]. Recent data also suggest that the frequency of invariant NKT (iNKT) cells in the peripheral blood of HAM/TSP patients is significantly decreased when compared with healthy subjects and/or ACs [53, 54]. These findings indicate that the activity of the NK or NKT cell response was associated with the absence of HAM/TSP. Interestingly, a previous uncontrolled preliminary trial of treatment of HAM/TSP with fermented milk containing viable *Lactobacillus casei* strain Shirota resulted in a significant increase in NK cell activity, with improvements in clinical symptoms [55]. Thus, circulating NK and NKT cells might also play an important role in the disease progression and the pathogenesis of HAM/TSP. Recently, it has been reported that in addition to the previously described $CD8^+$ T-cell spontaneous proliferation [56], $CD56^+$ NK cells also spontaneously proliferated *in vitro*, and spontaneous NK cell proliferation positively correlated with HTLV-1 PVL but not with the presence of HAM/TSP [57]. A hallmark of HTLV-1 infection is the *in vitro* proliferation of PBMCs when cultured in the absence of exogenous antigen or mitogen, referred to as spontaneous lymphocyte proliferation (SLP), and in HAM/TSP patients, the levels of SLP reflect the severity of the disease [58, 59]. Most of the high SLP observed in PBMCs from HAM/TSP patients is likely to be explained by a greater spontaneous expression of the provirus and consequently a greater proliferation of responding $CD8^+$ T cells in culture [56]. The greater proviral expression may be partly attributable to the impaired function and decreased number of NK cells in HAM/TSP patients. Although further

studies are required to clarify the role of NK cells in HTLV-1 infection and HAM/TSP pathogenesis, NK cells might be also an interesting candidate for future immunotherapy.

4.1.2. Interferons. Type I interferon (IFN) is a key innate immune cytokine produced by cells in response to viral infection. The type I IFN response protects cells against invading viruses by inducing the expression of interferon-stimulated genes (ISGs), which execute the antiviral effects of IFN [60]. The ISGs then generate soluble factors including cytokines that activate adaptive immunity or directly inhibit the virus itself [61]. To date, IFN-α is not only one of the effective therapeutic agents for HAM/TSP, but also known as an only therapeutic agent whose efficacy was demonstrated in randomized placebo-controlled trials [62, 63]. However, the therapeutic benefit is small, and IFN-α is not in general use in the treatment of HAM/TSP. The combination of the antiretroviral agent zidovudine (AZT) and IFN-α is also beneficial for overall survival in smoldering and chronic (i.e. indolent) ATL [64] although its efficacy has not yet been confirmed in well-designed prospective studies. It might be interesting to analyse which ISGs are changed in the course of IFN-α treatment and the functional role of ISGs as potential targets for therapy. In PBMCs of HTLV-1-infected individuals, the level of HTLV-1 mRNA is very low, and viral protein is not detectable, but these molecules are rapidly expressed after a short time in culture *in vitro* [42]. However, the mechanisms of this phenomenon are largely unknown. Recently, it has been reported that HTLV-1 expression in HTLV-1-infected T-cells is suppressed by stromal cells, that is epithelial cells and fibroblasts, in culture through type I IFNs [65]. Namely, HTLV-1 Gag protein expression was suppressed when contacted with stromal cells and restored when separated from the stromal cells. Although neutralizing antibodies against human IFN-α/β receptor only partly abrogated this phenomenon, the results indicate that the innate immune system suppresses HTLV-1 expression *in vitro* and *in vivo*, at least through type I IFN.

4.2. Antibody Response to HTLV-1. In 2002, it was reported that antibodies that recognize HTLV-1 Tax protein can cross-react with a heterogenous-nuclear-riboprotein (hnRNP-) A1, suggesting intriguing evidence for antigen mimicry in HTLV-1 infection [66]. However, subsequent analysis using Japanese samples under fully masked conditions indicated that there was no difference in the incidence of anti-hnRNP A1 Abs between HAM/TSP and other neurological diseases [67]. It is unlikely that anti-Tax Ab explains the onset or initial tissue damage of HAM/TSP, as the host protein hnRNP-A1 is not confined to the central nervous system but is widely expressed [68] and is not normally accessible to Ab attack. Anti-Tax Ab might be associated with subsequent inflammation following initial tissue damage and disruption of blood brain barrier, which is probably caused by the antiviral immune responses to HTLV-1 and induces the release of autoantigens.

In HTLV-1 infection, HAM/TSP patients generally have a higher anti-HTLV-1 Ab titer than ACs with a similar HTLV-1 proviral load [69–71]. These anti-HTLV-1 Abs often include IgM in both ACs and patients with HAM/TSP [70, 71]. These findings suggest that there was persistent expression of HTLV-1 proteins *in vivo* and the existence of an augmented humoral immune response to HTLV-1 in HAM/TSP patients. Although Ab responses to the immunodominant epitopes of the HTLV-1 envelope (Env) proteins were similar in all of three clinical groups (HAM/TSP, ATL, and ACs), reactivity to four Tax immunodominant epitopes was higher in HAM/TSP patients (71%–93%) than in ATL patients (4%–31%) or ACs (27%–37%) [72]. Among these anti-HTLV-1 antibodies, anti-EnvAb is particularly important since some anti-Env Abs have neutralizing activity against HTLV-1. Antisera raised against recombinant HTLV-1 Env polypeptides [73, 74], vaccinia virus containing HTLV-1 env gene [75, 76], immunization with neutralizing epitope peptides [77], and passive transfer of human IgG that has neutralizing activity [78, 79] were all shown to neutralize HTLV-1 infectivity. In HTLV-1 infection, the roles of HTLV-1 neutralizing Ab *in vivo* are still largely unknown. It will be interesting to examine whether HTLV-1 neutralizing Ab titres correlate with disease status and PVL in infected individuals. Since the mutation rate of HTLV-1 provirus is significantly lower than HIV-1, passive immunization with human monoclonal Ab may be beneficial and effective method to prevent HTLV-1 infection.

4.3. Cytotoxic T-Lymphocyte (CTL) Response to HTLV-1. Previous reports indicated that the HTLV-1-specific $CD8^+$ CTLs are typically abundant, chronically activated, and mainly targeted to the viral trans activator protein Tax [80]. Also, as already mentioned, the median PVL in PBMCs of HAM/TSP patients was more than ten times higher than that in ACs, and a high PVL was also associated with an increased risk of progression to disease [36, 37]. Furthermore, HLA-A*02 and HLA-Cw*08 genes were independently and significantly associated with a lower PVL and a lower risk of HAM/TSP [37, 41], and $CD8^+$ T cells efficiently kill autologous Tax-expressing lymphocytes in fresh PBMCs in HTLV-1-infected individuals [42]. These data have raised the hypothesis that the class I-restricted $CD8^+$ CTL response plays a critical part in limiting HTLV-1 replication *in vivo* and that genetically determined differences in the efficiency of the CTL response to HTLV-1 account for the risk for developing HAM/TSP. Indeed, as mentioned above (Section 3.1), MacNamara et al. [45] have shown that HLA class 1 alleles which strongly bind oligopeptides from the HBZ protein enable the host to make a more effective immune response against HTLV-1; therefore, such individuals have a lower PVL and are more likely to be asymptomatic. Moreover, another recent report showed the presence of HBZ-specific $CD4^+$ and $CD8^+$ cells *in vivo* in patients with HAM/TSP and in ACs and a significant association between the HBZ-specific $CD8^+$ cell response and asymptomatic HTLV-1 infection [81]. These findings provide strong evidence to support the hypothesis of the crucial role of CTLs and also confirm the importance of HBZ for persistent infection.

Since the frequency of HTLV-1-specific $CD8^+$ T cells was significantly higher in HAM/TSP patients than ACs [82, 83], and these cells have the potential to produce

proinflammatory cytokines [84], there is a debate on the role of HTLV-1-specific-CD8$^+$ T cells, that is, whether these cells contribute to the inflammatory and demyelinating processes of HAM/TSP, or whether the dominant effect of such cells *in vivo* is protective against disease. The analysis of gene expression profiles using microarrays in circulating CD4$^+$ and CD8$^+$ lymphocytes indicated that granzymes and perforin are more highly expressed in individuals with a low PVL [43], suggesting that a strong CTL response is associated with a low PVL and a low risk of HAM/TSP. Indeed, the lytic capacity of HTLV-1-specific CTL in patients with HAM/TSP and ACs, quantified by a CD107a mobilization assay, showed significantly lower CD107a staining in HTLV-1-specific CTL in HAM/TSP than ACs [85]. Recently, it has been reported that the high CTL avidity, which is closely associated with the lytic efficiency of CTL, correlates with low PVL and proviral gene expression [44], indicating that the efficient control of HTLV-1 *in vivo* depends on the quality of CTL, which determines the position of virus-host equilibrium and also the outcome of persistent HTLV-1 infection. However, two caveats must be made here. First, a protective role and a pathogenic role of CTLs are not mutually exclusive. Indeed, there are other examples of viral infections in which the virus-specific CTLs exert both beneficial (antiviral) and detrimental (inflammatory) effects, such as lymphocytic choriomeningitis virus (LCMV) infection in the mouse [86]. Second, it is difficult to separate cause and effect in analyzing the association between T-cell attributes and the efficiency of viral control in a persistent infection at equilibrium.

4.4. CD4$^+$ Helper T-Cell Response to HTLV-1. Antiviral CD4$^+$ T-cell responses are of central importance in driving B-cell and CD8$^+$ T-cell responses *in vivo*. The most common HTLV-1 antigen recognized by CD4$^+$ T-cells is the Env protein [87, 88], in contrast with the immunodominance of Tax in the CD8$^+$ T-cell response [89–91]. At a similar PVL, patients with HAM/TSP had significantly increased frequency of virus-specific CD4$^+$ T cells compared to ACs [88, 92]. The antiviral T-helper (Th)1 phenotype is also dominant among HTLV-1-specific CD4$^+$ T cells in both ACs and patients with HAM/TSP [93], and there is a higher frequency of IFN-γ, TNF-α, and IL-2 production by CD4$^+$ T cells in patients with HAM/TSP compared to AC of a similar PVL [93, 94]. A role for CD4$^+$ T cells in initiating and causing HAM/TSP is also consistent with the immunogenetic observations that the possession of HLA-DRB1*0101, which restricts the immunodominant epitope of HTLV-1 Env gp21, was associated with susceptibility to HAM/TSP in independent HTLV-1-infected populations in Southern Japan [37, 41] and Northeastern Iran [40]. Accordingly, a synthetic tetramer of DRB1*0101 and the immunodominant HTLV-1 Env380-394 peptide was used to analyze Env-specific CD4$^+$ T cells directly *ex vivo* [92]. The results showed that the frequency of tetramer$^+$CD4$^+$ T cells was significantly higher in HAM/TSP patients than ACs with similar PVL. Furthermore, direct ex vivo analysis of tetramer$^+$CD4$^+$ T cells from two unrelated DRB1*0101-positive HAM/TSP patients indicated that certain T-cell receptors (TCRs) Vβs were utilized and antigen-specific amino acid motifs were identified in complementarity determining region (CDR) 3 from both patients. These results suggest that the observed increase in virus-specific CD4$^+$ T cells in HAM/TSP patients, which may contribute to CD4$^+$ T cell-mediated antiviral immune responses and to an increased risk of HAM/TSP, was not simply due to the rapidly growing HTLV-1-infected CD4$^+$ T cells but was the result of *in vivo* selection by specific MHC-peptide complexes, as observed in freshly isolated HLA-A*0201/Tax11-19 tetramer$^+$CD8$^+$ T cells [95] and muscle-infiltrating cells from HAM/TSP patients and HTLV-1-infected polymyositis patients [96].

4.5. Regulatory T Cells (Tregs) in HTLV-1 Infection. Regulatory T cells (Tregs) are important mediators of peripheral immune tolerance and also play an important role in chronic viral infections. In HTLV-1 infection, it has been reported that HTLV-1 preferentially and persistently infects CD4$^+$CD25$^+$ lymphocytes *in vivo* [97], which contain the majority of the Foxp3$^+$ Tregs [98]. In HAM/TSP patients, the frequency of Foxp3$^+$ expression in CD4$^+$CD25$^+$ cells is lower than that in ACs and uninfected healthy controls [97, 99]. This is probably due to the fact that CD25 is transcriptionally induced by HTLV-1 Tax [100], which may result in the reduced proportion of Foxp3$^+$ cells in the CD4$^+$CD25$^+$ population in HTLV-1-infected individuals, especially HAM/TSP patients. It is important to note that the CD4$^+$CD25$^+$ population contains a mixture of Tregs and activated non-Tregs. Therefore, it is inappropriate to use CD25 as a marker of Tregs in HTLV-1 infection: the best current working definition of Treg phenotype is CD4$^+$Foxp3$^+$. Reports from different geographic regions indicate that the percentage of CD4$^+$Foxp3$^+$ cells is higher in the HAM/TSP patients than in ACs [101–103]. It has been reported that the high frequency of CD4$^+$Foxp3$^+$T cells in HTLV-1-infected individuals is maintained by CCL22 produced by HTLV-1-infected PBMCs [104]. The frequency of HTLV-1-negative CD4$^+$Foxp3$^+$ cells was positively correlated with the HTLV-1 proviral load [102, 105], and the CTL activity was negatively correlated with the frequency of HTLV-1-negative CD4$^+$Foxp3$^+$ cells [102], suggesting that CD4$^+$Foxp3$^+$ Tregs may impair the CTL surveillance of HTLV-1. If this is the case, activity of CD4$^+$Foxp3$^+$ cells may also determine the risk of developing HAM/TSP via increasing the HTLV-1 PVL.

4.6. Dendritic Cells (DCs). Dendritic cells are antigen-presenting cells which play a critical role in the regulation of the adaptive immune response. In HTLV-1 infection, it has been shown that the DCs from HAM/TSP patients were infected with HTLV-1 [106], and the development of HAM/TSP is associated with rapid maturation of DCs [107]. As already mentioned, one of the hallmarks of HTLV-1 infection is the spontaneous lymphocyte proliferation (SLP). Interestingly, depletion of DCs from the HAM/TSP patient's PBMCs abolished SLP, whereas supplementing DCs restores proliferation [106]; supplementing B cells or macrophages had no effect. A DC-dependent mechanism of SLP was further supported by data showing that antibodies to MHC

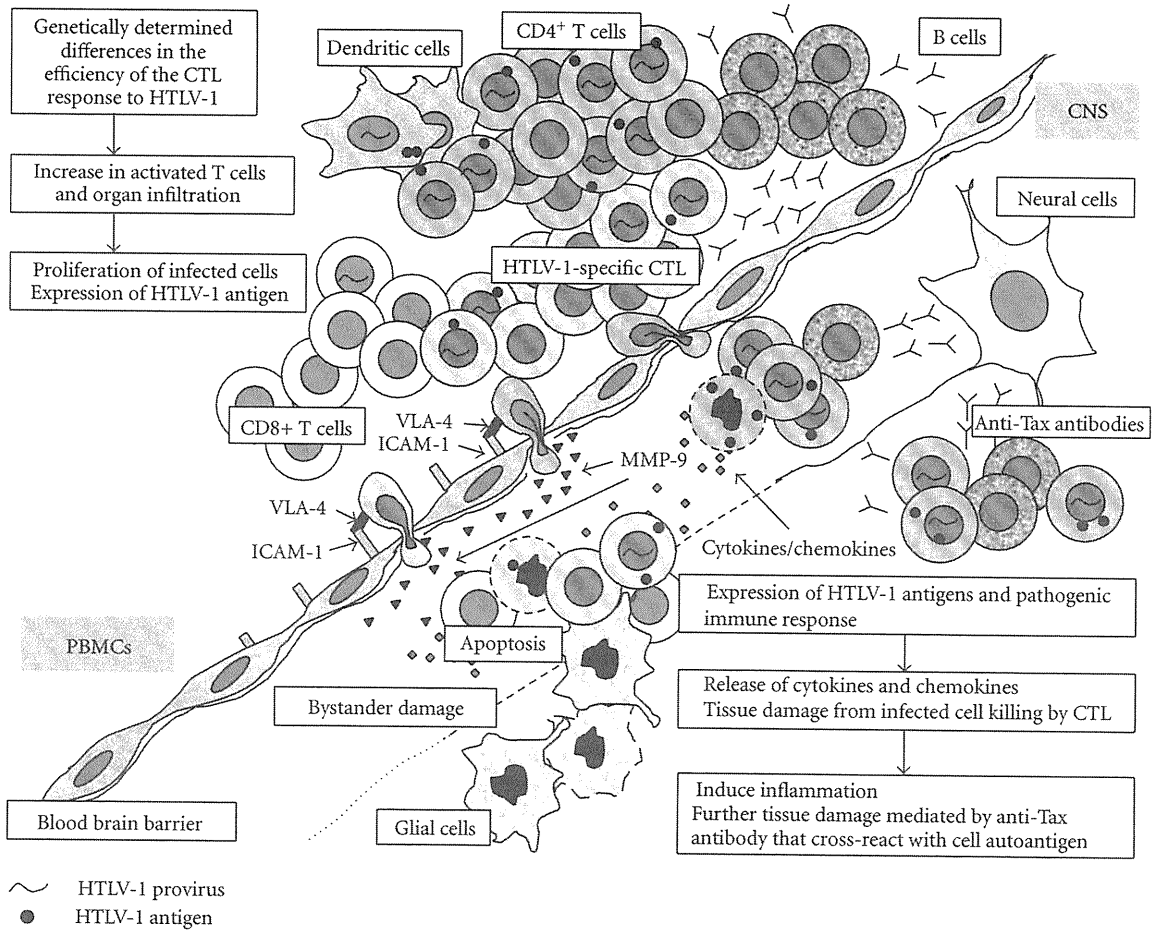

FIGURE 1: Hypothesis for the pathogenesis of human T-cell leukemia virus type-1 (HTLV-1) -associated myelopathy/tropical spastic paraparesis (HAM/TSP). Accumulating evidence suggests that the virus-host immunologic interactions play a pivotal role in HAM/TSP pathogenesis. Genetically determined less efficient CTL response against HTLV-1 may cause higher proviral load and antigen expression in infected individuals, which lead to activation and expansion of antigen-specific T-cell responses, subsequent induction of large amounts of proinflammatory cytokines and chemokines, and progression of HAM/TSP development. It is also possible that the immunoglobulin G specific to HTLV-1-Tax, which cross-react with heterogeneous nuclear ribonuclear protein-A1 (hnRNP-A1), is associated with subsequent inflammation following initial tissue damage.

class II, CD86, and CD58 can block SLP [108]. Recently, it has been demonstrated that both myeloid and plasmacytoid DCs are susceptible to infection with cell-free HTLV-1, and HTLV-1-infected DCs can rapidly transfer virus to autologous primary $CD4^+$ T cells [109]. In addition, other groups have obtained evidence that HTLV-1 transmission from DCs to T cells was mediated primarily by DC-SIGN [110], and DCs play a major part in generating and maintaining the Tax-specific $CD8^+$ T cells both *in vitro* and *in vivo* [111]. Moreover, using transgenic mouse models that permit conditional transient depletion of $CD11c^+$ DCs, and a chimeric HTLV-1 that carries the envelope gene from Moloney murine leukemia virus, Rahman et al. demonstrated the critical role of DCs in their ability to mount both innate and adaptive immune responses during early cell-free HTLV-1 infection [112, 113]. Since HTLV-1 can impair the differentiation of monocytes into DCs [114], the interaction of DCs with HTLV-1 plays a central part in the persistence and pathogenesis of HTLV-1.

5. Concluding Remarks

As shown in Figure 1, accumulating evidence suggests that the host immune response, especially the CTL response, plays a critical role in determining the risk of HAM/TSP. A less efficient CTL response against HTLV-1 may cause a higher PVL and higher antigen expression in infected individuals, which in turn lead to activation and expansion of antigen-specific T-cell responses, subsequent induction of large amounts of proinflammatory cytokines and chemokines, and progression to HAM/TSP. Since HLA class 1 genotype determines only up to 50% of HAM/TSP risk in infected people [41], it is important to discover other factors that determine the efficiency of the CTL response

to HTLV-1 and the outcome of HTLV-1 infection. Studies of the HTLV-1 receptor and DCs are also critical in the development of vaccine approaches to elicit cellular immune responses to key viral proteins such as Tax and Env to ablate HTLV-1-infected T cells. Newer approaches using genetically engineered and/or humanized mouse models for HTLV-1 infection will help to develop effective treatment and prevention of HAM/TSP in the future.

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Review Article

Double control systems for human T-cell leukemia virus type 1 by innate and acquired immunity

Mari Kannagi,[1,3] Atsuhiko Hasegawa,[1] Shuichi Kinpara,[1] Yukiko Shimizu,[1] Ayako Takamori[1] and Atae Utsunomiya[2]

[1]Department of Immunotherapeutics, Tokyo Medical and Dental University, Graduate School, Tokyo; [2]Department of Hematology, Imamura Bun-in Hospital, Kagoshima, Japan

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Human T-cell leukemia virus type 1 (HTLV-1) is the causative retrovirus of adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1-specific T-cell responses elicit antitumor and antiviral effects in experimental models, and are considered to be one of the most important determinants of the disease manifestation, since they are activated in HAM/TSP but not in ATL patients. The combination of low T-cell responses and elevated HTLV-1 proviral loads are features of ATL, and are also observed in a subpopulation of HTLV-1 carriers at the asymptomatic stage, suggesting that these features may be underlying risk factors. These risks may potentially be reduced by vaccination to activate HTLV-1-specific T-cell responses. HAM/TSP and ATL patients also differ in their levels of HTLV-1 mRNA expression, which are generally low *in vivo* but slightly higher in HAM/TSP patients. Our recent study indicated that viral expression in HTLV-1-infected T-cells is suppressed by stromal cells in culture through type-I IFNs. The suppression was reversible after isolation from the stromal cells, mimicking a long-standing puzzling phenomenon in HTLV-1 infection where the viral expression is very low *in vivo* and rapidly induced *in vitro*. Collectively, HTLV-1 is controlled by both acquired and innate immunity *in vivo*: HTLV-1-specific T-cells survey infected cells, and IFNs suppress viral expression. Both effects would contribute to a reduction in viral pathogenesis, although they may potentially influence or conflict with one another. The presence of double control systems for HTLV-1 infection provides a new concept for understanding the pathogenesis of HTLV-1-mediated malignant and inflammatory diseases. (*Cancer Sci* 2011; 102: 670–676)

It has been three decades since the discovery of human T-cell leukemia virus type 1 (HTLV-1) as the causative retrovirus of adult T-cell leukemia (ATL).[1,2] ATL develops during middle age or later mainly in a small portion of vertically HTLV-1-infected populations.[3,4] HTLV-1 also causes HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in another small population of infected individuals.[5,6] Some other inflammatory diseases such as uveitis and arthritis are also associated with HTLV-1 infection.[7,8] New therapeutic approaches such as hematopoietic stem cell transplantation (HSCT),[9,10] an antibody therapy targeting CCR4,[11] and antiviral therapy with interferon-alpha and zidovudin[12] partly improved the prognosis of ATL. However, ATL still shows high mortality, and HAM/TSP remains to be an intractable disease.

Enormous amounts of research findings have been accumulated regarding the virus-mediated pathogenesis. HTLV-1 Tax, a virus-encoded regulatory gene product, mediates cell activation, proliferation and resistance to apoptosis by transactivation through NF-κB, cAMP response element binding protein (CREB) and serum response factor (SRF), and by inactivation of tumor suppressors,[13–15] which would be involved in leukemogenesis and inflammation in HTLV-1 infection. Another minus-strand HTLV-1-encoded gene product, HTLV-1 basic leucine zipper factor (HBZ), is continuously expressed in infected cells *in vivo* regardless of the disease and may also be involved in the growth ability of infected cells.[16]

However, many unsolved questions still remain regarding the pathogenesis of HTLV-1 infection, for example, how the same virus causes totally different diseases such as ATL and HAM/TSP, why only small portions of HTLV-1-infected populations develop diseases, and why it takes more than 40 years to develop ATL. The answers to these questions would provide hints for predicting disease risks as well as aiding the development of prophylactic and therapeutic strategies.

HTLV-1-specific T-cell responses that contribute to antiviral and antitumor surveillance could be one of the most important determinants of the diseases. In fact, HTLV-1-specific T-cells are activated in HAM/TSP but not in ATL.[17–19] Oral HTLV-1 infection induces T-cell tolerance to HTLV-1 and increased proviral loads,[20,21] consistent with the epidemiological finding that vertical HTLV-1 infection is one of the risk factors for ATL.[3] Therefore, the individual status of HTLV-1-specific T-cell responses is expected to be an indicator of risk for ATL.[22] Although the pathological significance of HTLV-1-specific T-cells in HAM/TSP remains controversial,[23,24] advantages for HLA-A02-positive individuals in protection against HAM/TSP have been reported, and interpreted through the association of this HLA with strong CTL responses to a major epitope of HTLV-1 Tax.[25]

Elevation of proviral loads is also a risk factor for ATL. Given the fact that HTLV-1-specific CTLs have antiviral effects, these CTLs are likely to be one of the determinants of proviral loads.[26] However, proviral loads are also increased in HAM/TSP patients, and the correlations between proviral loads and HTLV-1-specific T-cell responses vary among studies,[27,28] suggesting the presence of additional factors for determining individual proviral loads.

Another curious finding in HTLV-1 infection is the scarcity of viral antigen expression in the peripheral blood, although the viral mRNA is barely expressed.[29] The transcription of HTLV-1 is mainly regulated by CRE-like repeats in the HTLV-1 LTR.[30] Involvement of inducible cAMP early repressor (ICER) and transducers of regulated CREB 2 (TORC2) in the inhibition of HTLV-1 transactivation has been suggested.[31,32] However, the mechanism involved in suppressing viral expression only *in vivo* has remained obscure. It is a paradox that HTLV-1 Tax contributes to the pathogenesis while Tax protein is undetectable *in vivo*. Expression of HBZ in the absence of Tax may partly

[3]To whom correspondence should be addressed. E-mail: kann.impt@tmd.ac.jp

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explain the growth advantage of infected cells,[33] but not all of HTLV-1-mediated leukemogenesis. In addition, it does not make sense that Tax-specific T-cell responses are maintained if Tax is not expressed *in vivo*. The paradox will remain until the state of viral expression and the mechanisms for suppressing HTLV-1 expression *in vivo* are clarified.

We recently found that innate immune responses, especially type-I interferons (IFNs), suppress HTLV-1 expression.[34] This integrates the issue of viral expression and the host defense system against HTLV-1, which includes innate immunity as well as acquired immunity. The presence of double control systems explains some of the paradox in persistent HTLV-1 infection, and adds new aspects to the pathogenesis of HTLV-1-mediated diseases.

Control of HTLV-1 by HTLV-1-specific T-cell responses

Antitumor surveillance by HTLV-1-specific T-cells. $CD8^+$ HTLV-1-specific CTL responses are found in many HAM/TSP patients and asymptomatic carriers (AC), but rarely in ATL patients.[17–19,35,36] These CTLs kill HTLV-1-infected cells *in vitro*, and mainly recognize HTLV-1 Tax.[18,37] The HTLV-1 envelope is also a popular target, especially for $CD4^+$ CTLs.[38] Other viral antigens, including polymerase,[39] ROF (p12) and TOF (p30/p13),[40] and HBZ,[41] have also been shown to be targets of CTLs. Elimination of $CD8^+$ cells among PBMCs from HAM/TSP patients induces HTLV-1 expression during subsequent cell culture,[42] clearly indicating that $CD8^+$ HTLV-1-specific CTLs contribute to the control of HTLV-1-infected cells.

A series of animal model experiments indicated that HTLV-1-specific T-cell responses limit the expansion of HTLV-1-infected cells *in vivo*. Oral HTLV-1 infection induced insufficiency of HTLV-1-specific T-cell responses in rats, and the HTLV-1 proviral loads were inversely correlated with HTLV-1-specific T-cell responses.[21] Re-immunization of these rats with mitomycin C-treated HTLV-1-infected cells restored HTLV-1-specific T-cell responses and reduced the proviral loads[43] (Fig. 1). In another rat model of HTLV-1-induced tumors, the otherwise fatal HTLV-1-infected lymphomas in T-cell-deficient rats were eradicated by transfer of T-cells from syngeneic rats that had been vaccinated with a Tax-encoding DNA or peptides corresponding to a major epitope for Tax-specific CTLs.[44,45]

Recent clinical reports have indicated that HTLV-1-carrying recipients after liver transplantation developed ATL under the administration of immunosuppressants.[46,47] In contrast, Tax-specific CTL responses were strongly activated in some ATL patients who obtained complete remission after HSCT, but were not observed in the same patients before transplantation.[48] These findings suggest that HTLV-1-specific T-cells, including Tax-specific CTLs, play important roles in antitumor surveillance against HTLV-1 leukemogenesis.

Insufficient HTLV-1-specific T-cell responses as a potential risk for ATL. Most HTLV-1-infected individuals are asymptomatic, and only about 5% develop ATL and <1% develop HAM/TSP.[3,49] The epidemiological risk factors for ATL include vertical transmission and increases in the number of abnormal lymphocytes or HTLV-1 proviral loads.[3,50,51] HTLV-1 proviral loads are also elevated in HAM/TSP patients.[52]

Immunological studies have suggested that insufficiency in host T-cell responses against HTLV-1 might be another risk factor for ATL.[22] A small-scale survey measuring Tax protein-specific IFN-γ production revealed a wide variety in the strengths of HTLV-1-specific T-cell responses among HTLV-1 carriers.[53] The combinations of HTLV-1-specific T-cell responses and proviral loads categorize HTLV-1 carriers into the following four groups: (i) low proviral loads with HTLV-1-specific T-cell responses; (ii) elevated proviral loads with HTLV-1-specific T-cell responses; (iii) low proviral loads with low T-cell responses; and (iv) elevated proviral loads with low T-cell responses (Fig. 2).

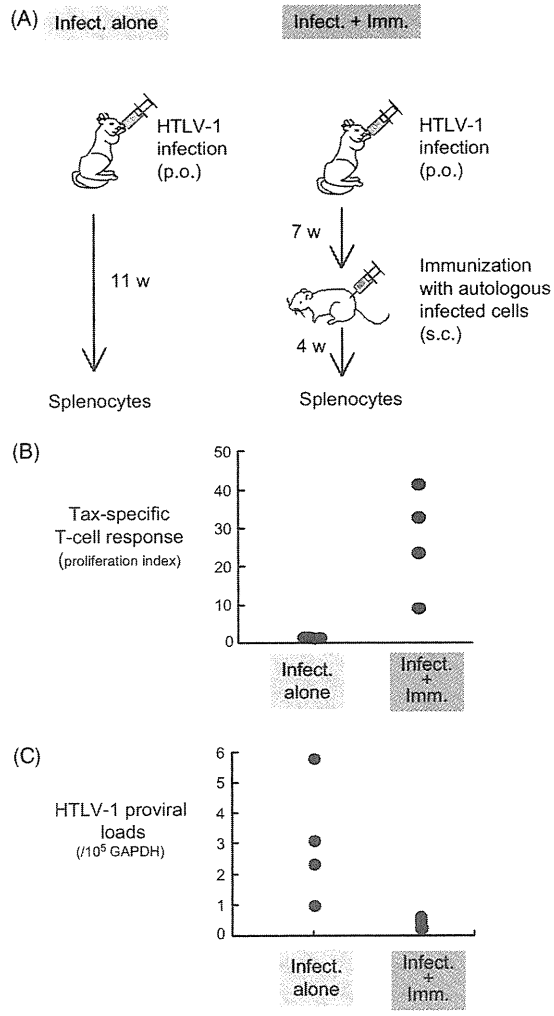

Fig. 1. Recovery of human T-cell leukemia virus type 1 (HTLV-1)-specific T-cell responses and reduction of proviral loads by re-immunization. Eight rats orally infected with HTLV-1 were divided into two groups. (A) One group was left untreated (Infect. alone) and the other was subcutaneously immunized with mitomycin C-treated HTLV-1-infected syngeneic rat T-cells (Infect. + Imm.) at 4 weeks. Spleen T-cells were harvested at 7 weeks after infection. (B,C) T-cells from the re-immunized rats (Infect. + Imm.) show elevated levels of Tax-specific T-cell proliferative responses (B) and lower proviral loads (C), compared with untreated rats (Infect. alone).[43]

Regarding these groups, ATL patients exhibit elevated proviral loads with low T-cell responses, while many, but not all, HAM/TSP patients show elevated proviral loads with high HTLV-1-specific T-cell responses. ACs are found in all four categories. It is noteworthy that small subgroups of ACs and smoldering ATL patients share a common feature with ATL patients. This indicates that the insufficiency of HTLV-1-specific T-cell responses is not merely the result of malignancy but is an underlying problem before the stage without apparent lymphoproliferation. Further follow-up studies are required to clarify whether the extent of the combination of elevated proviral loads with low T-cell responses could be a diagnostic indicator for risk of ATL.

Dissociation between proviral loads and T-cell responses. Although HTLV-1-specific T-cells have the potential to control infected cells, there are no clear correlations between

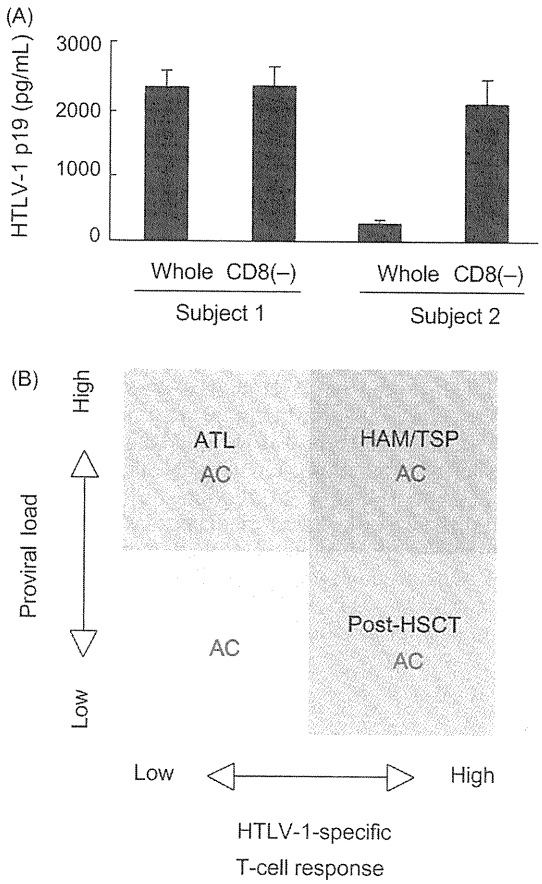

Fig. 2. Diversities in Tax-specific T-cell responses and dissociation with proviral loads in human T-cell leukemia virus type 1 (HTLV-1)-infected individuals. (A) Diversity in $CD8^+$ T-cell functions in two representative HTLV-1-infected individuals at the asymptomatic stage. Abundant amounts of HTLV-1 p19 were produced in PBMC cultures with or without $CD8^+$ T-cells in subject 1, but only after $CD8^+$ T-cell depletion in subject 2.[53] (B) A general image for the categories of HTLV-1-infected individuals at various stages according to the combinations of HTLV-1-specific T-cell responses (*x*-axis) and proviral loads (*y*-axis) is shown schematically. AC, asymptomatic carriers; ATL, adult T-cell leukemia; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HSCT, hematopoietic stem cell transplantation.

proviral loads and HTLV-1-specific T-cell responses among HTLV-1-infected individuals. This is not surprising because both the proviral loads and T-cell responses are high in HAM/TSP patients. The proviral loads may be negatively correlated with T-cell responses only within an individual but not among individuals. Several other reports have indicated various findings concerning this issue. For example, a study measuring IFN-γ-producing $CD8^+$ HTLV-1-specific CTLs indicated a positive correlation with proviral loads in HAM/TSP patients but not in ACs,[28] while a study evaluating $CD8^+$ CTL function by *ex vivo* clearance of infected cells showed negative correlations with low proviral loads within an AC or a HAM/TSP group,[42] and another study indicated an association of higher frequency of tetramer-binding Tax-specific CTLs with low proviral loads in ACs.[27] Such inconsistent results suggest the presence of certain other determinants of proviral loads in addition to HTLV-1-specific CTLs.

The HTLV-1 proviral loads reflect the number of infected cells in the peripheral blood. Expansion of HTLV-1-infected cells *in vivo* occurs through both *de novo* infection and proliferation of infected cells.[54] The number of $CD4^+$ $FoxP3^+$ cells,[55] the frequency of iNKT cells,[56] or MHC-I favorable for HBZ-specific T-cell responses[41] have been suggested to influence HTLV-1 proviral loads.

In HTLV-1-infected rats, however, the proviral loads are inversely correlated with HTLV-1-specific T-cell responses.[21] One reason for the discrepancy between humans and rats may be the genetic heterogeneity in humans. It appears that, under the homogeneous genetic background in the experimental rat system, the influence of insufficient HTLV-1-specific T-cell responses may appear more clearly than in humans, allowing *de novo* infection and proliferation of HTLV-1-infected cells *in vivo*. The dissociation of proviral loads and HTLV-1-specific T-cell responses in humans suggests that additional determinants of proviral loads may vary genetically among individuals. As described in the next section, we suppose that innate immunity could be a candidate for this effect.

Control of HTLV-1 by innate immunity

Status of HTLV-1 expression *in vivo*. Since HTLV-1-specific antibodies and T-cells are maintained in HTLV-1-infected individuals, viral expression must occur somewhere *in vivo*. This notion is further supported by the emergence of Tax-specific CTL responses in HTLV-1-uninfected donor-derived hematopoietic systems reconstituted in recipient ATL patients after HSCT.[48,57] However, HTLV-1 mRNA but not viral proteins are detectable in PBMCs freshly isolated from HTLV-1-infected individuals. The levels of HTLV-1 mRNA are higher in HAM/TSP patients than in ACs,[58] but viral proteins are still undetectable. Only a few reports have indicated HTLV-1 protein expression *in situ*.[59]

HTLV-1 expression in ATL cells immediately after isolation from the peripheral blood is very low, and becomes significantly induced after culture for some hours *in vitro*.[60,61] This phenomenon is observed in about one half of ATL patients regardless of the disease severity.[62] Viral induction after *in vitro* culture does not occur in the other one half of ATL patients, probably because of genetic and epigenetic changes in the viral genome.[63–65] Rapid induction of viral expression after *in vitro* culture has also been observed in PBMCs from HAM/TSP patients and ACs,[66] indicating that there must be a common mechanism for transiently suppressing HTLV-1 expression *in vivo* regardless of the diseases.

Suppression of HTLV-1 expression by type-I IFN responses. Recently, we found that type-I IFN responses are involved in the suppression of HTLV-1 expression.[34] When HTLV-1-infected T-cell line cells were co-cultured with stromal cells such as epithelial cells and fibroblasts, HTLV-1 mRNA and proteins were markedly decreased in HTLV-1-infected cells. Similarly, induction of HTLV-1 expression in cultures of primary ATL cells was also suppressed by co-culture with stromal cells. Type-I IFNs were involved in the stromal cell-mediated suppression of HTLV-1 expression, because it was partly neutralized by anti-IFN-α/β receptor antibodies. Since efficient HTLV-1 expression is dependent on transactivation of its own LTR by Tax protein,[30,67] limitation of this protein below a certain level will lead to the maintenance of HTLV-1 expression at low levels. Stromal cells reduced viral expression via type-I IFNs, but did not reduce cell growth and even supported it by unknown mechanisms.[34,68]

It has been reported that plasmacytoid dendritic cells (pDCs), a major producer of type-I IFNs, are susceptible to HTLV-1 infection.[69,70] In ATL patients, pDCs are decreased in number and also lack the ability to produce IFN-α.[69] A recent report indicated that pDCs generate type-I IFNs mainly through TLR7 recognition of HTLV-1 RNA.[71] The precise mechanisms of the HTLV-1-mediated IFN responses remain to be clarified.

In addition to recombinant IFN-α and IFN-β, recombinant IFN-γ was also capable of reducing HTLV-1 expression to

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lesser extents in HTLV-1-infected cell lines.[34,72] Participation of type-II IFN-producing cells other than stromal cells in HTLV-1 suppression *in vivo* is also conceivable.

Potential involvement of type-I IFNs in HTLV-1 suppression *in vivo*. In *in vitro* experiments, co-cultured stromal cells suppressed viral expression in HTLV-1-infected cells. Interestingly, when infected cells were re-isolated from the co-cultures, viral expression was restored to the original level over the following 48 h (Fig. 3).[34] This observation shows a striking similarity to the rapid induction of HTLV-1 expression in freshly isolated ATL cells after culture *in vitro*.

Involvement of type-I IFN responses in the suppression of HTLV-1 expression *in vivo* was confirmed using interferon regulatory factor-7-KO mice, which are deficient in most type-I IFN responses. Viral expression in HTLV-1-infected cells was significantly suppressed when the infected cells were intraperitoneally injected into WT mice but not into interferon regulatory factor-7-KO mice.[34]

It is speculated that the levels of viral expression in HTLV-1-infected lymphocytes may differ among various tissues depending upon the strength of IFN responses. Thus far, there is little information regarding HTLV-1 expression in various tissues. In transgenic mice with an HTLV-1 LTR-driven construct of the pX gene, expression of the transgene was only observed in limited organs including the central nervous system, eyes, salivary glands and joints.[73] It is intriguing that all of these tissues are involved in human inflammatory diseases related to HTLV-1 infection. Such coincidences suggest the involvement of HTLV-1 gene expression in the pathogenesis of these inflammatory diseases.

Double control of HTLV-1 by innate and acquire immunity

Relationship between acquired and innate immune control in HTLV-1 infection. At the primary infection, type-I IFNs generally play a critical role in limiting viral replication, and have positive effects on antigen presentation by activating DCs, inducing type-II IFN, and upregulating MHC-I, which subsequently augments T-cell responses.[74] However, the role of type-I IFNs in the chronic phase of viral infection may not always be positive. In HIV-1 infection, type-I IFNs may be a progressive factor for the disease by accelerating T-cell exhaustion.[75]

Suppression of HTLV-1 expression by type-I IFNs may reduce the efficacy of T-cell-mediated surveillance against HTLV-1-infected cells, because T-cells require viral proteins for recognition. On the contrary, if the IFN-mediated suppressive system is insufficient, HTLV-1-specific T-cell responses will be activated in response to viral antigens.

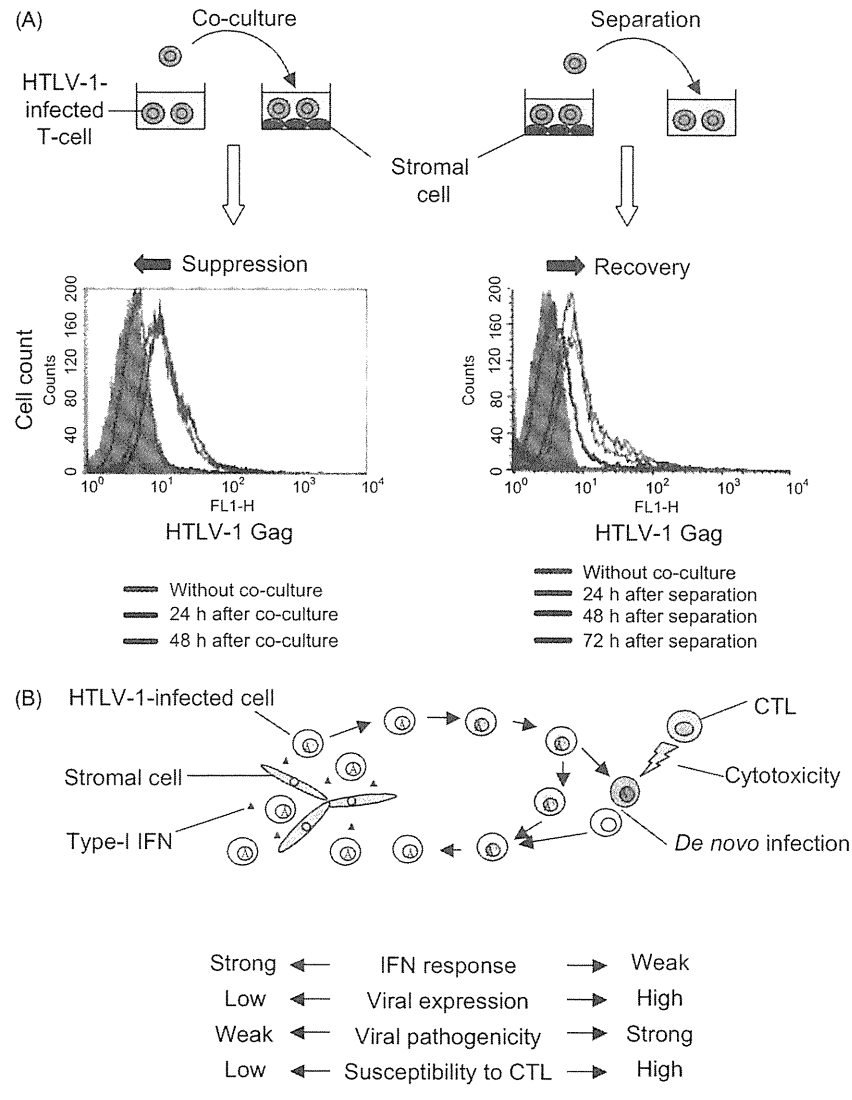

Fig. 3. Reversible suppression of human T-cell leukemia virus type 1 (HTLV-1) expression by innate immunity. (A) When IL-2-dependent HTLV-1-infected cells are co-cultured with 293T cells, intracellular HTLV-1 Gag proteins in the infected cells are decreased within 48 h (left panel). When the infected cells are re-isolated and further cultured on their own, Gag expression is recovered within 48 h (right panel).[34] (B) Scheme of the presumed status of HTLV-1-infected cells *in vivo*. Viral expression (indicated as pink) would be suppressed in tissues with strong IFN responses (left) and increased in tissues with weak IFN responses (right). CTL function, if any, is only effective upon viral expression, resulting in an infected cell reservoir without viral expression (left) and a T-cell surveillance system with low efficiency (right).

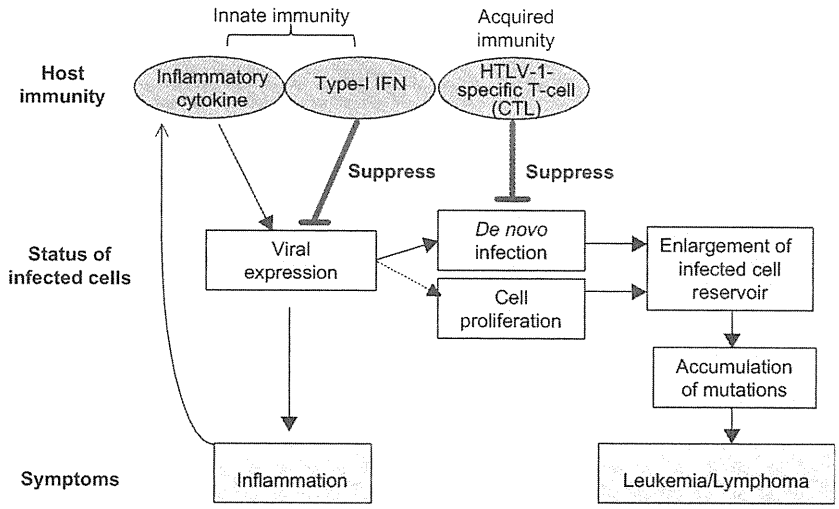

Fig. 4. Hypothetical relationships among the host immunity, status of human T-cell leukemia virus type 1 (HTLV-1)-infected cells and symptoms. HTLV-1-infected cells are controlled by at least two systems: type-I IFNs (innate immunity) and HTLV-1-specific T-cells (acquired immunity). The former suppress viral expression and the latter kill infected cells. An increase in viral expression would accelerate inflammation, increase the number of infected cells through *de novo* infection and activate HTLV-1-specific T-cells that determine an equilibrium level of proviral load within an individual. Viral expression may be a positive, but not absolute, factor for cell proliferation. When the viral expression is well controlled, the viral pathogenesis will proceed slowly, and may not be apparent until infected cell clones with a malignant phenotype finally emerge from the enlarged infected cell reservoir. Without proper T-cell responses, the emergence of such clones may occur earlier, because they would have more chance to survive.

The relationship between innate and acquired immunity may also differ among tissues. In tissues with strong IFN responses, viral expression in the infected cells would be suppressed and CTLs would ignore these cells. However, in tissues with weak IFN responses, infected cells would express viral antigens to be recognized by CTLs (Fig. 3). These presumptions can explain the status of HTLV-1-infected cells *in vivo*, which comprises a large reservoir of infected cells without viral expression and a low-efficiency surveillance system by CTLs that can only work on limited occasions.

Potential relationship between disease manifestation and innate and acquired host immunity in HTLV-1 infection. Although suppression of HTLV-1 expression may partly interfere with the efficacy of T-cell immunity, it may contribute to a slowing down of the Tax-mediated pathogenesis, tumorigenesis and inflammation (Fig. 4). In a rat model, shRNA-mediated suppression of Tax in HTLV-1-transformed cells rendered these cells resistant to Tax-specific CTLs but also reduced their ability for tumorigenesis *in vivo*.[76] Continuous suppression of HTLV-1 expression in humans may have a similar decelerating effect against Tax-mediated tumorigenesis. This might be a reason why it takes so long for ATL to develop. So long as the viral expression is well controlled, the viral pathogenesis may not be apparent until malignant cell clones finally come through the process of clonal evolution in the infected cell reservoir. Without proper T-cell responses, the emergence of such clones may occur earlier, because they would have more chance to survive.

HAM/TSP patients show elevated levels of viral expression for an unknown reason. Increased levels of inflammatory cytokines could be either a cause or a result of this phenomenon. The involvement of HTLV-1 proviral integration sites in transcription units in elevated viral expression has also been suggested.[77] An experimental rat model of HAM/TSP using a certain WKAH strain exhibits increased Tax mRNA expression in the spinal cord without T-cell infiltration,[78] suggesting that viral expression is a primary event while T-cell responses are not. Further studies revealed that this particular rat strain contains mutations in the promoter region of the IL-12 receptor, which potentially lead to reduced IFN-γ production in the spinal cord.[72] The associations of genetic factors related to the IFN system with HAM/TSP patients have remained obscure. Very recently, a gene expression profiling study indicated that expression of suppressor of cytokine signaling 1 (SOCS1) is upregulated in HAM/TSP patients and ACs, and is positively correlated with high HTLV-1 mRNA loads.[79]

Conclusions

HTLV-1 is controlled by both acquired and innate immunity. HTLV-1-specific T-cells contribute to antitumor surveillance, and type-I IFNs contribute to silencing viral expression. The presence of the double control systems with partial conflicts would explain some of the puzzles in HTLV-1 infection, such as the transient suppression of viral expression *in vivo*, apparently reciprocal occurrence of ATL and HAM/TSP, inconsistent correlations of proviral loads with T-cell responses, and a long incubation period.

Insufficient T-cell responses are regarded as a risk factor for ATL, and vaccines that augment HTLV-1-specific T-cell responses would be beneficial in reducing the risk in a subpopulation of HTLV-1 carriers exhibiting insufficient T-cell responses and elevated proviral loads.

Innate immune responses in HTLV-1 infection should be further investigated, because they could be another important determinant of disease manifestation and represent therapeutic targets in HTLV-1-related diseases.

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Functional impairment of Tax-specific but not cytomegalovirus-specific CD8$^+$ T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers

Ayako Takamori[1], Atsuhiko Hasegawa[1*], Atae Utsunomiya[2], Yasuhiro Maeda[3,9], Yoshihisa Yamano[4], Masato Masuda[5], Yukiko Shimizu[4], Yotaro Tamai[1], Amane Sasada[1], Na Zeng[1], Ilseung Choi[6], Naokuni Uike[6], Jun Okamura[7], Toshiki Watanabe[8], Takao Masuda[1] and Mari Kannagi[1]

Abstract

Background: Human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in a small percentage of infected individuals. ATL is often associated with general immune suppression and an impaired HTLV-1-specific T-cell response, an important host defense system. We previously found that a small fraction of asymptomatic HTLV-1-carriers (AC) already showed impaired T-cell responses against the major target antigen, Tax. However, it is unclear whether the impaired HTLV-1 Tax-specific T-cell response in these individuals is an HTLV-1-specific phenomenon, or merely reflects general immune suppression. In this study, in order to characterize the impaired HTLV-1-specific T-cell response, we investigated the function of Tax-specific CD8$^+$ T-cells in various clinical status of HTLV-1 infection.

Results: By using tetramers consisting of HLA-A*0201, -A*2402, or -A*1101, and corresponding Tax epitope peptides, we detected Tax-specific CD8$^+$ T-cells in the peripheral blood from 87.0% of ACs (n = 20/23) and 100% of HAM/TSP patients (n = 18/18) tested. We also detected Tax-specific CD8$^+$ T-cells in 38.1% of chronic type ATL (cATL) patients (n = 8/21), although its frequencies in peripheral blood CD8$^+$ T cells were significantly lower than those of ACs or HAM/TSP patients. Tax-specific CD8$^+$ T-cells detected in HAM/TSP patients proliferated well in culture and produced IFN-γ when stimulated with Tax peptides. However, such functions were severely impaired in the Tax-specific CD8$^+$ T-cells detected in cATL patients. In ACs, the responses of Tax-specific CD8$^+$ T-cells were retained in most cases. However, we found one AC sample whose Tax-specific CD8$^+$ T-cells hardly produced IFN-γ, and failed to proliferate and express activation (CD69) and degranulation (CD107a) markers in response to Tax peptide. Importantly, the same AC sample contained cytomegalovirus (CMV) pp65-specific CD8$^+$ T-cells that possessed functions upon CMV pp65 peptide stimulation. We further examined additional samples of two smoldering type ATL patients and found that they also showed dysfunctions of Tax-specific but not CMV-specific CD8$^+$ T-cells.

Conclusions: These findings indicated that Tax-specific CD8$^+$ T-cells were scarce and dysfunctional not only in ATL patients but also in a limited AC population, and that the dysfunction was selective for HTLV-1-specifc CD8$^+$ T-cells in early stages.

* Correspondence: hase.impt@tmd.ac.jp
[1]Department of Immunotherapeutics, Tokyo Medical and Dental University, Tokyo, Japan
Full list of author information is available at the end of the article

Background

Human T-cells leukemia virus type 1 (HTLV-1) is the causative agent of a highly aggressive $CD4^+$ T-cell malignancy, adult T-cell leukemia (ATL)[1,2]. As many as 10 million individuals are thought to be infected worldwide, in southern Japan, the Caribbean basin, South America, Melanesia, and equatorial Africa[3]. Unlike human immunodeficiency virus (HIV), the majority of HTLV-1-infected individuals are clinically asymptomatic during their lifetime. However, approximately 5% develop ATL, and another 2-3% develop a variety of chronic inflammatory diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[4-8].

HTLV-1-specific cytotoxic T-lymphocytes (CTLs) are thought to play a pivotal role in containing the proliferation of HTLV-1-infected T-cells[9,10]. Tax is known to be the dominant target antigen for HTLV-1-specific CTLs[10-13], and a high frequency of Tax-specific CTLs can be detected in HAM/TSP patients and some asymptomatic HTLV-1 carriers (ACs)[10-14]. However, ATL patients show general immune suppression[15], reduced frequency and dysfunction of Tax-specific CTLs[16,17]. Regulatory T cell (Treg)-like function of $FoxP3^+$ ATL cells and diminished function of dendritic cells may be involved in the immune suppression in ATL patients [18,19], but the precise mechanism is not yet clarified. We previously demonstrated that a fraction of ACs also exhibit reduced T-cell responses against Tax protein [20]. These observations suggest that the reduced HTLV-1-specific T-cell response might be an underlying risk of ATL development, but not the result of ATL. However, it is unknown how the function of HTLV-1-specific $CD8^+$ T-cells becomes impaired in a small percentage of ACs and whether its dysfunction is specific for HTLV-1 antigen or due to general immune suppression.

During chronic stage of infection with several viruses, such as HIV and hepatitis C virus (HCV), virus-specific CTLs gradually lose their cytotoxic activity, the ability to proliferate and secrete a diverse profile of cytokines, ultimately leading to exhaustion, anergy or even deletion of these cells[21-26]. Programmed death-1 (PD-1), a negative regulator in the CD28 superfamily, has recently been shown to be highly expressed on virus-specific T-cells during many chronic viral infections[27-29]. It has also been reported that the interaction of PD-1 with PD-ligand 1 (PD-L1) negatively regulates cytokine production and proliferation of T-cells[30,31]. A previous report indicates that PD-1 is up-regulated on the dominant Tax-specific CTLs in ATL patients and ACs and that immune regulation through the PD-1/PD-L1 pathway may be involved in the dysfunction of HTLV-1-specific CTLs in ATL patients[32].

Studies on memory T-cell differentiation have shown that phenotype, function, and homeostasis of memory T-cells vary for different persistent virus infections[33]. Central memory T-cells (T_{CM}; $CD45RA^-CCR7^+$) are elicited by non-persisting virus that provide transient antigen stimulation, such as in Influenza virus infection. In contrast, effector memory T-cells (T_{EM}; $CD45RA^-CCR7^-$) predominate when relatively high levels of antigen persist, such as in HIV infection. Terminally differentiated memory (T_{Diff}; $CD45RA^+CCR7^-$) can be seen when antigen persists at a low level, such as in cytomegalovirus (CMV) infection. In HTLV-1 infection, it has been reported that dominant Tax-specific CTLs in HAM/TSP patients consist of T_{EM} and T_{Diff} compartments[34].

We previously identified some major epitopes recognized by HTLV-1-specific CTLs in infected individuals carrying HLA-A2, -A11, or -A24[12,35,36]. These allowed us to monitor HTLV-1-specific CTLs and analyze their functions *ex vivo*, by using antigen/HLA tetrameric complexes. In this study, we demonstrate that IFN-γ production and proliferative capacity of tetramer-binding Tax-specific $CD8^+$ T-cells were severely impaired not only in ATL patients but also in a minor population of asymptomatic HTLV-1 carriers (ACs). Importantly, the T-cell dysfunction at the asymptomatic stage was selective for HTLV-1 but not for CMV antigen. In addition, severely impaired HTLV-1-specific but not CMV-specific $CD8^+$ T-cells responses were also observed in patients diagnosed as smoldering ATL, the clinical condition of which is close to that of AC. The dysfunction of HTLV-1-specific $CD8^+$ T-cells in an early clinical stage implies HTLV-1-specific immune suppressive mechanism might be an underlying risk for ATL.

Results

Incidence and frequency of Tax-specific $CD8^+$ T-cells in ACs, and HAM/TSP and cATL patients

In 23 ACs and 18 HAM/TSP and 21 cATL patients carrying HLA-A2, -A11 and/or -A24 alleles, we evaluated the frequencies of Tax-specific $CD8^+$ T-cells by using cognate Tax/HLA tetramers (Figure 1 and Table 1). Tax-specific $CD8^+$ T-cells were detected in 87.0% of ACs and all HAM/TSP patients tested. In contrast, only 38.1% of cATL patients have detectable frequencies of Tax-specific $CD8^+$ T-cells (Table 1). Figure 1B shows that the average frequency of Tax-specific $CD8^+$ T-cells in the $CD8^+$ T-cells of cATL patients (n = 21, 0.90% range: 0%-9.45%) was significantly lower than that in ACs (n = 23, 2.37%, range: 0%-8.23%, $P = 0.0023$). HAM/TSP patients had the highest average frequency of Tax-specific $CD8^+$ T-cells among the three groups (n = 18, 8.88%, range: 1.86%-29.9%, $P = 0.0001$; vs. AC, $P < 0.0001$; vs. cATL patients), which is consistent with

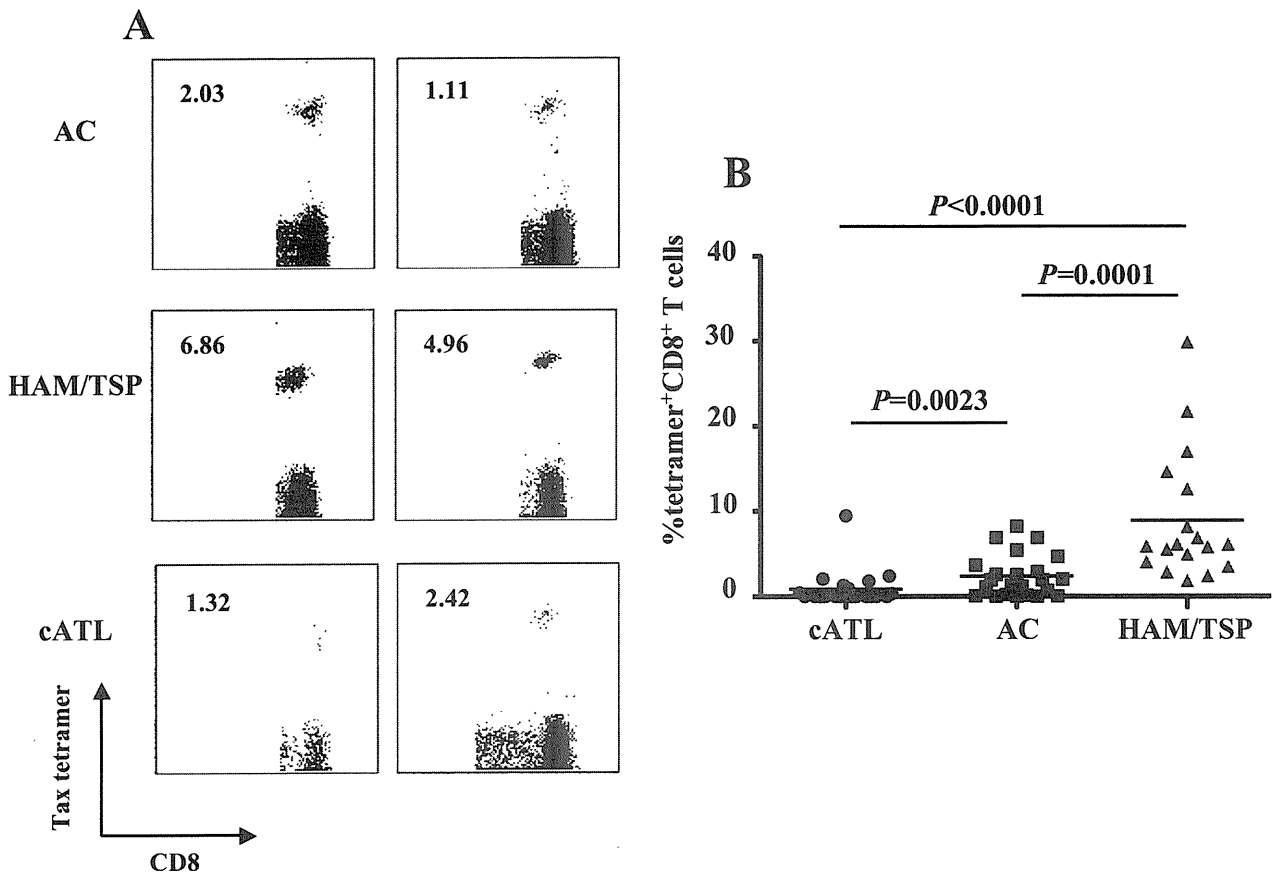

Figure 1 Incidence and frequency of Tax-specific CD8$^+$ T-cells in ACs, and HAM/TSP and cATL patients. (A) Whole blood or PBMCs from AC (top), and HAM/TSP (middle) and cATL (bottom) patients were stained with Tax/HLA tetramer. Number indicates the percentage of tetramer$^+$ cells in CD8$^+$ T-cells. (B) The percentage of Tax-tetramer$^+$ CD8$^+$ T-cells in AC (n = 23), and HAM/TSP (n = 18) and cATL (n = 21) patients. *P* value was determined by the Mann-Whitney *U* test. Horizontal bars indicate the average percentage of Tax-tetramer$^+$ CD8$^+$ T-cells for the group.

previous reports [10,17,37]. It is of note that Tax-specific CD8$^+$ T-cells are detectable even in cATL patients, although the frequency is very low.

Impaired cell proliferation and IFN-γ production of Tax-specific CD8$^+$ T-cells in cATL but not HAM/TSP patients

We next examined IFN-γ production and cell proliferation of Tax-specific CD8$^+$ T-cells in HAM/TSP and cATL patients (Figure 2A). Intracellular IFN-γ staining showed that Tax-specific CD8$^+$ T-cells in all HAM/TSP patients tested produced IFN-γ when stimulated with Tax peptide (Figure 2A). Tax-specific CD8$^+$ T-cells in those HAM/TSP patients proliferated regardless of stimulation with Tax peptide (Figure 2B). In contrast to HAM/TSP patients, IFN-γ production from Tax-specific CD8$^+$ T-cells in a cATL patient was hardly detectable even when stimulated with Tax peptide (4.8%, Figure 2A). In the same donor, Tax-specific CD8$^+$ T-cells

Table 1 The number of blood samples with detectable Tax-specific CD8$^+$ T-cells in all samples tested in this study

Tax/HLA tetramers used in this study	Disease Status		
	AC	HAM/TSP	cATL
HLA-A*0201/Tax11-19	12/14[1]	7/7	2/11
HLA-A*1101/Tax88-96	4/4	4/4	3/5
HLA-A*2402/Tax301-309	13/15	13/13	5/16
No. of tetramer$^+$ samples/total no. of blood samples[2]	20/23 (87.0%)	18/18 (100%)	8/21 (38.1%)

[1] No. of samples with detectable Tax-specific CD8$^+$ T-cells/total no. of samples carrying each HLA allele. When the frequency of tetramer$^+$ cells was more than 0.04% of CD8$^+$ T-cells, the sample was regarded as detectable.

[2] In case Tax-specific CD8$^+$ T-cells was detectable by either tetramer in a sample carrying two of three HLA-A alleles above, the sample was regarded as positive.