Plasma Soluble CD30 as a Possible Marker of Adult T-cell Leukemia in HTLV-1 Carriers: a Nested Case-Control Study

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Abstract

Elevated levels of soluble CD30 (sCD30) are linked with various T-cell neoplasms. However, the relationship between sCD30 levels and the development of adult T-cell leukemia (ATL) in human T-cell leukemia virus type 1 (HTLV-1) carriers remains to be clarified. We here investigated whether plasma sCD30 is associated with risk of ATL in a nested case-control study within a cohort of HTLV-1 carriers. We compared sCD30 levels between 11 cases (i.e., HTLV-1 carriers who later progressed to ATL) and 22 age-, sex- and institution-matched control HTLV-1 carriers (i.e., those with no progression). The sCD30 concentration at baseline was significantly higher in cases than in controls (median 65.8, range 27.2-134.5 U/mL vs. median 22.2, range 8.4-63.1 U/mL, P=0.001). In the univariate logistic regression analysis, a higher sCD30 (≥30.2 U/mL) was significantly associated with ATL development (odds ratio 7.88 and the 95% confidence intervals 1.35–45.8, P = 0.02). Among cases, sCD30 concentration tended to increase at the time of diagnosis of aggressive-type ATL, but the concentration was stable in those developing the smoldering-type. This suggests that sCD30 may serve as a predictive marker for the onset of aggressive-type ATL in HTLV-1 carriers.

Keywords: Adult T-cell leukemia (ATL) - CD30 - human T-cell leukemia virus type 1 (HTLV-1)
that CD30 expression has been noted in approximately one-fourth of ATL cells (Ohtsuka et al., 1994). We also reported that ATL cells produced sCD30 and elevated serum sCD30 levels correlated with the aggressiveness of the acute type of ATL and the chemotherapy resistance (Nishioka et al., 2005). Furthermore, it was suggested that analysis of sCD30 levels may be useful for prediction of overall survival in ATL patients, especially before allogeneic hematopoietic stem cell transplantation (Pornkuna et al., 2014a). These observations suggest that elevated circulating sCD30 levels may be related to the future risk of developing ATL among asymptomatic HTLV-1 carriers. However, so far, no study has provided data on the impact of sCD30 on the development of ATL in asymptomatic HTLV-1 carriers.

Thus, the aim of this study was to investigate whether circulating sCD30 levels are associated with the development of ATL in HTLV-1 carriers, by conducting a nested case-control study within an existing prospective study of asymptomatic HTLV-1 carriers in Japan.

Materials and Methods

Data source
Anonymized demographical data, laboratory data, clinical data, and stored plasma samples from HTLV-1 carriers were obtained from a nationwide cohort study of HTLV-1 carriers in Japan [Joint Study on Predisposing Factors of ATL Development (JSPFAD)] (Iwanaga et al., 2010), after approval by the JSPFAD committee (approved number 2012-5) and by the Institutional Review Board of the National Hospital Organization Kumamoto Medical Center (No. 391 on May 21, 2012). Details of the JSPFAD have been previously reported (Iwanaga et al., 2010). In brief, the project was established in August 2002, consisting of 14 university hospitals and 27 educational hospitals located in a wide range of areas in Japan for the purpose of investigating risk factors for the development of ATL by prospectively following a large number of participants of asymptomatic HTLV-1 carriers. All the Institutional Review Boards at collaborative institutions approved the JSPFAD protocol, and all participants provided written informed consent to participate in accordance with the Declaration of Helsinki. In the present study, we used a fixed dataset of the JSPFAD with 1218 asymptomatic HTLV-1 carriers by the end of 2008 as data source. Cases and controls were selected from the database and were individually matched to cases (1:2 ratio) by age at the entry (±5 years) into the JSPFAD (i.e., baseline), sex, and institution. Because only 11 cases were available, 22 controls were selected.

Laboratory and virological data
Data other than demographics (sex and age at baseline) obtained were comorbidities, laboratory data, such as lactate dehydrogenase (LDH) (IU/L) and soluble interleukin-2 receptor alpha chain (sIL-2R) (U/mL), and HTLV-1 proviral load in peripheral blood mononuclear cells (PBMCs) (copies/100 PBMCs, i.e. % of PBMCs) (Iwanaga et al., 2010). Detailed methods for the measurement of HTLV-1 proviral load were described in a previous JSPFAD report (Iwanaga et al., 2010). In this study, plasma sCD30 concentration was measured, after plasma samples were provided by the biomaterial bank of the JSPFAD, by using the Human sCD30 Platinum ELISA kit (eBioscience, Vienna, Austria) according to the manufacture’s protocol at the Research Laboratories of Kyowa Medex Co., Ltd., Shizuoka, Japan. The sCD30 measurement was blinded with regard to case or control status. For cases, laboratory data and plasma samples pre- and post-ATL development were obtained. For controls, only baseline data and plasma samples were obtained.

Statistical analysis
To reduce skewness of the distribution of continuous raw data, LDH, sIL-2R, and sCD30 were log10 transformed, and proviral load were square-root-transformed (SQRT). To dichotomize the continuous data, cut-point values were chosen based on the median value of a total number of subjects. The Wilcoxon signed-rank test was used to test for significant differences in baseline data of age and laboratory data among the matched pairs of cases and controls. Univariate correlation analysis of continuous baseline variables was carried out with the Spearman’s rank test. Categorical data were compared with the chi-square test or Fisher’s exact test. Odds ratios (ORs) and the 95% confidence intervals (CIs) related to dichotomized variables and ATL risk were computed using univariate logistic regression analyses. All statistical tests were two-tailed, with a P value<0.05 considered to indicate statistical significance. Statistical analyses were performed using SAS software (version 12, SAS Institute, Japan) and the graphical presentations were produced using Prism software (GraphPad Prism 5.0, San Diego, CA).

Results

Baseline characteristics of case and control subjects
The baseline data of 11 cases (HTLV-1 carriers who later developed ATL) and 22 controls (HTLV-1 carriers who did not develop ATL) are summarized in Table 1. There were no significant differences between the cases and controls on age at baseline (P=0.82) and sex (P=1.0). Thus, age and sex were exactly matched between cases and controls. As expected, proviral load (P<0.001) and sIL-2R (P=0.009) were significantly higher in cases than controls (Table 1 and Figure 1a and 1c). The plasma concentration
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The concentration of sCD30 was also significantly higher in cases (median 65.8, range 27.2-134.5, P<0.001) than controls (median 22.2, range 8.4-63.1) (Table 1 and Figure 1b). There was no significant difference in the concentration of LDH (IU/L) between the cases (median 234, range 158-509) and controls (median 205, range 129-459) (P=0.41) (Table 1 and Figure 1d).

Association between sCD30 level and ATL risk

To evaluate the association between the plasma level of sCD30 and the risk of the developing ATL from HTLV-1 carrier status, we performed logistic regression analyses by dichotomizing continuous data based on the median value of a total number of subjects. The cut-off point for sCD30, LDH, and sIL-2R were set at log10-transformed values of 1.48, 2.31, and 2.74, respectively, and that for proviral load was set at SQRT value of 1.92, based on results in Table 1. The frequency distribution of each variable in cases and controls is shown in Table 2. Univariate logistic regression analyses disclosed that both sCD30 and sIL-2R, but not LDH, were significantly associated with the risk of developing ATL from HTLV-1 carrier status (Table 2).

Table 1. Baseline Characteristics of Case and Control Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (N=33)</th>
<th>Cases (N=11)</th>
<th>Controls (N=22)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry (years)</td>
<td>Median (range)</td>
<td>64.1 (41.0-78.9)</td>
<td>62.2 (41.0-75.8)</td>
<td>64.8 (41.2-78.9)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male / Female, n (%)</td>
<td>9 (27) / 24 (73)</td>
<td>3 (27) / 8 (73)</td>
<td>6 (27) /16 (73)</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>Median (range)</td>
<td>205 (129-509)</td>
<td>234 (158-509)</td>
<td>205 (129-459)</td>
</tr>
<tr>
<td>Log10 value</td>
<td>2.31 (2.11-2.71)</td>
<td>2.37 (2.20-2.71)</td>
<td>2.31 (2.11-2.66)</td>
<td></td>
</tr>
<tr>
<td>sIL-2R (U/mL)</td>
<td>Median (range)</td>
<td>546.5 (235-2660)</td>
<td>948.5 (349-2660)</td>
<td>457.0 (235-1650)</td>
</tr>
<tr>
<td>Log10 value</td>
<td>2.74 (2.37-3.42)</td>
<td>2.98 (2.54-3.42)</td>
<td>2.66 (2.37-3.22)</td>
<td></td>
</tr>
<tr>
<td>HTLV-1 PVL (%)</td>
<td>Median (range)</td>
<td>3.7 (0.04-28.6)</td>
<td>10.6 (3.5-28.6)</td>
<td>1.5 (0.04-14.7)</td>
</tr>
<tr>
<td>SQRT value</td>
<td>1.92 (0.20-5.35)</td>
<td>3.25 (1.87-5.35)</td>
<td>1.21 (0.20-3.84)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>sCD30 (U/mL)</td>
<td>30.2 (8.4-134.5)</td>
<td>65.8 (27.2-134.5)</td>
<td>22.2 (8.4-63.1)</td>
</tr>
<tr>
<td>Log10 value</td>
<td>1.48 (0.92-2.13)</td>
<td>1.82 (1.43-2.13)</td>
<td>1.35 (0.92-1.80)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Relationship between Baseline Viral Markers and the Development of ATL

<table>
<thead>
<tr>
<th>Markers†</th>
<th>No.</th>
<th>Univariate OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2.31</td>
<td>5 / 10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2.31≤</td>
<td>6 / 10</td>
<td>1.20 (0.27 - 5.25)</td>
<td>0.81</td>
</tr>
<tr>
<td>sIL-2R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2.74</td>
<td>2 / 14</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2.74≤</td>
<td>8 / 8</td>
<td>7.00 (1.19 - 41.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>PVL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.92</td>
<td>1 / 15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1.92≤</td>
<td>10 / 7</td>
<td>21.4 (2.28 - 202)</td>
<td>0.007</td>
</tr>
<tr>
<td>sCD30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.48</td>
<td>2 / 14</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1.48≤</td>
<td>9 / 8</td>
<td>7.88 (1.35 - 45.8)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*HTLV-1, human T-cell leukemia virus type 1; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor alpha chain; PVL, proviral load; sCD30, soluble CD30; OR, Odds ratio; CI, confidence intervals; † The cut-point values for dichotomization were chosen based on the median value of the log10 transformed LDH, sIL-2R, and sCD30, and the median value of the square-root-transformed proviral load.
regression analyses revealed that the higher levels of sIL-2R and proviral load were significantly associated with the increased risk of the developing ATL in the univariate analyses. The higher level of sCD30 was also associated with a significantly increased risk of the developing ATL (OR=7.88, 95%CI=1.35-45.8, P=0.02).

The correlation between baseline sCD30 level and viral markers

To elucidate whether the higher level of sCD30 is associated with the development of a specific subtype of ATL from HTLV-1 carrier, we evaluated the correlation between sCD30 concentration and other viral markers by plotting data individually.

The relationship between individual values of log-transformed sCD30 and SQRT proviral load was plotted in Figure 2a (control group) and Figure 2b (case group, where the colored markers indicated the transformed subtype of ATL from HTLV-1 carrier status). In the control group, there was a significant correlation between the two values (correlation coefficient, r=0.49, P=0.02). However, in the case group, there was no significant correlation between the two values (correlation coefficient, r=-0.14, P=0.67). Among cases, two subjects who later developed lymphoma-type ATL (orange quadrangle) and one who later developed smoldering-type ATL (blue triangle) showed the very high concentration of sCD30 despite the relatively lower proviral load.

The relationship between log-transformed sCD30 and sIL-2R concentrations was plotted in Figure 2c (control group) and Figure 2b (case group, correlation coefficient, r=0.61, P=0.003) and in case (d). In cases (b and c), individual value was plotted using different marks based on the transformed subtype of ATL from HTLV-1 carrier status, such as red circle for acute-type ATL, orange quadrangle for lymphoma-type ATL, and blue triangle for smoldering type of ATL. The marked elevation of level of sCD30 was observed in those who developed acute-type ATL (case 2) and the lymphoma-type ATL (case 5 and 7). The marked elevation of level of sCD30 was also associated with a significantly increased risk of the developing ATL in the univariate regression analyses. The higher level of sCD30 was also associated with a significantly increased risk of the developing ATL (OR=7.88, 95%CI=1.35-45.8, P=0.02).

Sequential change of sCD30 levels in cases

To assess changes of sCD30 level before and after development of ATL in case group, the sCD30 concentrations were plotted over time from the baseline at entry into the cohort to the date of the development of ATL. Abbreviations: HTLV-1, human T-cell leukemia virus type 1; sIL-2R, soluble interleukin-2 receptor alpha chain; PVL, proviral load; sCD30, soluble CD30; r, Spearman correlation coefficient

Figure 2. Correlation between Baseline Values of sCD30, sIL2R, and Proviral Load in Cases and Controls. Correlation analyses were performed between sCD30 and PVL in controls (a) and in cases (b), and between sCD30 and sIL-2R in controls (c) and in cases (d). In cases (b and c), individual value was plotted using different marks based on the transformed subtype of ATL from HTLV-1 carrier status, such as red circle for acute-type ATL, orange quadrangle for lymphoma-type ATL, and blue triangle for smoldering type of ATL. *square-root-transformed PVL value. **log-transformed sCD30 or sIL-2R value. Abbreviations: HTLV-1, human T-cell leukemia virus type 1; sIL-2R, soluble interleukin-2 receptor alpha chain; PVL, proviral load; sCD30, soluble CD30; r, Spearman correlation coefficient

Figure 3. Sequential changes in soluble CD30 and sIL-2R levels in cases from baseline to the time of diagnosis of ATL. (a) Change of sCD30 levels of three cases who developed the aggressive-type ATL. (b) Change of sIL-2R levels of three cases who developed the aggressive-type ATL. In (a) and (b), Case 2 progressed to acute-type ATL. Case 5 progressed to lymphoma-type ATL, but the sampling data was after receiving chemotherapy. Case 7 progressed to lymphoma-type ATL. (c) Change of sCD30 levels of five cases who developed smoldering ATL. (d) Change of sIL-2R levels of five cases who developed smoldering ATL. The values of sCD30 and sIL-2R were log-transformed. X-axis indicates years from the entry date of the cohort to the date of the development of ATL. Abbreviations: HTLV-1, human T-cell leukemia virus type 1; ATL, adult T-cell leukemia; sIL-2R, soluble interleukin-2 receptor alpha chain; sCD30, soluble CD30

Sequential change of sCD30 levels in cases

To assess changes of sCD30 level before and after development of ATL in case group, the sCD30 concentrations were plotted over time from the baseline at carrier status to the time of diagnosis of ATL, along with the change in sIL-2R levels for reference. The line plots in Figure 3a and 3b indicate the plasma concentrations of sCD30 and sIL-2R, respectively, at entry into the cohort as a HTLV-1 carrier status and at the development of the acute type of ATL (case 2) and the lymphoma-type ATL (case 5 and 7). The marked elevation of level of sCD30 was observed in those who developed acute-type ATL (case 2, raw data from 57.6 to 619.4 U/mL or the log-transformed data from 1.76 to 2.69) and lymphoma-type ATL (case 7, raw data from 117.3 to 2724.7 U/mL) group and Figure 2d (case group). There was a significant correlation between sCD30 and sIL-2R in control group (correlation coefficient, r=0.61, P=0.003) and in case group (correlation coefficient, r=0.84, P=0.002). In case group (Figure 2d), two subjects who later developed lymphoma-type ATL (orange quadrangle) and another two later developed smoldering-type ATL (blue triangle) showed the very higher concentration of both the baseline sCD30 and sIL-2R at HTLV-1 carrier status.

There was no significant correlation between sCD30 concentration and LDH in both the control and case groups (data not shown).
or the log-transformed data from 2.07 to 3.44). In a case that progressed to lymphoma-type ATL (case 5), sCD30 level decreased from 118.9 to 69.6 U/mL raw data (the log-transformed from 2.07 to 1.84) during chemotherapy. Sequential changes of sIL-2R levels were also similar with the sCD30 patterns of the three cases (Figure 3b). However, in cases that progressed to smoldering types of ATL (Figure 3c and 3d), changes in levels of both sCD30 and sIL-2R were not clear before and after diagnosis of ATL.

Discussion

The results of the present study demonstrated a possible association between the plasma levels of sCD30 in HTLV-1 carriers and the development of ATL. The univariate logistic regression analysis indicated that a higher sCD30 concentration (≥30.2 U/mL) in the plasma of asymptomatic HTLV-1 carriers was associated with the development of ATL. The data also showed that the sCD30 concentration markedly increased after the onset of acute or lymphoma type of ATL in HTLV-1 carriers. This suggests that sCD30 may serve a predictive marker for aggressiveness of ATL cells, as we have reported previously (Nishioka et al., 2005; Pornkuna et al., 2014a; Takemoto et al., 2014).

In case group in the present study, correlation analyses indicated that the relationship between sCD30 level and proviral load was not statistically significant, but the relationship with sIL-2R level was significant (Figure 2). Moreover, sequential changes of sCD30 levels and sIL-2R levels in cases were almost parallel to each other (Figure 3). These results were interesting because, at cell line levels, elevated CD30 expression is considered to be one of the causes of constitutive NF-kappa B activation in ATL cells, thereby resulting in ATL development (Higuchi et al., 2005). Tang et al reported that CD30-CD30L signaling are important for T-cell regulation (Tang et al., 2008). Based on these results, in the microenvironment, it is conceivable that ATL stem cells produce sIL-2R and sCD30 to prevent activating T cell death by excess IL-2R signaling and cell death caused by CD30-CD30L interaction (Pornkuna et al., 2014b). However, CD30 expression was not always observed in lymph nodes of all ATLs (Takeshita et al., 1995). While our results provide a new perspective on the mechanism of ATL leukemogenesis in HTLV-1 carriers, the role of the CD30 expression on ATL remains to be elucidated.

A higher expression of CD30 on CD4-positive cells has been reported in many autoimmune diseases and chronic inflammatory disorders (Horie et al., 1998; Gerli et al., 1995; Calgaris-Cappio et al., 1995; Krams et al., 1996; Giacomelli et al., 1997). Chronic inflammation is generally known to be one of the risk factors for many cancers, including lymphoid malignancies (Trinchieri et al., 2012). Even in the field of HTLV-1 researches, there has been clinical evidence that HTLV-1 carriers with chronic Strongyloides infection frequently progress to overt ATL (Nakada et al., 1984). In the present study, in fact, cases had comorbidity with a variety of conditions at the entry of HTLV-1 carrier cohort, including pneumonia in one; hypertension in one; rash in one; eye disease in one; therapy resistant atopic dermatitis in one; and prostatitis in one. Some molecular evidence has been also reported suggesting that chronic inflammation may trigger the proliferation of HTLV-1-infected T cells as followed. Zhao et al. (2012) reported that the HTLV-1 bZIP factor (HBZ) gene, which is known to induce chronic inflammation, play a significant role in the pathogenesis of ATL (Zhao et al., 2012). Although the relationship between HBZ expression and sCD30 on ATL cells remains unknown, we speculate that sCD30 may be a marker that reflects the inflammation-related tumorigenesis of ATL cells, based on our findings that there was a higher sCD30 concentration in cases than in controls.

Aggressive-type ATL has a distinctive clinical course with lymphadenopathy, hepatosplenomegaly, and visceral invasion by malignant cells types. The mechanism of the migration of leukemic cells into various organs remains to be elucidated. In the early stage of ATL, the presence of oligoclonal proliferation of infected cells and the clonal change at the time of crisis has been reported (Takekoto et al., 1994; Tsukasaki et al., 1997). In the present study, high levels of sCD30 (65.8 U/mL), sIL-2R (948.5 U/mL), and HTLV-1 proviral load (10.6%) at baseline were shown in the cases developed to ATL. Although sCD30 concentration increased after developing acute and lymphoma types of ATL in HTLV-1 carriers, no clear evidence of a relationship between sCD30 level and the aggressiveness of ATL was found. Our findings suggest that the clonal change occurred even in the clinical HTLV-1 carrier stage. Additional studies should be performed to further clarify the role of sCD30 in the peripheral blood on the clonal proliferation of HTLV-1 infected cells.

The main limitation of this study was the small number of subjects, which meant we were unable to perform multivariate analysis. The small number of subjects was mainly due to the very low incidence of ATL with a long latency period among HTLV-1 carriers. Further longitudinal large-scale studies are warranted to confirm our results.

In conclusion, our results suggest that an elevated plasma level of sCD30 is a possible progression marker for the development of aggressive-type ATL in asymptomatic HTLV-1 carriers. Measurement of the level of sCD30 may be useful to identify high-risk HTLV-1 carriers.

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References


