New targets for adult T cell leukemia/lymphoma (ATLL): a map for ATLL immunotherapy

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Introduction

Human T-cell lymphotrophic virus type 1 (HTLV-1) belongs to the Retroviridae family in the genus of Deltaretrovirus. Almost, 10–20 million people are infected with HTLV-1 worldwide that mostly remain in an asymptomatic carrier state (ACs). However, about 3-5% of infected subjects may develop two life threatening aggressive diseases, a clonal expansion of mature activated CD4+ T-cell malignancy called adult T cell leukemia/lymphoma (ATLL) or a neurodegenerative inflammatory disease, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The interaction of virus and host were evaluated in chemokine level.

Material and Methods

The expression of CCR6, CXCR-3, HTLV-1 PV, HTLV-1-Tax, and HBZ were assessed in 12 asymptomatic HTLV-1 carriers (ACs), 12 healthy controls (HCs) and 12 ATLL patients. We applied quantitative real-time PCR (qRT-PCR) and TaqMan method.

Ten ml vein blood was collected from each subjects and ficoll (Cedercorporation, Canada) density gradient was used for isolation of Peripheral blood mononuclear cells (PBMC). RNA extracted using Trizol solution (Tripure, Roche, Germany) and was then reversed transcribed to cDNA using Thermo Fisher Scientific’s cDNA synthesis kit (Thermo Fisher, USA).

Real time PCR for gene expression assessments

RT-qPCR was performed on a Q6000 machine and analyzed by Rotor Gene 6000 Software (Qiagen, Hilden, Germany). To assess the expression of each gene of interest and reference in each sample, the standard curves, relative quantification.

HTLV-1 PV measurement

Because HTLV-1 in DNA form integrated to the host genome, extraction of genomic DNA was necessary for absolute measuring of the load of provirus (PV). The genomic DNA was extracted from PBMCs using Favorgen extraction kit (Taiwan).

A commercial kit for assessing of HTLV-1 PV was used (Amits Gene, Iran) and PV was quantified using this equation.

HTLV-1 PV in 104 PBMCs = (Copies of HTLV-1 provirus/ copies of Albumin/2) x 104

Statistical analysis

The results were analyzed by SPSS software version 11.5 (SPSS, Chicago, IL, USA) according to the variables distributions.

Results

The study findings showed strong suppression of CXCR3 gene expression in ATLL patients compared to HTLV-1 virus carriers and healthy people showed a significant difference (P=0.00 and P=0.008). Although, the mean expression of CCR6 genes in ATLL patients decreased compared to ACs (P=0.04), no significant difference was seen to the healthy group. Furthermore, the findings showed that Tax protein did not expressed in ATLL patients. However, HTLV-1-HBZ protein expression in ATLL group was nearly 4 times more than ACs (P=0.001). Finally, HTLV-1 proviral load (PVL) was 15 times more than ACs.

Conclusion

Our study results illustrates that the expression of chemokine receptors is directly related to the course and stages of the disease as well as the prognosis of the disease. In carriers, compared to healthy individuals, we still have a higher level of expression of chemokine. In addition, with the progression of the conflict and the progression to malignancy in TCD4 cells, we can conclude that with decreased levels of these chemokine receptors can lead to malignancy with a poor prognosis. Furthermore, in the absence of Tax, HTLV-1-HBZ protein implicated in the maintenance of malignancy and HTLV-1 escape from the host cell immune system. Therefore, HTLV-1-HBZ and host CXCR3 might be good target for ATLL therapy.

Table 1. HTLV-1 PV gene expression in ATLL-1 infected subjects, ATLL patients and asymptomatic carriers

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>SEM± mean</th>
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<tbody>
<tr>
<td>ATLL patients</td>
<td>12</td>
<td>2.0±0.87</td>
</tr>
<tr>
<td>HTLV-1 asymtomatic carriers</td>
<td>12</td>
<td>0.8</td>
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