HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP): Still an Obscure Disease

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Abstract: Human T-cell leukemia virus type 1 (HTLV-1) is the ethiologic agent of the neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the majority of HTLV-1–infected individuals remain asymptomatic during their lifetime, approximately one percent of this population develops a myelopathy consisting of a chronic inflammation of the white and gray matter of the spinal cord. Glucocorticoids are widely used for treatment because of their anti-inflammatory properties, improving symptoms mainly in those patients with only a few years from onset of the disease, when inflammation is more prominent. Interferon-alpha and vitamin C are other therapies presenting some benefits in clinical practice, probably due to their anti-viral and immunomodulatory activities observed ex vivo. Furthermore, inhibitors of histone deacetylase, which increase virus expression but result in a substantial decline in the proviral load, have also been proposed. This review is intended to bridge the gap between clinical and basic science by presenting recent findings on HAM/TSP disease, mechanisms of drug action, and benefits of these therapies in HAM/TSP patients.

Keywords: HTLV-1, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), glucocorticoids; neuroinflammation, pathogenesis, HAM/TSP therapy.

INTRODUCTION

Retroviruses are important causes of human morbidity and mortality and have evoked pandemics in the last three decades. Among these retroviruses, the human T-lymphotropic type 1 (HTLV-1) and type 2 (HTLV-2) have long-term silent viral persistence in the host for several decades. It is estimated that 10 to 20 million people are infected worldwide [1], and Brazil has about two million people infected with HTLV-1, corresponding to up to 2% of the infected blood bank samples in some regions of the country [2]. Although this virus has worldwide distribution, Japan, Africa, the Caribbean Basin and South America are considered to be endemic areas for HTLV-1 infection [3].

Although most HTLV-1-infected carriers remain healthy, 2.5% will eventually develop HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), adult T-cell leukemia/lymphoma (ATL) and inflammatory disorders correlated with HTLV-1 infection, such as myositis, arthritis, dermatitis, uveitis and alveolitis [4, 5]. There is no accurate number of HAM/TSP or ATL cases since these diseases are not considered reportable by the World Health Organization (WHO), although Japan reports approximately 800 cases of ATL yearly [4]. The major histopathological characteristic of HAM/TSP is a chronic inflammation of the white and gray matter of the spinal cord followed by a degenerative process that preferentially affects the white matter in the lower spinal cord [3, 6]. HAM/TSP is characterized by a chronic slowly progressive spastic paraparesis with bladder disturbances, absent or mild sensory loss and low back pain, with seropositivity for HTLV-1 antibodies, in the absence of spinal cord compression [4, 7]. Despite the more usual presentation characterized by a slow progression, 21.5% of the patients may experience a rapid progression, with severe disability two years after the onset of symptoms [5]. This phenomenon is related to older age of onset, parenteral HTLV-1 transmission route, high viral loads, and high antibody titers [8, 9].

In contrast to ATL, the incidence of HAM/TSP decreases with age. Thus, HAM/TSP progress is similar to multiple sclerosis (MS), where few cases have been reported among people over 60 years of age [10]. This could be explained by age-related lack of CD8+ cell hyperactivity and associated lower risk for clinical development of HAM/TSP [11].

HAM/TSP PATHOGENESIS

Although previous studies have investigated the potential underlying factors for the HAM/TSP pathogenesis, no consensus has been reached so far. There have been many tentative explanations, but HTLV-1 tax viral load, genetic background or immune disturbances have been implicated as
likely major causes. Fig. (1) summarizes the changes in blood and spinal cord of HAM/TSP patients. There is a mounting evidence for high levels of serum/cerebrospinal fluid (CSF) inflammatory cytokines in HAM/TSP patients. In fact, MIP-1α levels are higher in HAM/TSP patients than in asymptomatic carriers [12]. Thus, in addition to the proinflammatory cytokines, including IFN-γ, IL-2, and IL-15 [13], and the high activity of cytotoxic T lymphocytes (CTL), MIP-1α released by peripheral blood mononuclear cells (PBMC) may have an important role in HAM/TSP pathogenesis. This process can also influence the inflammatory activity in the spinal cord and thus contribute to the HAM/TSP progress. Alternatively, type 3 CXC chemokine receptors (CXCR3), which are expressed at high levels on activated and memory T lymphocytes, selectively respond to some of these chemokines. Those memory cells are inducible by IFN-γ, which is found at high levels in HTLV-1-infected subjects [14]; these cells may in turn migrate to the spinal cord, leading to a potential damage to the myelin membrane [15]. Thus, two pathways could be implicated in HAM/TSP development, including: (i) memory cells directly involved with cytolytic damage driven by IFN-γ, and (ii) RANTES and MIP-1α acting as chemotactic factors, all produced mainly by CD8+ T cells. We have observed that proinflammatory soluble factors, such as MIP-1α, may have an important role in mediating tissue-specific leukocyte recruitment and T-cell stimulation. This may result in damage to the myelin membrane, a major characteristic of myelopathy in HTLV-1-infected patients. A chemokine-induced activation of T cells via CCR5 leads to activation of focal adhesion kinases, which also have important roles in cell motility including cell spread and migration [16]. These CCR5 ligands are critical for T-cell proliferation and the transcriptional activation of cytokine genes [17]. This mechanism may be directly involved as a cytolytic damage in the adult T cell leukemia (ATL), since high levels of RANTES were described in these patients [18], and one in vitro study showed a role of tax induction of MIP-1α [19]. The predilection of neuroinflammation by HTLV-1 in the thoracic cord may be due to slower local blood flow, allowing a better opportunity for cells expressing adhesion molecules to transmigrate [20], while other, more watershed areas of the central nervous system usually remain clinically silent.

**TREATMENTS/STRATEGIES**

There is no standard therapy for HAM/TSP. Several studies have proposed the use of anti-inflammatory drugs, immune modulator drugs and others. In our experience in the last 15 years in a clinical setting in São Paulo (Brazil), corticosteroids have yielded better results in patients with less than 5 years from HAM/TSP onset.

**Glucocorticoids**

Despite no standard therapy for HAM/TSP is available, glucocorticoids (GCs) are the most widely prescribed class of drugs for its treatment. Their clinical efficacy is attributed to their ability to reduce the expression of proinflammatory genes, resulting in several benefits including a reduction of inflammatory cells migration, regulation of Th1/Th2 cytokine balance and potential reduction in HTLV-1 proviral load.

Most GC-related effects are mediated by the intracellular receptors (GRs) which are ligand-activated transcription factors. The GRs can act either by enhancing or repressing transcription of target genes [21, 22]. Genes that are negatively regulated by GR often involve the negative interference of GR with the activity of other transcription factors, such as NF-κB, CREB, STAT, GATA-3, interferon regulatory factor 3 (IRF3), activating protein (AP)-1, nuclear factor of activated T cells (NFAT), and T-box expressed in T cells (T-Bet). Typical target genes that are negatively regulated include several inflammatory proteins such as IL-1β, IL-2, IL-6, IL-8, IL-12, IL-5, IL-18, COX-2, E-selectin, interferon-γ (IFN-γ), TNFα, and intercellular adhesion molecule (ICAM), monocyte chemoattractant protein 1 (MCP-1) chemokine (C-C motif), vascular cell adhesion molecule (VCAM) [reviewed by [21, 23]. On the other hand, there are several recently characterized anti-inflammatory genes
whose expression is up-regulated by GR, such as iKB, IL-10, the genes coding for MKP-1, lipocortin-1, secretory leukocyte protease inhibitor (SLPI), annexin A1, and GC-induced leucine zipper (GILZ) [23, 24].

As discussed above, HAM/TSP is a chronic inflammation as long as patients show high levels of proinflammatory proteins and some of them may be inhibited by GC. These proteins include IFN-γ, TNFα, IL-2, MIP1α, IL-15, IL-6, and IL-16 and may have direct effects in white matter lesions [13, 25-27]. TNF-α, for example, induces cytotoxic damage to endothelial cells. MIP-1α and IFN-γ can enhance transendothelial migration of lymphocytes into the nervous system, IL-16 is a chemoattractant for CD4 + cells, and CD4 + cells produce IL-2 that is required by IL-2 non-producer CD8 + cells for proliferation [28]. In this context, one study showed that IFN-γ and MIP1α levels decreased after treatment with prednisolone in PBMCs from HAM/TSP patients while IL-4 and IL-10 levels increased [25].

In our experience, GCs have been used with better results in patients with less than five years of HAM/TSP onset. This possibly occurs because of changes in inflammatory context during HAM/TSP disease. In patients with a clinical history of up to three years it has been observed a high expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelium, TNF-α, IL-1β and IFN-γ in perivascular infiltrating cells in spinal cord and IL-1α predominantly expressed in the infiltrating macrophage and parenchymal astrocytes [29, 30]. However, in those patients with longer time of disease, the myelin and axons are equally degenerated and lost and there is a small number of inflammatory cells (mostly CD8 + cells) with downregulation of proinflammatory cytokine expression (with the exception of IFN-γ) [3, 31]. Therefore, therapies with GCs present better result in patients with less than 5 years of HAM/TSP onset, probably because of stronger inflammatory context in the early phase of disease.

The GCs also suppress the T lymphocyte proliferation mainly through its role in lowering the synthesis of IL-2 [21, 32]. These drugs may also reduce spontaneous proliferation of peripheral blood mononuclear cells (PBMCs) from patients with HAM/TSP [33]. This is important since the quantification of provirus reflects the number of HTLV-1-infected cells, which defines the proviral load. Therefore, suppressing the counts of HTLV-1-infected cells reduces the proviral load and may provide an improvement of clinical symptoms [34].

However, previous studies addressing the clinical efficacy of corticosteroids have yielded conflicting results. A study conducted in Japan, where several therapies have been tested in a group of 200 patients with HAM/TSP, revealed improved motor activity in 69% of patients with orally administered prednisolone, in 40% of those who underwent an intrathecal injection of hydrocortisone, and in 30% of those who underwent pulse therapy with methylprednisolone (10 patients) [35]. In Brazil, Araújo and colleagues conducted a clinical trial with 23 patients in Rio de Janeiro, with improvement for only one patient who reported shorter time of illness [36]. In Japan, a study conducted with patients with radiological changes (leukoencephalopathy on MRI) following intravenous injection of methylprednisolone (1 g / day for 3 days) revealed therapeutic effects in the short term, but without any radiological and clinical benefits in the long-term [37]. Recently, we reported a study with 39 patients with HAM/TSP treated with methyl-prednisolone (1g/day bolus for 3 days). They received an average of 3.4 pulses during follow-up. Some patients also received concomitant physical therapy, and symptomatic drugs such as baclofen, tricyclic antidepressants, vitamin B and oxbutidine. During this period we noticed a 24.5% improvement from baseline on the ISS scale, after a mean of 2.2 years of follow-up [38]. To date, data describing the real clinical benefits of GC treatment for HAM/TSP in the literature are limited. This is partially explained by the fact that few studies have had a longer follow-up time. It is possible that corticosteroids produced only a limited improvement, as suggested by a better performance in the scores only up to visit two. However, HAM/TSP is a chronic and progressive illness. One study showed that the median times from onset of the disability until the assignment of scores 6, 6.5, and 8 on the Disability Status Scale (DSS) scale were 6, 13 and 21 years, respectively [39]. We believe that slowing down the disability progression in the long run may itself represent a clinical benefit. An explanation for the apparent efficacy of corticosteroids could be that HAM/TSP is considered an immune-mediated disease, mainly driven by IFN-γ overproduction. Thus, the anti-inflammatory properties of corticosteroids may have exerted a significant impact on the myelin membrane inflammation process, improving some symptoms, mainly in those patients with only a few years from onset, when inflammation is more prominent [36]. In addition, corticosteroids decrease the number of mononuclear cells and α-2 microglobulin in the CSF, and probably decrease the HTLV-1 DNA proviral load [40, 41].

It should be kept in mind that clinical efficacy of GC treatment is related to tissue sensitivity to these hormones. Several mechanisms of tissue sensitivity and resistance to GCs have been described and include altered expression or distribution of GRs on peripheral lymphocytes, activation of mitogen-activated protein (MAP) kinases, cytokine-mediated pathways, activation of the transcription factor activator protein 1 (AP1), histone deacetylase-2 (HDAC2) expression, and increased P-glycoprotein-mediated drug efflux [42]. HTLV-1 (tax or HBZ proteins) activates several signaling pathways that have the potential to alter the sensitivity to GCs, such as mitogen-activated protein (MAP) kinase p38, AP-1 and pathways activated by IL-2 [43, 44]. However, despite some evidence, no study has showed the direct action of these factors in altering tissue sensitivity to GCs in HAM/TSP. In addition, previous studies demonstrated an overexpression and higher activity of P-glycoprotein, encoded by the multidrug resistance gene (MDR1), in T lymphocytes from HTLV-1-infected subjects (asymptomatic individuals and HAM/TSP and ATL patients) [45, 46]. The P-glycoprotein is a drug efflux pump for xenobiotic compounds including GCs. It is responsible for decreasing intracellular drug accumulation and often mediates the development of resistance to drugs, including resistance to GCs. Moreover MDR1 gene promoter is transcriptionally activated by the HTLV-1 tax protein [45]. These observations open up the possibility of new therapeutic approaches for HAM/TSP through the use of P-glycoprotein inhibitors along with GCs.
Histone Deacetylase Inhibitor

Histone deacetylation or acetylation is an important mechanism of gene expression regulation. Acetylation of the lysine residues at the N-terminus of histone proteins removes positive charges, reducing the affinity between histones and DNA (negatively charged) facilitating access of the promoter region by RNA polymerase and transcription factors. Therefore, histone acetylation enhances transcription and histone deacetylation represses transcription. Histone acetylation is catalyzed by histone acetyltransferases (HATs) enzyme and histone deacetylation is catalyzed by histone deacetylase (HDAC). Inhibition of HDAC activity therefore results in histone hyperacetylation and an increase in gene expression. Some different forms of HATs and HDACs have been identified, including CBP/p300 forms. Some studies showed that Tax recruitment of p300 (a HAT enzyme) to the HTLV-1 promoter enhances the level of viral transcription in vitro and is directly correlated with histone acetylation [47, 48]. Other studies showed Tax excludes HDAC enzyme from HTLV LTR DNA sequence preventing histone deacetylation and chromatin inactivation [49]. So, histone acetylation is an important mechanism of HTLV-1 gene expression regulation.

Interestingly HTLV-1 proviral expression is stable over periods of several years [8]. This equilibrium, appears genetically determined and is tightly controlled by host immune responses [50]. The selective pressure of immune response maintaining this equilibrium seems effective since the viral load consists basically of infected cells containing transcriptionally silent viruses [51, 52]. In support of this, very little viral expression has been detected in the peripheral blood [53], but HTLV is expressed during short-term culture ex vivo [54]. So, there is a hypothesis that infected cells escape from immune surveillance after silencing virus transcription. Thus, the utilization of a HDAC inhibitor, that increase virus expression [55], would result in a better immune response against HTLV.

Based on this, a study showed that treatment of 16 HAM/TSP patients with a standard clinical dose of valproate (HDAC-1 inhibitor) produces a substantial decline in the proviral load [52]. Although HTLV-1 proviral load is transiently increased as expected, a significantly decreased viral load is observed following that (mean of 24 fold) in all patients. Valproate treatment also induced the reduction of spasticity in all patients. Other study showed that inhibition of HDAC doubled Tax expression in naturally infected lymphocytes after overnight culture [55]. However, the rate of CD8+ cell-mediated lysis of Tax-expressing cells ex vivo was halved. In addition, valproate treatment was mildly toxic to lymphocytes from HTLV-1–infected individuals. It is important to mention that the impact of this inhibitor on HTLV-1 proviral load in vivo cannot be accurately predicted and that caution is recommended when using HDAC inhibitors for nonmalignant cases of HTLV-1 infection [55].

Interferon-Alpha

It is well accepted that interferon-alpha (IFN-α) is a cytokine with powerful antiviral, antiproliferative and immunomodulatory activities. IFN-α appears to be the major cytokine responsible for the amplification of the CD8+ T cell response and resistance to viral infections and additionally regulate the CD4+ Th1 cells, which are both crucial to development of protective immune responses [56]. Its endogenous production has been shown to occur during exposure of cells to viruses, double-stranded RNA, and other cytokines [57]. IFN-α exerts its therapeutic effects both via direct cellular effects via action on cell surface receptors and by indirect mechanisms involving induction of host antiviral responses. Among the mechanisms of direct inhibition of viral replication, it is the induction of cellular enzymes such as 2’5’oligoadenylate synthetase resulting in viral RNA degradation [58]. IFN-α also inhibits viral replication indirectly, by altering cytokine synthesis, which can amplify the cytotoxic T cell and natural killer (NK) cell response against infected cells.

IFNs were able to prevent virus production and release from a cell line chronically infected with HTLV-1 [59]. IFN-α also revealed an in vitro modulation of the spontaneous proliferation, and this phenomenon coincided with a clinical improvement in patients with HAM/TSP treated with this cytokine [33]. Another study showed that lymphocytes from patients with HAM/TSP cultured with IFN-α decreased the levels of proviral DNA and viral mRNA, and that this cytokine was able to modulate a proliferative response of CD8+ T cells [60]. Indeed, Saito et al. [61] showed that HTLV-1 proviral load was significantly decreased concomitantly with the reduction of memory CD8+ T cells.

Interestingly, the IFN-α therapy in nine patients with HAM/TSP reduces CD4+ cell subsets (i.e. Th1 cells) in the cerebrospinal fluid [39, 62]. After therapy, the percentages of CXCR3+ and CCR5+ cells in CD4+ cells significantly decreased in the cerebrospinal fluid as well as in the blood. In addition, the therapy also lowered the intracellular IFN-γ/IL-4 T cell ratio in the blood [62]. These results suggest that IFN-α suppresses Th1 responses in HAM/TSP and that patients with higher Th1 immunity and proviral load may be better responders to the therapy [62]. One multicenter, randomized, double-blind and controlled clinical trial used three different doses (0.3; 1.0 ; 3.0 MU) of IFN-α, daily for 4 weeks, with 48 HAM/TSP patients. Clinical evaluation included motor dysfunction, urinary disturbances, and changes of neurologic signs. The frequency of therapeutic response judged as excellent to good, 4 weeks after starting therapy and 4 weeks after completion of therapy, were 66.7% [10 of 15] and 61.5% (8 of 13) for the 3.0 MU group. The therapeutic response in the 3.0 MU group was significantly higher than in others groups [63]. These data are important, demonstrating that IFN-α is effective in the treatment of HAM/TSP.

The HTLV-1 infection seems to result in low levels of endogenous IFN-α production. Smith et al. [64] have demonstrated low or undetectable levels of this cytokine from five HTLV-1 infected lines. Another study demonstrated that HTLV-1 infection results in the inhibition of type I IFN gene expression (that includes IFN-α) [65]. In this same study, suppressor of cytokine signaling (SOCS1) was highly expressed in CD4+ T cells from HAM/TSP and AC patients, but not in ATL. Subsequent analysis demonstrated that HTLV-1-induced SOCS1 expression played a positive role in viral replication through inhibition of the IFN response.
SOCS1 directly interacted with IRF3 (interferon regulatory factor 3) and promoted its proteasomal degradation, thus blocking IFN-α synthesis [65]. Therefore, in this context, IFN-α therapy seems to be still more important in HAM/TSP treatment. However, experimental evidence has been obtained that SOCS1 can suppress IFN signaling (including exogenous IFN-α) by direct binding to phosphorylated type I IFN receptor [66]. This would be a possible explanation for the observation that some patients do not present a significant improvement with IFN-α therapy.

Vitamin C

Vitamin C supplementation has been shown to be of therapeutic value in specific clinical conditions, and devoid of serious side effects. The first study about vitamin C and HAM/TSP evaluated seven patients. Grades of the disability showed a modest clinical benefit in HAM/TSP patients after therapy with ascorbic acid. Motor disability grades were improved by more than one grade in 20.0% (4/20) of patients and 50% (10/20) showed a fair response to treatment [35]. In another recent study ex vivo, ascorbic acid induced a dramatic 95% decrease in spontaneous proliferation, a decrease in tax and LTR transcription, and a decrease in IFN-γ production in HAM/TSP PBMC [68]. Obviously, the combination therapy with high-dose vitamin C in HAM/TSP should be further explored.

CONCLUSIONS

In our experience, GCs may improve the neurological symptoms and therefore, the quality of life of HAM/TSP patients. More important, they can prevent the HAM/TSP disease progression that can occur if no therapeutic measure is taken. Another major advantage of this therapy is its feasibility, especially for developing countries. The low cost, the relatively easy administration, and the reversible side effects indicate that this therapy is a promising strategy protecting a certain number of patients against more severe disabilities. Anyway, only a double-blinded placebo-controlled clinical trial can ultimately determine the potential role of GCs in HAM/TSP, a disease for which very few clinical trials have been published. Additionally, physical and psychological rehabilitation should be performed for all patients, regardless of their clinical status. Furthermore, new approaches such as immune therapies like those used for multiple sclerosis, rather than anti-retroviral therapy, may be used for treating this condition.

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