Serum Levels of Soluble Fas/APO-1 Receptor in Human Retroviral Infection and Associated Diseases

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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 signal system, suggesting a relationship between some disorders and sFas function. We measured sFas-levels in sera from normal controls and patients with disorders linked to human retroviral infection of human immunodeficiency virus (HIV) and human T-cell leukemia virus type-1 (HTLV-1). The sFas level of normal controls, HTLV-1 carriers seronegative for HIV, and patients with HTLV-1 associated myelopathy/tropical paraparesis (HAM/TSP), adult T-cell leukemia (ATL), and AIDS was 1.62±0.49, 1.90±0.49, 2.00±0.59, 3.32±2.05, and 3.06±0.92 ng/ml, respectively. Although the level of sFas in patient groups with HAM/TSP, ATL, and AIDS was significantly high in comparison to that of normal controls (p<0.01), the individual values were highly variable within the groups. The sFas level was statistically correlated to the soluble interleukin-2 receptor (sIL-2R) level, as well as to cells expressing membrane Fas (mFas), indicating the same cellular origin. In some ATL cases, however, serum sFas levels and mFas expression density on leukemic T-cells were discrepant, with especially high levels of the soluble form and a lack of expression of the membrane form observed in 2 cases. sFas detection could serve as a putative marker for active diseases in patients with ATL and AIDS.

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Introduction

Membrane Fas (mFas)/APO-1, a member of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily, is a glycosylated 48 kD cell surface receptor containing a single transmembrane domain (1, 2). This mFas, triggered by its ligand or the monoclonal antibody (CD95) of anti-Fas, can induce apoptosis or programmed cell death during mammalian lymphocyte development (3). Abnormal signaling via mFas, however, appears to be associated with human lymphoproliferative syndrome and autoimmunity such as lymphoma, systemic lupus erythematosus, and angioimmunoblastic lymphadenopathy (4, 5). Furthermore, the human Fas molecule may be implicated in the lymphocytopenia seen in patients with viral infections (6), especially human immunodeficiency virus (HIV). The signal transduction between mFas and its ligand has been investigated. A soluble isoform (sFas) derived from the mRNA as the membrane form seems to play an important role in the signaling. Namely, they may modify ligand concentrations by stabilizing binding protein, downregulate membrane receptor numbers as a mechanism of genesis, and specifically inhibit ligand-receptor association in the extracellular space (7).

This prompted us to first explore the clinical significance of serum sFas/APO-1 levels in various conditions associated with human retroviral infection of HIV and human T-cell leukemia virus type-1 (HTLV-1) in the Nagasaki district, one of the most endemic areas for HTLV-1 and subsequent adult T-cell leukemia (ATL) in Japan (8).
Materials and Methods

Samples
Serum and lymphocytes from 25 healthy persons seronegative for HIV and HTLV-1 (normal controls), 15 healthy HTLV-1 carriers seropositive for HTLV-1 but not HIV, 20 HTLV-1 associated myelopathy/tropical paraparesis (HAM/TSP) patients, 31 ATL patients, and 14 AIDS patients were collected between February 1993 and June 1996. sFas was flow cytometrically detected on fresh cells from heparinized peripheral blood, and then the sera were stored at −80°C until the measurement of sFas. Normal controls and HTLV-1 carriers corresponded to blood donors and normal volunteers in our institute. AIDS patients comprised of 9 hemophiliacs and 5 non-hemophiliacs. ATL was subtyped into 20 acute ATL and 11 smoldering and chronic ATL. The median age in controls, HTLV-1 carriers, HAM/TSP, ATL, and AIDS was 36, 38, 59, 58, and 40 years old, respectively. The male to female ratio in each group was 1.3:1, 1.2:1, 0.3:1, 1.2:1, and 13:1, respectively.

Detection of sFas
A sandwich enzyme immunoassay (sFas ELISA kit, MBL Inc, Nagoya, Japan) was carried out to determine sFas levels in serum. The sFas present in the test samples bound to anti-Fas polyclonal antibodies specific to the first epitope of the intracytoplasmic domain (a.a No 305–319) of the Fas molecule which was coated on the microplate well. A horseradish peroxidase-conjugated anti-Fas monoclonal antibody against the second epitope of the extracytoplasmic domain (a.a No 110–120) bound to the sFas captured by the first antibody and completes the sandwich. After washing to remove unbound materials, a substrate solution was added to each well. The reaction was then stopped and the absorbance determined at 450 nm. The value (ng/ml) was calculated from a standard curve. The reliability of the kit was confirmed by using recombinant Fas control samples.

Similarly, soluble interleukin-2 receptor (sIL-2R) levels also were detected according to methods described in detail elsewhere (9).

Detection of mFas
mFas was immunophenotyped using flow cytometry (FCM) as described previously (10). Briefly, 5 μl of anti-Fas monoclonal antibody (MBL, Nagoya, Japan) was applied to 100 μl heparinized whole blood as the first layer. After washing, a FITC-conjugated goat anti-mouse immunoglobulin F(ab)'2 fragment (Ortho Diagnostic System Inc., Raritan, NJ) was used as the second layer. Finally, after lysing erythrocytes with lysing solution buffer (Ortho Diagnostic System Inc., Raritan, NJ), the cells gated in an area of lymphocyte on scatter cytogram were analyzed by Cytoron flow cytometry (Ortho Diagnostic System K.K., Tokyo, Japan). The positive % and mean fluorescent intensity for mFas were calculated using the manufacturer’s program. Control preparations were set up by replacing the MoAb with a mouse Ig of the same isotype.

Results
Serum sFas values for all samples are given in Fig. 1A. Six patients with AIDS, all hemophiliacs, were coinfected with HIV and HTLV-1, while all patients with HAM/TSP and ATL were positive for HTLV-1 alone. There was no correlation between sFas levels and coinfection in patients with AIDS. The values for ATL patients tended to be highly variable. The mean sFas (ng/ml) was 4.20 in acute ATL, and 2.12 in smoldering and chronic ATL. Among those cases with 20 acute type ATL, 4 had extremely high sFas levels of 5 ng/ml or more. The individual value of sFas correlated significantly with that of serum sIL-2R, as shown in Fig. 1B (Y=2.544 + 0.2204X, r=0.5297, p<0.0001). The mean serum sFas and sIL-2R levels of each group are
Table 1. Comparison of sFas and sIL-2R Levels in Sera, and mFas Expression and Fluorescent Intensity

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No</th>
<th>sFas ng/ml</th>
<th>sIL-2R U/ml</th>
<th>mFas %</th>
<th>Intensity MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls seronegative for HIV and HTLV-1</td>
<td>25</td>
<td>1.62±0.49</td>
<td>362±64</td>
<td>30.1</td>
<td>74.9</td>
</tr>
<tr>
<td>Healthy HTLV-1 carriers</td>
<td>15</td>
<td>1.90±0.49</td>
<td>429±170</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>HAM/TSP</td>
<td>20</td>
<td>2.00±0.59</td>
<td>709±340</td>
<td>85.0</td>
<td>86.0</td>
</tr>
<tr>
<td>ATL</td>
<td>31</td>
<td>3.32±2.05</td>
<td>12,040±12,500</td>
<td>94.5**</td>
<td>90.0</td>
</tr>
<tr>
<td>AIDS</td>
<td>14</td>
<td>3.06±0.92</td>
<td>1,008±465</td>
<td>83.3</td>
<td>85.0</td>
</tr>
</tbody>
</table>

*including ATL cells in the case of ATL. **excluding 2 patients with no mFas expression on ATL cells.
sFas and sIL-2R levels in sera, and mFas expression (%) on lymphocytes, with intensity scored as the mean channel (MC) by flow cytometry. There were significant differences in sFas levels, sIL-2R levels, and mFas expression between the normal controls and the respective groups, HAM/TSP, ATL, and AIDS (p<0.05). The MC value of each group was not statistically different.

Figure 2. Representative histogram expressing mFas fluorescent density of normal sample with 98.2 MC (a), AIDS sample with 99.4 MC (b), and ATL samples with 76.7 (c) and 115.8 (d) MC, respectively.
summarized in Table 1. Compared to the controls, both levels were significant in HAM/TSP, ATL, and AIDS (p<0.05). In order to reveal the relationship between soluble and membrane isoforms of Fas, we examined mFas fluorescent density and positivity on lymphocytes and leukemic cells flow cytometrically. As shown in Fig. 2a and Table 1, the mFas positive rate in normal controls was 30.1% with the mean channel (MC) of 98.9 fluorescent intensity. On the other hand, most lymphocytes and leukemic T-cells from patients with HAM/TSP, ATL, and AIDS expressed mFas with a variable density. Especially, mFas of ATL-cells as shown in Fig. 2c (MC, 76.7) and 2d (115.8) was characterized to be varied in fluorescent intensity from weak to strong. Interestingly, 2 of the ATL cases in which no mFas was detectable on leukemic T-cells, had the highest sFas level, 9.85, and 4.07 ng/ml, respectively. Moreover, mFas positivity in each case was not consistently correlated to sFas level in patients with ATL, HAM/TSP, and AIDS.

Next, we compared sFas levels with standard disease parameters such as white blood cell counts, leukemic cells (tumor burden), and disease stage. Although the average leukocyte count in ATL was an elevation of 23,000/μl with 60% leukemic cells, the high level of sFas was distributed into progressive disease. The level, then, came down to within the normal range after successful anti-cancer chemotherapy in 3 cases serially measured.

In AIDS patients, hematological analysis in all cases revealed CD4 cytopenia of 200 μl or less with CD4 to CD8 ratio of 0.25, ranging from 0.01 to 0.58. Their sFas level was significantly higher than that of controls. Especially, the positive rate of CD4 cells was statistically lower in patients with a high sFas level of 3.5 ng/ml or more than in the remaining patients (4.2% vs 14.2%, p<0.05).

Discussion

In this study, using a sandwich ELISA, sFas was detectable in all samples, from healthy individuals to patients with disorders linked to retroviral infection of HIV and HTLV-1. In addition, we compared sFas levels in normal healthy sera with that in sera from patients associated with the viruses. sFas was detectable even in the normal healthy controls, the mean sFas level of which was considered reliable because it approximated the 2 ng/ml previously reported by Knipping et al (11). The individual values of sFas in ATL and AIDS patients was widely distributed, with only a small number of cases with a high value (greater than 3 ng/ml). The clinical significance of such variations among patients with the same disease is not known.

We compared sFas levels with sIL-2R levels, cell surface membrane Fas, and certain clinical parameters. Our results revealed a good correlation between sFas and sIL-2R levels, suggesting that the two receptors are of the same origin, and were possibly derived from activated-T cells or leukemic-T cells (11). However, as shown in the results, sFas levels were high and discrepant irrespective of lymphopenia in the AIDS group and weak or absent mFas expression in some ATL cases. This indicates that serum sFas levels do generally correlate with mFas expression on activated-T cell and leukemic-T cell surfaces, but sFas in serum is not always derived from their cells. Therefore, other producers also seem to be involved in the hypersecretion of sFas. A soluble form of Fas is known to be generated by differential splicing of the mRNA, yielding a secreted molecule lacking the transmembrane domain (TM), that does not parallel mFas expression. Interestingly, we have established an ATL cell line previously reported (12), in which the TM domain of Fas has been spliced out of the mRNA, that does not express mFas (data not shown). The discrepancy between the soluble and membrane isoforms in our 2 ATL cases may represent a similar phenomenon in vivo. Thus secretion from the leukemic T-cells may be independent of mFas expression.

Next, the clinical significance of sFas was examined. sFas levels could serve as a putative marker for an active persisting leukemia of ATL. Although sFas levels were high at the time of diagnosis, they became within normal limits at remission after successful chemotherapy in patients with aggressive forms of ATL.

In cases of AIDS, it was difficult to evaluate the relationship between the level of sFas and the disease state. mFas expression is reported to be significantly increased in the CD4 and CD8 cells of HIV+ persons in comparison to HIV- controls (13). Thus, the origin of sFas is speculated to be derived from a membrane form expressed on activated-lymphocytes with HLA-DR and IL-2R. However, there have been only few reports the concerning serum sFas level, and the physiologic mechanism by which sFas affects AIDS pathology remains to be elucidated. In this study, our data indicate that a high level of more than 3.5 ng/ml tends to correlate with a reduction of CD4 cell count and disease progression.

Finally, although the study of soluble and membrane Fas isoforms has only just begun, it is expected to eventually disclose the pathogenesis in intractable diseases, especially those of the human retrovirus-associated disorders.

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References


