Detection of the soluble form of the Fas/Apo-1 receptor in patients with human T-lymphotropic virus type 1-associated myelopathy


Abstract

Objective
Fas/Apo-1 mediates apoptosis via Fas and Fas ligand transduction. Recently, a soluble form of Fas (sFas) was described which seems to be functionally implicated in the Fas signaling system, suggesting a relationship between some disorders and sFas function.

Materials and Methods
To determine if sFas is involved in the pathogenesis of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), we measured sFas-levels in sera from normal controls, human T Lymphotropic virus type-1 (HTLV-1) healthy carriers and patients with HAM/TSP using a highly sensitive enzyme-linked immunosorbent assay (ELISA).

Results
The sFas level of normal individuals, HTLV-1 carriers, and patients with HAM/TSP, was 0.14 ± 0.11, 0.26 ± 0.25, and 0.39 ± 0.35 ng/ml, respectively. Although the level of sFas in patient's group with HAM/TSP, was significantly higher in comparison to that of normal controls (p <0.05), the individual values were highly variable within the groups.

Conclusion
These results suggest that sFas may play an important role in the pathogenesis of HAM/TSP and that serum sFas may be related to clinical activity in HAM/TSP patients.

Keywords: Apoptosis, Fas/FasL, HAM/TSP, HTLV-1, sFas.
Introduction
Human T-cell lymphotrophic virus type-1 (HTLV-1) is a retrovirus that resides in and functionally alters immune cells of central importance for immunoregulation (1). Ninety-five percent of infected people remain life-long asymptomatic carriers of the virus. About 2–3% of infected people develop an aggressive T-cell tumor, adult T cell lymphoma/leukemia (ATLL), and another 2–3% develop chronic inflammatory diseases, of which the best known is HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1). In northeast of Iran (Mashhad), is a new endemic area for HTLV-1 infection (2). The prevalence of HTLV-1 infection in Iran is estimated to be 2-3 % among the whole population and 0.7 % in blood bank donors (2).

Patients with HAM/TSP show high titers of HTLV-1 antibody, high proviral load and a high frequency of HTLV-1-specific cytotoxic T lymphocyte (CTL) compared with HTLV-1 carriers (2,3). The CTL response is known to be a key factor in HTLV-1 infection but the frequency and efficiency of HTLV-1-specific CTLs, is higher in HAM/TSP patients than in asymptomatic carriers (2,4). It is not clear whether the HTLV-1-specific CTL response is protective or destructive. The CTL response against HTLV-1 in asymptomatic carriers is more efficient than in HAM/TSP patients (2). Therefore the HTLV-1-specific CD8+ cells are able to prevent viral replication and spread. However, a high CTL response may also lead to the production of inflammatory cytokines and bystander damage (2,5). Though the causative virus for central nervous system (CNS) destruction is HTLV-1, the direct infection is not critical (6) and apoptosis may play an important role for the pathogenesis (7). Apoptosis (or Programmed Cell Death) is a physiological process of cell death that normally occurs when cells are damaged or no longer needed (8). The dysfunction of an apoptotic pathway, resulting in either “too much” or “too little” apoptosis, may lead to the development of many different disease states, such as cancer, various neurodegenerative and cardiovascular diseases, and autoimmunity, for which the therapeutic options have until now remained limited (8). Apoptosis is triggered by an apoptotic stimulus, and signal transduction after an apoptotic stimulus occurring at the plasma membrane via specific death receptors (8). The prototypical death receptors are tumor necrosis factor receptor-1 (TNF-R1) and Fas (CD95; Apo-1), and their corresponding ligands are TNF-α and Fas ligand (FasL) respectively (9).

CTLs can kill their targets by three distinctive mechanisms: (a) secretion of cytokines such as TNF-α and interferon (IFN)-γ, (b) secretion of perforin and granzyme, (c) a nonsecretory pathway involving Fas and its specific ligand (FasL) (2).

The Fas/Apo-1 antigen, (designated as CD95), is a member of the tumor necrosis factor receptor (TNFR) superfamily (TNFRSF6) (10, 13) which is expressed on the surface of a number of normal and malignant cells (24) and is responsible for inducing apoptosis when bound to Fas ligand (FasL) and monoclonal antibody (MAb) anti-Fas IgM (15-18). Fas may also play an important role in viral infections such as influenza virus (19) and HTLV-1 (20). Thus, mFas/Fas-L interaction in vivo controls the peripheral lymphocyte life span under the influence of sFas and plays a role in developing and eliminating autoreactive cells (21,22). However, a soluble isoform (sFas) is derived from the same mRNA as the membrane isoform by alternative splicing (23, 24). It also seems to play an important role in receptor triggering (25-27). Further investigations revealed that sFas was abundant in serum from patients with autoimmune diseases (28), hematopoietic...
Diagnosis of disability grading was based on the criteria proposed by Kagoshima-Osame. Physical examination and laboratory tests were done at the time that blood samples were taken and all clinical status and laboratory findings were evaluated according to the prepared clinical questionnaires of patients. Informed consent was obtained from all patients, carriers and healthy individuals. All samples were quickly frozen and stored at -70°C until analysed.

Detection of sFas: A sandwich enzyme immunoassay (sFas ELISA kit, Bender Med System, Vienna, Austria) was carried out to determine sFas levels in serum. Briefly, serum samples diluted 1:10 with Sample Diluent. The sFas present in the test sample bound to anti-sFas murine monoclonal antibodies specific to one epitope of sFas molecule, which was coated on the microplate well. A biotin-conjugated anti-Fas murine monoclonal antibody against the other epitope of sFas was bound to the sFas captured by the first antibody and completed the sandwich. After an incubation (1 hour at room temperature) followed by a wash step (3 times) Streptavidin-HRP (horseradish peroxidase) was added to bind to the biotin-conjugated anti-Fas antibody (incubation for 1 hour at RT). After washing (3 times) to remove unbound materials, a substrate solution (tetramethylbenzidine and H2O2) was added to each well (incubation for 15 minutes at RT). The enzyme reaction was then stopped with stop solution (1 M phosphoric acid). The absorbance of each sample was measured at 450 nm. A standard curve was used to determine the sFas concentration (ng/ml) in each sample.

A statistical analysis test was done by using student's t test to compare serum levels of sFas between the various clinical groups. Values of p<0.05 were considered to be statistically significant. Values of soluble Fas given in the text are mean ± standard deviation.

Materials and Methods

Patients and Samples: Twenty patients with HAM/TSP, 20 asymptomatic HTLV-1 carriers and 10 healthy controls, who were followed at HTLV-1-Clinic of Ghaem Hospital, Mashhad, Iran, were analyzed in this study. All individuals were from Khorasan province, which is an endemic area for HTLV-1 infection in Iran. HTLV-1 infection was documented in all patients and carriers by ELISA and polymerase chain reaction (PCR) (or Western Blot). The clinical diagnosis of HAM/TSP was based on the criteria proposed by Osame (37).
Results
HAM/TSP was diagnosed in 20 patients (4 male and 16 female; age range, 20-60 years; mean age, 44.1 years) according to the criteria of Osame. 90% of patients were female with mean age, 45.8 years. Birthplace of patients was, 4 from Neishabour, 1 from Bejestan, 1 from Ghouchan, 1 from Torbat Heidarieh, 2 from Torghabeh and 11 from Mashhad. All of the patients were Fars racialy. All the patients had been breast fed for 2 years when they were newborn. 90% of patients got married, 50% of them had sort of surgery and 25 of them had taken blood transfusion. 2 of the patients had close relatives with the same disease. Disability grade range of patients and symptoms duration range were, 2-5 grades and 2-20 years, respectively. 20 asymptomatic HTLV-1 carriers (AC) and 10 healthy volunteers were used as controls. Although the levels of sFas in the sera of patients with HAM/TSP (0.39±0.35 ng/ml) (range, 0.054-1.030) were significantly higher than healthy individuals (0.14±0.11 ng/ml) (range, 0.019-0.294) (p<0.05) but the individual values were highly variable within the groups (Figure 1 and Figure 2).

Figure 1: Mean sFas serum level in Patients with HAM/TSP, asymptomatic carrier (AC) and control individuals.

There were no statistically significant differences between HAM/TSP patients and AC (0.26±0.25 ng/ml) (range, 0.048-0.903) (p=0.18) and also no significant differences between AC and healthy groups (p=0.15). There was no significant correlation between sFas serum levels in patients group with duration and severity of myelopathy. There were no statistically significant differences between HAM/TSP patients and asymptomatic carrier (AC) (0.26±0.25 ng/ml) (p=0.18) and also no significant differences between AC and healthy groups (p=0.15).

Figure 2: The levels of sFas in sera of patients with HAM/TSP (0.39±0.35 ng/ml) were significantly higher than control individuals (0.14±0.11 ng/ml) (p<0.05).

Discussion
In the present study, we demonsrated that sFas was increased in sera of patients with HAM/TSP, which is characterized histologically by gliosis and mononuclear cell infiltrates around blood vesels (7). As far as we know, this is the first report in Iran that examined the soluble Fas antigen in sera of patients with HAM/TSP. For several years, the only two factors unequivocally associated with a higher risk of HAM/TSP were a high...
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proviral load and being female (38). In our study majority of patients were female (80%), too. We also found that in our study 100% of the patients had been breast fed for 2 years when were newborn, 66.7% of patients got married, 52.4% of them had sort of surgery and 23.8 of them had taken blood transfusion, which are the most common ways of HTLV-1 transfer (34, 49).

HTLV-1 infects CD4+ T cells and incorporates into the genome as a provirus. Whereas more than 10% of CD4+ T cells may be infected in vivo, only a small fraction of productively infected T cells cause activation of the immune system (34). While an activated immune system is needed to eradicate the infection, the spread of the virus is also accelerated under these conditions (34). The interactions between HTLV-1 and the cellular immune system can be divided into viral interference with functions of the infected host T cell and the subsequent interactions between the infected T cell and the cellular immune system (34). HTLV-1-mediated activation of the infected host T cell is induced primarily by the viral protein Tax, which influences transcriptional activation, signal transduction pathways, cell cycle control, and apoptosis. These properties of Tax recapitulate T-cell activation events during the G1 phase of the cell cycle and may well explain the ability of Tax to immortalize T cells (34). By virtue of their activated state, HTLV-1-infected T cells can nonspecifically activate resting, uninfected T cells via virus-mediated upregulation of adhesion molecules. This may also favor viral dissemination, since transmission of HTLV-1 usually requires T-cell-T-cell interaction (40). Moreover, the induction of a remarkably high frequency of antiviral CD8+ T cells does not appear to eliminate or control the HTLV-1 infection. Indeed, individuals with a high frequency of virus-specific CD8+ T cells have a high viral load, indicating a state of chronic immune system stimulation (34). The resulting high antigen load would make a powerful stimulus to the CTLs whereas in healthy carriers a brisk CTL response would be maintained by a lower antigen load (1). As a result, the frequency of HTLV-1-specific CTLs at equilibrium would differ little between HAM/TSP patients and asymptomatic carriers.

It is not understood why such a high frequency of antiviral CD8+ T cells (1 in 4 CD8+ T cells may be specific for a single viral epitope) can coexist with such a high frequency of virus-infected CD4+ T cells. Perhaps the circulation of infected CD4+ T cells allows them to escape CD8+-T-cell-mediated killing (34). Thus, the complex interaction of HTLV-1 with T cells allows the virus to persist in the host by expanding the population of infected T cells and by enhancing the spread of the virus to uninfected resting T cells. The host responds to the infection by a vigorous education of virus-specific CD8+ T cells but fails to eliminate the virus (34). Despite the fact that HTLV-1 has been established as the etiologic agent in HTLV-1 myelopathy, the pathogenesis remains unknown (39). There are two major hypothesis. In the first hypothesis, HTLV-1 infects gelial cells in the central nervous system, and a subsequent cytotoxic immune response against the infected cells results in demyelination (41). In the second hypothesis, HTLV-1 infection leads to the activation of autoreactive T cells and the induction of an autoimmune process (39). Recent data demonstrating that circulating CD8+ cytotoxic T lymphocytes reacted against HTLV-1 protein products in a manner restricted by MHC class I (HLA-A2) antigens in patients with HTLV-1 myelopathy (2) but not in a carrier or a patient with adult T-cell leukemia favor the first hypothesis (42). Furthermore, a high proportion of cerebrospinal fluid T cells in patients with HTLV-1 myelopathy are CD8+ and cytotoxic to cells infected with HTLV-1.
Demyelination mediated by cytotoxic T cells could occur in two ways: by the direct killing of HTLV-1-infected glial cells in a fashion restricted by MHC class I antigens or by the secretion of cytokines from cytotoxic T cells that recognize viral protein products or are directly activated by the HTLV-1 infection (39). Although there is circumstantial evidence of a demyelination mediated by CD8+ T cells in patients with HTLV-1 myelopathy, an autoimmune mechanism is still an alternative hypothesis. The autoimmune model would predict that autoreactive T cells became activated, resulting in a T-cell-mediated destruction of the myelin. This activation could be initiated by HTLV-1 infection of the autoreactive T cells, or nonspecifically by other infected immunoregulatory T cells (39).

We have also shown high levels of sFas in sera of patients with HAM/TSP as it's been showed by previous studies (7,44,45). In viral diseases, Fas-induced apoptosis play an important role in the disease progression (7). Fas antigen was found to express on some Epstein-Barr virus infected T or B cell populations. Influenza virus infection causes apoptotic death of cultured cells. Fas molecule has been implicated in the abnormality high levels of lymphocyte apoptosis seen in HIV-infected humans. In human T cell lines transformed by HTLV-1 and cultured cells from patients with adult T cell leukemia (ATL), Fas was strongly expressed on both types of cells (7). Incubation of these cells with anti-Fas resulted in inhibition of proliferation and apoptosis. These reports suggest that Fas-mediated apoptosis play a critical role in the pathogenesis of HAM/TSP. Fas was shed from these cells and these events may lead to the increased level of sFas in sera. In our study, sFas levels of HAM/TSP patients varied among patients and no statistically significant differences were observed between patients with a different clinical status of infection (Fig 1).

In HTLV-1 infection, several lines of evidence suggest that the Fas/FasL system may contribute to the elimination of virus-infected T cells (45). In HAM/TSP patients, apoptotic CD45RO+ T cells have been reported in the spinal cord (46). This raised the possibility that apoptosis of T lymphocytes is a mechanism by which HTLV-1 infected cells are eliminated from the CNS lesions of HAM/TSP (46). Recently, reported findings showed that sFas inhibited the cytotoxicity of membrane-bound Fas (26). If sFas inhibits the Fas-mediated apoptosis of HTLV-1 infected T cells, it is possible that severe inflammation is induced by the surviving HTLV-1 infected and activated T cells. In this case, increased levels of sFas would act as an exacerbating agent in HAM/TSP (47). These observations suggested that activated Fas/FasL system may be responsible for the tissue damage associated with HAM/TSP. Although further studies are needed to elucidate the detailed mechanism of Fas/FasL-mediated cell death to the pathogenesis of HAM/TSP, our present findings suggest that the soluble form of Fas also plays a role in HAM/TSP pathogenesis. Because it was mentioned that resistance to apoptosis is one of the pathogenic mechanisms for HAM/TSP, presence of high-level sFas in sera and implication of that in apoptosis transduction can be a good explanation of pathogenesis in this disorder.

Since both the soluble and membrane isoforms of Fas are derived from the same precursor mRNA, their generation should be tightly inter-regulated (47). However, the mechanism of such regulation and the physiologic significance of the soluble isoform remain to be elucidated. In general, tumor and abnormal cells have a higher tendency than non-malignant cells to produce and shed soluble forms (48). Moreover, the
increase in the levels of the soluble forms and the change in those of the membrane forms suggest a role in the malignancies (17). Thus, abnormal cells might switch from producing mFas to producing sFas in order to escape Fas-induced apoptosis. sFas secreted into the serum may regulate immune function in patients and immortalization of the cell lines. Copeland et al. examined the sensitivity of HTLV-1-infected T-cell lines to anti-CD95 antibody-mediated apoptosis (34). Despite expression of high levels of CD95, the HTLV-1-infected cell lines showed reduced susceptibility to anti-CD95-induced apoptosis. Because of these findings we hypothesized that increased levels of sFas and its implication in Fas/FasL system, might be a good answer to reduction seen in T cells-Fas-mediated apoptosis.

In conclusion, alternative splicing may be an important event in the regulation of Fas/FasL interaction and thus in the regulation of immune responses by these receptor-ligand pairs and though the biological activity of sFas is not fully clarified and the significance of soluble Fas antigen in circulation and CSF is unclear, it is important to examine the pathogenesis of these diseases in terms of Fas mediated apoptosis.

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References
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