

## REVIEW ARTICLE

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# HTLV-I Infection: Virus structure, Immune Response to the Virus and Genetic Association Studies in HTLV-I-Infected Individuals

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## ABSTRACT

Although the structure of human T lymphotropic virus type I (HTLV-I) has been known well, the function of some proteins encoded by HTLV-I PX region is not fully understood. Furthermore, the responses of the immune system to HTLV-I remain still unknown. Most of HTLV-I-infected individuals show a strong and persistently activated cytotoxic T-cell (CTL) response to the virus. The frequency of HTLV-I specific CTL is higher in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) compared with HTLV-I carriers. However, the efficacy of the immune response determines the outcome of HTLV-I-associated diseases. Among the risk factors which contribute to the observed differences between HAM/TSP patients and HTLV-I carriers, the interaction between different genes and/or environmental factors seem to be important. These factors may also involve in outcome of HTLV-I infection in infected-individuals.

**Key words:** CTL response; Cytokine gene polymorphism; HAM/TSP; HTLV-I; MHC

## INTRODUCTION

HTLV-I is an exogenous, human retrovirus, which varies little in sequence compared with human Immunodeficiency virus type 1 (HIV-1). HTLV-I is associated with two distinct types of diseases: adult T-cell leukemia/lymphoma (ATL) and HAM/TSP in which lesions in the central nervous system (CNS) causes progressive weakness and paralysis. Studies have shown that this virus is also associated with other inflammatory diseases including: uveitis, arthritis, myositis, alveolitis and sjögren's syndrome. Among the populations infected with HTLV-I, only less than 5%

develop HAM/TSP or ATL and 95% remain asymptomatic for life long.

In many studies on HTLV-I virus, a great interest is focused on why certain individuals develop ATL or HAM/TSP. This review aims to explain briefly the structure of HTLV-I and immune response to it. We attempt to mention some of the host genetic factors which may be involved in the predisposition of HAM/TSP in HTLV-I-infected individuals.

## Genetic Structure of HTLV-I

From the recent classification, HTLV-I is classified as a complex retrovirus, in the genus delta-retrovirus of the subfamily Orthoretrovirinae.<sup>1,2</sup> Retroviruses contain RNA as the genetic material in their virion and DNA as their genetic material in the cell. The length of the HTLV-I genome is 9.032 basepair (bp).<sup>3</sup> The group

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antigens are just the same as other retroviruses-(gag), polymerase (pol), and envelope (env) genes are flanked by long terminal repeats (LTR) (Figure 1).<sup>4</sup> The LTR consists of U3, R and U5 regions. The U3 region of HTLV-I controls the virus transcription. It contains essential elements such as, the TATA box which is necessary for viral transcription, a sequence that causes termination and polyadenylation of the RNA messenger and Tax responsive elements (TRE) involved in Tax protein transcription which regulates the transcription of the HTLV-I provirus.<sup>5-9</sup> The R region overlaps the 3' of the U3 region and contains the majority of the Rex-response element. The "gag" gene encodes the virus core protein which is initially synthesized with approximately molecular weight of 53 kD (pr 53). During viral maturation this precursor is cleaved to form the matured matrix P19 (MA), the capsid P24 (CA) and the nucleocapsid P15 (NC).<sup>10,11</sup>

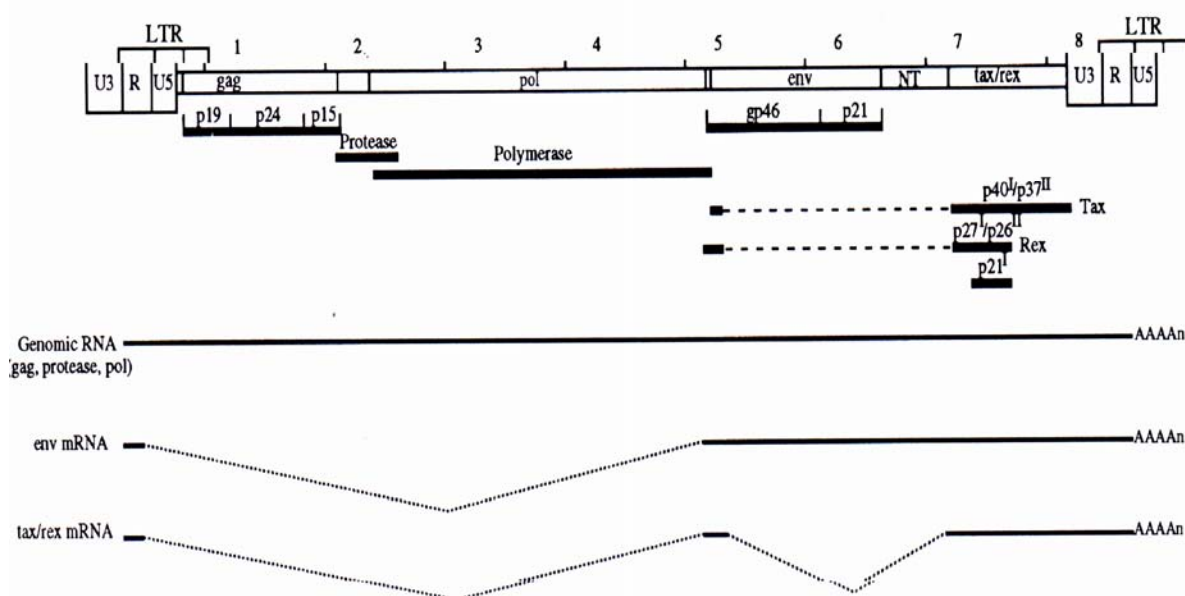
HTLV-I protease spans the 5' end of the pol region and the end of the gag region. This gene encodes a P234 amino acid protein with molecular weight of 14 kD.<sup>1</sup> The 5' end of the pol gene overlaps with the 3' end of the protease. The pol gene encodes several enzymes including: the reverse transcriptase (RT), integrase and RNAase H. The RT is essential for synthesis of viral DNA. RNAase H is responsible for degradation of RNA template and primer tRNA. The

integrase is necessary for the integration of the viral DNA into the target cell.<sup>12</sup>

The envelope proteins mediate association of the virion with the host cell and enter into it. This protein is synthesized as a precursor of 62 kD, which is cleaved into a gp45 surface protein (SU) and a gp20 transmembrane protein (TM).<sup>13</sup> The SU is a hydrophobic 45 kD glycoprotein which is responsible for binding of the virus to its receptor. The TM is a 20 kD glycoprotein which anchors the surface protein and transmembrane complex at the surface of the infected cells or virion.<sup>14</sup>

The PX region of the HTLV-I encodes two important proteins: Tax and Rex. This region is located between the env gene and 3' LTR of HTLV-I genome (Figure 1). The PX region of HTLV-I contains four open reading frames (ORFs), X-I, X-II, X-III and X-IV.

The IV open reading frame encodes the Tax gene.<sup>15,16</sup> Tax is a 40 kD protein with 353 amino acids localized in the nuclei of HTLV-I infected cells.<sup>17</sup> Tax is a trans-activating nuclear phosphoprotein, which regulates HTLV-I transcription by interacting with TRE1 and TRE2 located in the U3 region of the proviral LTR. Tax does not bind directly to these DNA elements, but it activates other transcription factors, which binds to TRE1 and TRE2.<sup>18-20</sup>



**Figure1. Structure and organization of HTLV-I and its products.** The genome of the HTLV-I is indicated at the top of the figure. Locations of the genes are shown. Sizes and positions of the proteins encoded by the provirus are shown below the genome. The bottom of the figure shows the structure of the three species of mRNA products (adapted from reference number 4).

Three major pathways of Tax transactivation have been identified: a) c-AMP responsive element binding protein and activating transcription factor (CRE/ATF) pathway b) the nuclear factor kappa binding (NFkB/Rel) pathway c) the serum response factor (SRF) pathway.<sup>21,22</sup>

Figure 2 shows pleiotropic actions of Tax protein.<sup>23</sup> In addition to HTLV-I LTR, the promoter regions of many cellular genes which contain NF- $\kappa$ B binding sites are transactivated by Tax. These include: interleukin 1 (IL-1), IL-2, IL-3, granulocyte macrophage colony stimulation factor (GM-CSF), c-fos, c-sis, c-myc, vimentin, transforming growth-factor  $\beta$ 1 (TGF-  $\beta$  1), tumor necrosis factor  $\beta$  (TNF $\beta$ ), IL-6, IL-10 and IL-15.<sup>24-26</sup> Furthermore, other genes such as nerve growth factor (NGF), interferon gamma (IFN- $\gamma$ ), parathyroid hormone-related protein, major histocompatibility complex (MHC) class I, various transcription factors (Fos/Jun) are transactivated by Tax.<sup>27</sup> The only gene known to be suppressed by Tax is a DNA repair enzyme called DNA polymerase  $\beta$ .<sup>28</sup>

Rex is a 27 kD phosphoprotein, which plays a critical role as a regulator of viral structural gene expression, but it suppresses the transcription of the viral genome. This protein is encoded by ORF III. Rex protein is also associated with intracellular transcription of unspliced and singly spliced mRNA of the virus.<sup>29, 30</sup>

ORF II reading frame encodes P13<sup>II</sup> and P30<sup>II</sup> proteins. These proteins are not essential for viral replication *in vitro*. However, *in vitro* studies have suggested that ORF II is important for viral infectivity.<sup>31-33</sup> ORFI encodes a 12 kD protein named P12<sup>I</sup>. This protein shows a weak oncogenic activity, but it has a role in the activation of host cells in the early stages of infection where interaction of P12<sup>I</sup> with the IL-2-receptor  $\beta$  and  $\gamma$  chains may involve in host cell activation, resulting in an increased rate of the MHC heavy chain and  $\beta_2$ -microglobulin complex and its transportation to the cell membrane. So, P12<sup>I</sup> plays an important role during early stages of HTLV-I infection and might enable the virus to establish infection in the host.<sup>34</sup>

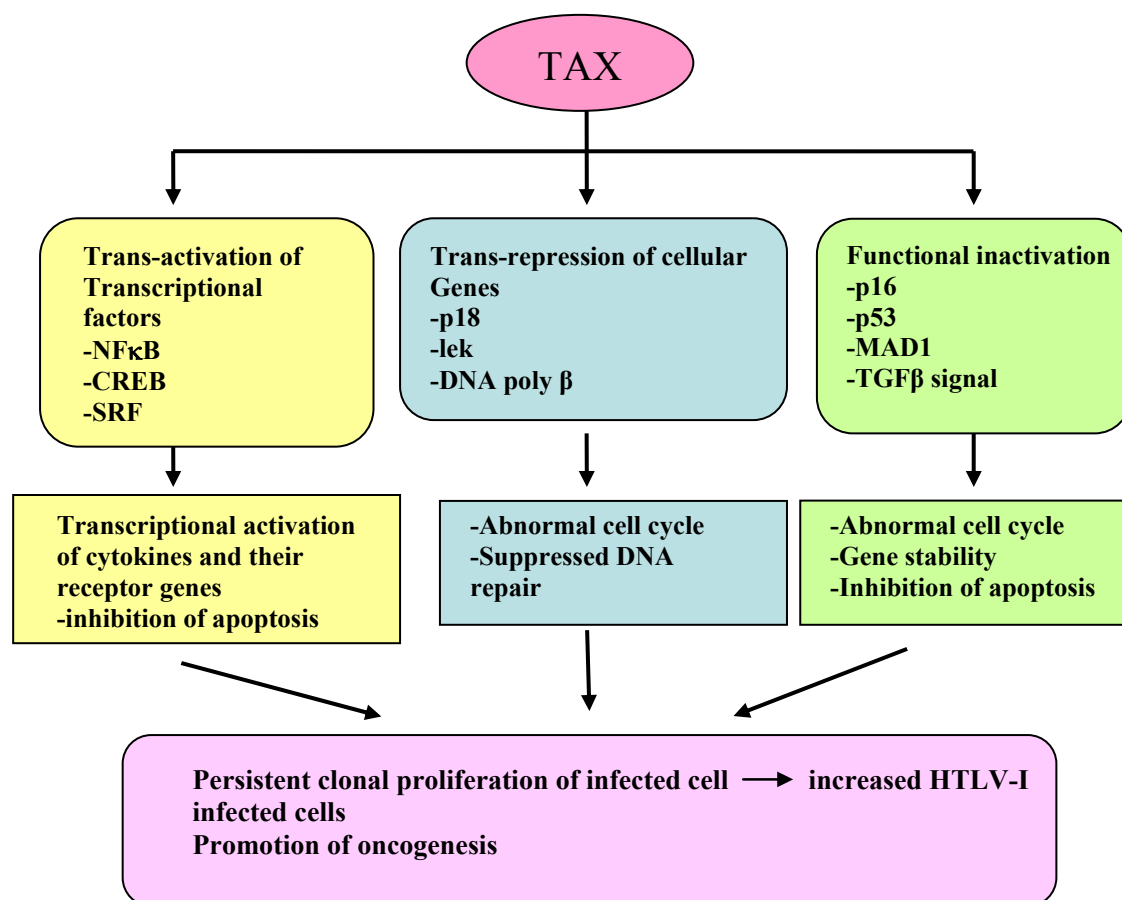


Figure 2. Summary of pleiotropic actions of Tax protein (adapted from reference number 23).

## Epidemiology

HTLV-I is endemic in many worldwide regions. It is estimated that between 10 and 20 million people of the world are infected by the virus.<sup>35</sup> There are some small clusters in the world with high HTLV-I prevalence such as: The South of Japan (Kyushu, Shikoku, Okinawa), Caribbean (Jamaica, Trinidad, Martinique, Barbados, Haiti), the equatorial regions of Africa (Ivory Coast, Nigeria, Zaire, Kenya, Tanzania), South America (Colombia), and the Middle East (north east Iran, Mashhad).<sup>36-42</sup>

One of the most important characteristics of HTLV-I is its restricted geographic seroprevalence that is remarkable in HTLV-I epidemiology. A low prevalence and sporadic cases of HTLV-I has been reported in many parts of the world such as: Taiwan, India, China, Korea, Iraq, Kuwait and the past Republics of the Soviets Union.<sup>37</sup> Seroprevalence of HTLV-I infection varies from 3-6% in Caribbean island to 6-37% in the south west of Japan.<sup>36, 43</sup> The highest geographic prevalence of HTLV-I infection in the world is the southwest district of Japan as 1.2 million people in this region have antibodies against HTLV-I.<sup>44, 45</sup>

It is known that HTLV-I seroprevalence increases with age and it is higher among subjects above 40 years of age.<sup>43, 46-48</sup>

A higher seroprevalence of about more than twice has been seen in females, because the virus transmission is more likely efficient from males to females during sexually active years.<sup>43, 48-50</sup>

The HTLV-I infection tends to be more within family members and three to four times greater than its rate in general population. It is suggested that repeated close contact and shared environment could be important in HTLV-I transmission.<sup>49, 51</sup>

## HTLV-I Infection in Iran

The sporadic cases of ATL seen among Jewish immigrants living in Israel originated from Mashhad firstly suggested a possibility of HTLV-I prevalence in this city.<sup>52</sup>

Further studies demonstrated that the presence of HTLV-I infection in this group was about 12%. The high risk of HTLV-I infection might be due to a closed and ethnically segregated population and high rate of interfamilial marriages among its members.<sup>53</sup> In a similar, study 23% of seropositivity and existence of cases of spastic para paresis was reported in Mashhadi-

born Iranian Jew.<sup>54</sup> The prevalence of HTLV-I infection in the Mashhad region is approximately 3% in the whole population and 0.7% in blood donors. So, this region of Iran was considered as a new endemic area for HTLV-I. The HTLV-I strain in Mashhad belonged to the cosmopolitan group.<sup>42,55</sup>

## Transmission

Transmission of HTLV-I occurs through three main routes:

1-transmission of HTLV-I from mother to child by breast-feeding is one the main modes of HTLV-I transmission. Studies in Japan showed that the prevalence of HTLV-I infection in children of carrier mothers was significantly higher (21%) than in children in the general population (1%). More than 85% of infected mothers had infected their children.<sup>56</sup>

The duration of breast-feeding affects the risk of HTLV-I transmission.<sup>57</sup> HTLV-I antigen in cord blood lymphocytes of babies born to healthy carriers raised a possibility to consider intrauterine transmission as an alternative pathway.<sup>58</sup> However, the HTLV-I provirus in the cord blood circulation is derived from migrated maternal cells that are not a part of blood circulation of the baby. Thus, intrauterine transmission could not be a major pathway of transmission.<sup>59</sup>

2- Another way of HTLV-I transmission is sexual contact. Heterosexual transmission is able to introduce HTLV-I infection into previously uninfected groups.<sup>60</sup> Transmission from man to woman is more frequent (60%) than woman to man (0.4%).<sup>61</sup> Like HIV, HTLV-I can be transmitted through homosexual activity.<sup>62</sup>

3- Blood transfusion is the third mode of HTLV-I transmission.<sup>63</sup> The proviral DNA in donor's blood lymphocytes acts as an infectious agent.

The probability of sero-conversion in a recipient of contaminated blood is about 44%.<sup>64</sup> Thus, it is essential to have an efficient blood screening system for HTLV-I in endemic areas to limit the HTLV-I transmission. Whole blood components, platelets and packed red blood cells, but not fresh frozen plasma, are the sources of virus transmission. Among blood cells, white blood cells are reservoirs of HTLV-I. If infected units of blood are stored for more than one week, then the probability of transmission of HTLV-I would be less.<sup>50, 65</sup> HTLV-I can also be transmitted by sharing of needles among drug addicts.<sup>66</sup>

## HAM/TSP

HAM/TSP is a chronic progressive demyelinating disease which predominately affects the spinal cord. The age of onset of disease is usually adulthood.

The symptoms of HAM/TSP are summarized by Hollsberg and Hafler as the following: "The initial symptoms of HTLV-I myelopathy include weakness and stiffness of the lower extremities, often associated with paresthesias, heaviness and persistent lower back pain. The leg weakness is usually symmetric and slowly progressive. Some patients also have weakness of the upper extremities.

Mild distal sensory loss in the lower extremities may be observed, and the paresthesias may be painful. Urinary urgency, incontinence, and bowel disturbances are also common. The sphincter impairments are probably caused by interruption of the descending sympathetic pathways in the thoracic cord, where autopsy studies have located most abnormalities associated with HTLV-I".<sup>67</sup>

## Immune Response to HTLV-I

### Humoral Immune Response

The immune response against HTLV-I is strong. In order to detect specific HTLV-I antibody against virus, screening tests such as enzyme linked immunosorbent assay (ELISA) or partial agglutination are performed. Positive results should be confirmed by western blot (WB) or polymerase chain reaction (PCR). In western blot, the reactivity of the test is examined against different products of HTLV-I such as, the products of gag (P19 or P24) and env (gp-21 or gp-46) genes.<sup>50, 68</sup> When HTLV-I infection occurs, different antibodies against gag proteins predominant with anti-24 appear within 30 to 60 days after primary infection and before the appearance of anti-19 antibodies. Most of the times, antibodies to P-21 envelop protein appear before gp-46 antibodies. Tax antibody is the latest antibody, which appears against HTLV-I virus.<sup>69</sup>

The serum antibody titer correlates with the proviral load of HTLV-I infection. Patients with HAM/TSP have high titers of specific IgG and IgA HTLV-I antibodies in sera and CSF of the HTLV-I carriers. According to western blot analysis anti-HTLV-I IgM antibodies appears in most patients and just a few of HTLV-I carriers, suggesting continuous replication of HTLV-I in HAM/TSP patients.<sup>70,71</sup> An important fact that remains unclear is: whether immune response,

antibody production and appearance of high titer of HTLV-I antibody contributes significantly either to the protection of the pathogenesis of HTLV-I or to control the equilibrium provirus load. It has been revealed that spreading of HTLV-I occurs from cell to cell without the need of extracellular enveloped virions. This fact suggests that during spreading of HTLV-I the immune response exerts a low pressure on the virus. However, env proteins are expressed on the surface of the infected lymphocytes at a low level, therefore anti HTLV-I antibody might reduce the efficiency of cell-to-cell of HTLV-I transmission.<sup>72,73</sup>

### T-Helper Lymphocyte Response to HTLV-I

It is difficult to study the CD4<sup>+</sup> response to HTLV-I infection, because the virus rapidly induces activation and proliferation of the CD4<sup>+</sup> T cells and expression of many host genes such as IFN- $\gamma$ . However, ELISPOT assay has shown that the median frequency of HTLV-I-specific CD4<sup>+</sup> T cells is 25 times greater in HAM/TSP patients compared with HTLV-I carriers with a similar proviral load.<sup>73, 74</sup> The high frequency of HTLV-I-specific CD4<sup>+</sup> cells supports the hypothesis that such cells activated by exposure to HTLV-I antigen *in vivo*, causes the inflammatory lesions which result in tissue damage in HAM/TSP.<sup>73</sup>

CD4<sup>+</sup> T cells recognize HTLV-I antigen such as env, gag, and pol proteins, respectively.<sup>74</sup> It is not clear if preferential infection of CD4<sup>+</sup> T cells by HTLV-I impairs the immune response to the virus or not. It has been reported that impaired Foxp3 expression in CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells may contribute to the development of inflammatory disease during HTLV-I infection.<sup>75</sup>

### CD8<sup>+</sup> T Cell Response to HTLV-I

CD8<sup>+</sup> T cells play a critical role in eliminating virally infected cells and limiting virus replication. Kannagi et al showed that HTLV-I infected T cells are susceptible to CD8<sup>+</sup> T cell-mediated lysis before the appearance of detectable env protein on the cell surface.<sup>76,77</sup>

HTLV-I specific cytotoxic T lymphocytes (CTL) have been detected in the blood of HAM/TSP patients and HTLV-I carriers. These cells are both CD8<sup>+</sup> human leukocyte antigen (HLA) class I and CD4<sup>+</sup> HLA class II restricted and recognize several epitopes of the HTLV-I Tax, Rex and env proteins.<sup>78, 79</sup> HTLV-I specific CD4<sup>+</sup> T cell lines have been generated after *in*

*vitro* stimulation by HTLV-I infected cells. Many of these cell lines were shown to be cytotoxic and HLA class II restricted. These CTLs recognize the HTLV-I env protein between amino acids 196 and 209. The frequency of these cells in the circulation is low; therefore, they are detected after repeated stimulation.<sup>76, 80</sup> Most patients with HAM/TSP show a high frequency of HTLV-I-specific cytotoxic T lymphocyte compared with HTLV-I carriers. HTLV-I specific CD8<sup>+</sup> T cells recognize a nine amino acid peptide of the Tax protein (Tax 11-19) associated with HLA-A<sub>2</sub> allele.<sup>81-83</sup> However, CTLs specific to other proteins such as gag, pol and env have also been identified.<sup>78, 81</sup>

Both HTLV-I carriers and HAM/TSP patients exhibit a Tax-specific response, but the frequency and efficiency of HTLV-I-specific CTLs are higher in patients with HAM/TSP than asymptomatic carriers.<sup>74, 84</sup> Circulating CD8<sup>+</sup> Tax 11-19-specific T cells were also found at high frequency in HAM/TSP Patients. These cells consist of a heterogeneous population with migratory capacity expressing different chemokine receptors such as CXCR3, IL-8 receptor A and B (CXCR1 and CXCR2) CCR5 and the IL-2R  $\beta$ -chain.<sup>85</sup>

HTLV-I Tax specific CD8<sup>+</sup> with the characteristic of memory and/or effector cells have been shown to increase in HAM/TSP patients compared with HTLV-I carriers. Chronic exposure to HTLV-I antigen may result in a sustained pool of T cell effector memory that migrate to the CNS and mediate the spinal cord injury seen in patients with HAM/TSP.<sup>86</sup> In addition, HTLV-I proviral load directly correlates with the frequency of these cells in HAM/TSP patients, suggesting that the proviral load promotes the Tax-specific cytotoxic response.<sup>87</sup> However, short-term culture of CD4<sup>+</sup> Tax expression cells showed that there is a negative correlation between Tax11-19-specific CD8<sup>+</sup> frequency and the percentage of CD4<sup>+</sup> T cells.<sup>88</sup>

The main mechanism of the CTL response against the virus remains still unknown. However, the CTL response to a persistent virus at equilibrium could lead to wide variation in the virus load between subjects whose frequency of specific CTL response is not significantly different.<sup>89</sup> This model suggests two predictions: first, polymorphism in gene such as MHC which affects the efficiency of CTL response may be associated with individual's variation in the HTLV-I proviral load and the risk of HAM/TSP. Second, CTL efficiency would be greater in individuals with a low

provirus load compared with those with a high provirus load.<sup>74</sup> Possessions of either the MHC class I HLA-A\*02 or HLA-Cw\*08 is associated with a significant reduction in both HTLV-I proviral load and the HAM/TSP risk.<sup>90</sup> The possible mechanism is that HLA-A\*02-restricted or HLA-Cw\*08 restricted CTLs are efficient at killing HTLV-I infected cell. Again these data support the idea that viral variation in CTL efficiency is associated with proviral load.<sup>91</sup> This hypothesis may raise this possibility that the immune response contributes to the tissue damage observed in the CNS. Activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells have been found in both white matter lesions and CSF of HAM/TSP patients. It is possible that HTLV-I specific antibody or T cells recognize cell HTLV-I-infected cells resident in the CNS, therefore this mechanism may contribute to the tissue damage in HAM/TSP patients.<sup>92, 93</sup>

### Clonal Expansion in HTLV-I Infection

The immune response recognizes and eliminates foreign pathogens through the actions of T cells and B cells. The diversity and specificity of both these cells enable them to react to a broad range of antigens. Accumulation of distinct  $\alpha/\beta$  TCR<sup>+</sup> T cell clonotypes after antigen stimulation have been demonstrated *in vitro* and *in vivo*. In healthy subjects, distinct clonal accumulation in peripheral blood mononuclear cell (PBMC) has been identified.<sup>94, 95</sup> CD8<sup>+</sup> T cells of HAM/TSP patients used a limited TCR V $\alpha$  and V $\beta$  genes.<sup>96</sup> Consistent with these results, Hara *et al.* showed the unique and restricted CDR3 motif in the TCR V $\beta$  gene sequence from lymphocytes in the spinal cord lesion from the autopsy of patients with HAM/TSP.<sup>97</sup> Analysis of TCRs HLA-A2 restricted HTLV-I Tax 11-19-specific CTL in HAM/TSP patients showed that there is no restricted variable region usage. However sequence analysis of the T cell TCR showed evidence for an oligoclonal expansion.<sup>98</sup> An accumulation of distinct clonotypes of  $\alpha/\beta$  TCR<sup>+</sup> peripheral blood lymphocyte (PBL) have been reported in HAM/TSP. Some of the accumulated T cells clones in the PBMC and CSF were HTLV-I Tax11-19 peptide specific. Such clones were expanded strongly after being cultured with an HTLV-I tax11-19 peptide. Expansion of immunodominant HTLV-I Tax11-19-specific T cell clones with strong cytotoxic activity has been shown in both PBL and CSF of patients with HAM/TSP.<sup>99</sup> Eriaku *et al.* demonstrated clonal

expansion in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in healthy carriers and HAM/TSP patients, but no significant differences were observed between these groups and also there was no obvious restriction in the TCR region.<sup>100</sup> The results of clonal expansion depend on the methods that have been used; suggesting that the outcome of experiments in specific diseases may be controversial. Ureta-Vidal *et al.* found that CTL directed against various epitopes of the immunodominant Tax protein significantly expanded in HAM/TSP patients,<sup>101</sup> however, another study demonstrated that TCR Vh7.2 was under-utilized and Vh12 was over-utilized in CD4<sup>+</sup> T cells of HTLV-I infected individuals compared with healthy, while there were no such differences in CD8<sup>+</sup> T cells.<sup>102</sup>

### HAM/TSP and Th1 and Th2 Balance

On the basis of immunoregulatory cytokines, T helper (Th) cells are classified in to two subtypes Th1 and Th2. Th1 cells are characterized by production of IL-2, IFN- $\gamma$ , and TNF- $\beta$ , whereas Th2 lymphocytes produce IL-4, IL-5, IL-9, IL-10 and IL-13.<sup>103</sup>

*In vitro* stimulation of CD4<sup>+</sup> circulating T cells from HAM/TSP patients makes these cells to produce a high intracellular IFN- $\gamma$ /IL-4 ratio; therefore the Th1/Th2 balance is shifted toward to the Th1 side in patients with HAM/TS.<sup>104</sup> Co-infection of HTLV-I with *strongyloides stercoralis* changes the polarization of Th2 to Th1 by increasing the level of IFN- $\gamma$  suggesting that HTLV-I induces Th1 responses.<sup>105</sup> Furthermore, short-term culture of CD4<sup>+</sup> T cells from patients with HAM/TSP and asymptomatic HTLV-I carriers demonstrated that the frequency of HTLV-I env and Tax-specific CD4<sup>+</sup> T cells was higher in HAM/TSP compared with carriers and these specific cells showed Th1 phenotype which may be involved in the pathogenesis of HAM/TSP.<sup>106</sup>

### Immunogenetic Analysis of HTLV-I Infection

Both viral factors and host genetic background may influence the outcome of HAM/TSP in HTLV-I carriers. There is no clear evidence for association between HTLV-I variants and susceptibility to HAM/TSP in carriers.<sup>107</sup> It has been suggested that an amino acid substitution in the Tax protein increases HAM/TSP risk in HTLV-I carriers, but more studies showed that mutation in the Tax gene is linked to HTLV-I subtype rather than risk of HAM/TSP.<sup>108</sup> More recently, Furukawa *et al.* reported a variant of the Tax

gene more frequently observed in patients with HAM/TSP compared with HTLV-I carriers.<sup>109</sup>

### Association of HLA with Outcome of HTLV-I Infection

Discovery of MHC restriction of the immune response to antigens has provided evidence that genetic diversity of HLA genes is related to this phenomenon. Recognition of MHC sequence and identification of allelic diversity in this system and improvements in molecular genotyping of HLA have led to analysis of the associations between HLA and diseases.

The risk factors which predispose HTLV-I carriers to develop HAM/TSP or ATL have been studied in Japanese population. Bangham *et al.*, examined the influence of HLA-DRB1 (DR1) and HLA class I in a population in an immunogenetics study in Japan. The result of HLA-DRB1 typing clearly showed that HLA-DRB1\*0101 predisposed HTLV-I carriers to develop HAM/TSP. In contrast, possession of the HLA class I alleles A\*02 and Cw\*08 show their strong effects on the outcome of HTLV-I infection through an effect on provirus load, whereas expression of HLA-B\*5401 was associated with higher proviral load, and increased the risk of developing HAM/TSP in HTLV-I infected patients.<sup>90,110</sup> The exact mechanisms responsible for the association of HLA-DRB1\*0101 and HLA-B\*54 and increase risk of HAM/TSP remain unknown. Although Jeffery *et al.* reported that HLA-A\*02 is a protective allele in HTLV-I carries, Yashiki *et al.* showed no significant difference in observed HLA-A\*02 frequencies between HAM/TSP patients and HTLV-I carriers in the same population. They suggested that this discrepancy might be attributed to either micro-heterogeneity in southern Japanese or in obtaining the samples at different times.<sup>111</sup> HLA-A\*24, Cw\*07 and B\*07 were more frequent in the HAM/TSP patients reported by Yashiki.

In a case control study we compared the frequencies of HLA-DRB1 locus alleles in Iranian patients with HAM/TSP, HTLV-I carriers and healthy controls. Our results showed that the frequency of the HLA-DRB1\*01 allele was higher in HAM/TSP patients compared with carriers.

Thus, possession of HLA-DRB1 not only predisposes Japanese's HTLV-I infected individuals, but also Iranian HTLV-I cases to develop HAM/TSP (personal communication).<sup>112</sup>

**Table 1. Significant gene polymorphisms association studies in HTLV-I-infected individuals in Japan and Iran.**<sup>124</sup>

Locus	Allele	Effect on HTLV-I infection	Ref
HLA-A	HLA-A*02 <sup>a</sup>	Decreases risk of HAM/TSP, reduces proviral load	110
HLA-B	HLA-B*54 <sup>b</sup>	Increases proviral load	110
HLA-C	HLA-Cw*08	Decreases risk of HAM/TSP, reduces proviral load	110
HLA-DR	HLA-DRB1*01	Increases risk of HAM/TSP	90, 112
TNF	TNFA -863A	Increases risk of HAM/TSP in individuals with a high proviral load	114
SDF-1 $\beta$	SDF-1 $\beta$ +801A	Decreases risk of HAM/TSP, no effect on proviral load	114
IL-15	IL-15 +191C	Reduces proviral load	114
IL-10	IL-10 -592A	Decreases risk of HAM/TSP, reduces proviral load in both HAM/TS and HTLV-I carriers	119
Perforin	Perforin +418 C	Increases risk of HAM/TSP	120
Vitamin D receptor (VDR)	VDR ApaI a	Increases risk of HAM/TSP, no effect on proviral load	121
matrix metalloproteinase-9 (MMP9)	MMP9 CA repeat	Increases risk of HAM/TSP	122
Aggrecan	Aegean variable number of tandem repeat (VNTR)	Increases risk of HAM/TSP	123

a: HLA-A\*02 allele is not associated with HAM/TSP in Iranian HTLV-I infected individuals.

b:HLA-B\*54 allele is absent in Iranian population

We also showed that the frequency of HLA-Cw\*08 was higher in HAM/TSP patients compared with the healthy controls, suggesting that this allele may be involved in outcome of HTLV-I infection in Iran. It has been shown that linkage disequilibrium of HLA class I vary among different ethnic groups and subgroups. In this case, this linkage is also more variable in HAM/TSP patients who belong to different ethnic groups,<sup>113</sup> which may suggest that in HLA association studies ethnic differences should be considered.

#### Association of Cytokine Gene Polymorphism with Outcome of HTLV-I Infection

Differences in cytokine production between HAM/TSP patients and HTLV-I carriers may be influenced by genetic polymorphisms. Such polymorphisms may identify genetic susceptibility to diseases. Cytokine gene polymorphisms, which affect the inter-individual production of the specific cytokine *in vitro*, have been candidates for many infectious and non-infectious diseases. According to this criterion, HAM/TSP patients, HTLV-I carriers and healthy controls can be classified as higher or lower producers

of a defined cytokine. Production of a higher or lower amount of a cytokine may be associated with an increased risk of HTLV-I carriers to develop HAM/TSP and cytokine production could provide evidence for the pathogenesis of HAM/TSP. Association studies such as case control studies, in which cases are compared with controls from the same populations possibly give a higher chance of detecting the influence of small genetic effects in HTLV-I infection.

A population immunogenetic study in Japanese infected individuals with HTLV-I revealed that single nucleotide polymorphisms (SNP) in cytokines and other genes related to the immune response are involved in HAM/TSP. The polymorphism in the TNF- $\alpha$  promoter at position -836 predispose towards HAM/TSP, whereas polymorphism in the 3' untranslated region in the chemokine gene stromal cell-derived factor-1 at position +801 confers protection.<sup>114</sup> More recently studies have described three new polymorphisms located in the 5'-flanking promoter/enhancer region of TNF- $\alpha$  at positions -1031\*C, -863\*A and -857\*T corresponding with



higher TNF- $\alpha$  production.<sup>115</sup> Nishimura *et al.* reported that the distribution of higher TNF- $\alpha$  producer allele – 857\*T is more frequent in HAM/TSP patients compared with controls. They also indicated that there is a linkage disequilibrium between the –857\*T allele with intermediate or low TNF- $\alpha$  production with the TNFa10 and TNFa11 microsatellites, suggesting that HAM/TSP patients genetically are not high TNF- $\alpha$  producers.<sup>116</sup> Consistent with this result, Seki *et al.* showed that there is an association between high TNF- $\alpha$  producer alleles, –1031\*C and –863\*A and the risk of HTLV-I associated disease, uveitis.<sup>117</sup> However, the frequency of the –857\*T allele is also increased in individuals with ATL compared with HTLV-I carriers<sup>118</sup> which may suggest that possession of this allele may increase the chance of HTLV-I carriers developing HAM/TSP or ATL.

We performed a case-controlled association study to determine the effect of cytokines gene polymorphism including: TNF- $\alpha$  (–308\*G/A), TGF- $\beta$ 1 (codon 10 and 25), IL-10 (–1082\*G/A), IFN- $\gamma$  +874\*T/A, IL-13 +2043\*G/A and IL-4 –590\*C/T and GM-CSF (–677\*A/C, –1440\*A/G and –1916\*T/C) on subjects with HTLV-I infection to analyse their role in HTLV-I infection. The analysis of genotype and allele frequencies of these polymorphisms showed no significant difference between HAM/TSP and HTLV-I carriers. On Japanese population it has been reported that the IL-10 –592\*A allele is associated with lower HTLV-I Tax-induced transcription activity and risk of HAM/TSP.<sup>119</sup>

In addition, we hypothesized that CTL-mediated killing through perforin may lead HTLV-I-infected individuals to remain carriers or to develop HAM/TSP. We detected a novel polymorphism in the first intron at position +418. Genotyping of patients with HAM/TSP and HTLV-I carriers showed that this polymorphism is associated with the outcome of HTLV-I infection.<sup>120</sup>

## CONCLUSION

One of the most important factors account for the variation in the proviral load between individuals infected with HTLV-I is the efficiency of the CTL response to the virus. The efficient CTLs response leads to the rapid killing of HTLV-I-infected lymphocytes and decreasing the proviral load. The factors which determine this high CTL-responsiveness to HTLV-I is not clearly known, although it is

associated with certain gene including HLA class I and II and other genetic factors, including non-HLA genes such as cytokines. Genetic background and environmental factors may determine the outcome HTLV-I associated diseases in Iranian and Japanese infected individuals. Therefore, further studies are required to clarify other gene and risk factors in both Iranian and Japanese patients.

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