In this last session, our current studies on adult T cell leukemia/lymphoma (ATLL) are introduced with the result of a survey in a highly ATLL-endemic area, this survey was conducted to elucidate the infectious mode of HTLV-I, which is closely related to the induction of ATLL.

I. Histologic feature and immune response in ATLL

The following two histological changes are noted in biopsy lymph nodes of patients with ATLL: 1) the disappearance of germinal centers and 2) the infiltration and proliferation of neoplastic cells showing peculiar deformation of nucleus.

The formation of the germinal center in lymph nodes bearing neoplastic cells shows striking contrast between AIDS with Kaposi's sarcoma and ATLL, both diseases caused by T cell tropic retroviruses and result in impairment of immune response. In AIDS with Kaposi's sarcoma, germinal centers of lymph nodes increased both in number and size, and sometimes cellular elements of Kaposi's sarcoma extended to the narrow inter-follicular spaces. On the contrary, germinal centers were absent or atrophic in 96% of ATLL biopsy lymph nodes before therapy. The following observations ruled out the possibility that the disappearance of germinal centers depends on the mechanical destruction of tissue components by proliferating neoplastic cells in the marginal
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cortex. 1) The structure of the marginal sinus was well-preserved even in
cases in which neoplastic cells markedly proliferated in the marginal cortex
\(^{2-4}\). 2) The formation of germinal centers was completely suppressed in the initial
stage of neoplastic cell infiltration in lymph nodes where the distribution
of a small number of neoplastic cells was restricted to the paracortical
area \(^{2-4}\). 3) ATLL neoplastic cells expressing helper/inducer phenotype
\((\text{OKT} \, 4^+/8^-)\) had a suppressor effect on pokeweed mitogen activated B cell
proliferation and/or generation of plaque forming cells in vitro, probably
as a result of proliferation of suppressor T cells in close proximity to
neoplastic cells \(^{5}\). These findings suggest that infiltrating or proliferating
neoplastic cells in lymph nodes suppress indirectly, through the suppressor
T cells, the proliferation of B cells which constitute the cellular elements
of germinal center. This effect by neoplastic cells is restricted to the T
cell-dependent proliferation of B cells in the definite area. Therefore,
humoral immune response is generally well-preserved in ATLL patients. Our
nationwide survey disclosed normal levels of serum IgG in many patients with
ATLL \((92\%)\) as well as with other lymphoid malignancies \(^{6}\).

The feature of immune deficiency in ATLL is the selective impairment of
cellular immunity, as is seen in AIDS. There is no evidence that such impair-
ment is induced directly by HTLV-I, which does not show cytopathic activity
to target cells such as helper or cytotoxic T cells. However, the following
observations suggest cellular immune impairment in many cases of ATLL. 1)
Half of the patients had pneumonia caused by opportunistic infection. In
pneumonia cytomegalovirus infection accounted for over 50\%, followed by
\(Pneumocystis \, \text{carinii}\) infection \((17\%)\) in autopsy cases of ATLL \(^{7}\). 2) Negative
and weakly positive PPD skin tests were found in 96\% of the patients with
ATLL \(^{6}\). In a recent second nationwide survey, 70\% of the ATLL patients had
negative PPD skin test, and this value was higher than the incidence of T cell
malignancies other than ATLL (46%) or lymphoid malignancies of non-T non-B cell type (36%)\(^8\). 3) Reduced reactivity for phytohemagglutinin (PHA) in peripheral blood T cells was found in most patients with ATLL\(^9-11\).

ATLL is morphologically characterized by the proliferation of neoplastic mature T cells (ATLL cells)\(^3\). These cells showing a remarkable variety of nuclear deformation and are called as flower cells (Fig. 1). Flower cells are predominant in 76% (N=183) of biopsy lymph nodes of patients with ATLL, and half of them were associated with neoplastic giant cells as classified to "diffuse lymphoma, pleomorphic type" according to the Japanese Lymphoma Study Group (LSG)\(^12\). Morphological identification of ATLL cells is not easy in all lymph nodes, as seen in 24% of ATLL biopsy lymph nodes that are classified as small, medium-sized, large and mixed types by LSG. Besides this, the peculiar nuclear deformation as seen in flower cell is not found in cultured cell lines established from ATLL patients using specific T cell stimulants such as T cell growth factor (IL-2), allogeneic cells and PHA or Concanavalin A. Therefore it is also difficult to establish whether T cell line obtained from patients with ATLL are derived from the neoplastic cell itself, even if T cell lines have HTLV-I genome products (ATLL virus related antigens, ATLA), because HTLV-I can infect normal T cells and the genome is randomly inserted into cellular DNA. Furthermore, ATLA cannot be detected in ATLL cells of fresh materials by immunofluorescence without culture at least one or two days. To identify the ATLL cells in tissue, we produced a monoclonal antibody specifically reacting with ATLL cells\(^13\). Among the T cell lines established by us from the blood of patients with ATLL using medium RPMI-1640 supplemented with 10% human cord serum without addition of any specific T cell stimulant, there was a cell line, KUT-2 exceptionally containing low percentage of ATLA positive cells (lower than 2%) in immunofluorescence. A monoclonal antibody, FTF-148, was prepared by hybridizing
murine myeloma NS-1 cells and spleen cells of BALB/c mice immunized with KUT-2 cells. This antibody reacted with over 90% of the cells in all of 5 T cell lines (MT-1, MT-2, KUT-1, KUT-2 and UW-4 cells) established from patients with ATLL, and HUT-2 cell obtained from a patients with serum anti-ATLA positive cutaneous T cell lymphoma, but not reacted with 3 T cell lines (HSB-2, CCRF-CEM, Molt-4 cells) or one non-T non-B cell line (K-562 cell) obtained from non-ATLL patients. The antigen detected by FTF-148 (FT antigen) was proved to be different from IL-2 receptor and Ia antigen, which were expressed on the surface of ATLL cells. The above results suggest that FTF-148 is a specific antibody for ATLL cells. This antibody also reacted with 3 B cell lines (KUT-3, ATL-B-1 and ATL-2) established from ATLL patients and infected with HTLV-I, resembling the reaction with ATLA. However, non-glycosilated FT antigen of 75 K and 50 K was different from known HTLV-I genome products and their precursors including p40 of pX products. It was also confirmed morphologically in immunofluorescence, that over 90% of MT-1 and KUT-2 cells reacted with FTF-148 antibody, however, ATLA positive cells accounted for less than several percent in both cell lines. In immunoelectron microscopy, FT antigen was detected on the cell surface in almost all KUT-2 cells and the distribution of this antigen was restricted to the cell surface of MT-2 cells, which had ATLA or HTLV-I particles in the cytoplasm and on the cell surface. These findings mean that FT antigen is expressed in cells infected with HTLV-I, but not in HTLV-genome products themselves.

Using FTF-monoclonal antibody, some neoplastic cells can be identified in smear preparations of the blood or bone marrow and in frozen sections of lymph nodes by immunofluorescence or immunoenzyme methods.
II. Intrafamiliar Infection of HTLV-I

In the past 8 years, Uwa district located in southwestern Shikoku island was found to be a highly ATLL-endemic area where 61 natives have been confirmed to have ATLL and all but one were over 40 years of age (Fig. 2). During the period from 1980 to 1984, the survey on ATLL was performed under the cooperation of 11 hospitals covering the entire Uwa district to clarify the infectious mode of HTLV-I\textsuperscript{14-16}. During the survey, 37 patients with ATLL were confirmed, giving an average of 9.3 patients per year and an annual incidence of patients with ATLL of 1 patient per 1633 individuals over 40 years of age showing positive serum anti-ATLA antibody (seropositive adults) (Table 1).

Among patients with ATLL in Uwa district, two patients with ATLL were identified in each of 9 families, suggesting the familial predisposition of HTLV-I infection\textsuperscript{15}. Among 98 individuals from 13 families of patients with ATLL, 49 were seropositive (50%). This value is significantly higher than that of seropositive adults in the entire Uwa district (8.0%) or in Uwajima City Hospital (6.4%)\textsuperscript{15}. Analysis of 36 seropositives among 75 children of 26 pairs of parents is shown in Table 2. The difference of seroconversion in children between seropositive mothers and fathers was significant. There was also a sex-difference in seroconversion in children from parent of patients with ATLL, 18% of the children of male patients were seropositive, but seropositives among children of female patients accounted for 62%\textsuperscript{16}. The family survey indicated that HTLV-I was transmitted mainly from mother to child. Fig. 3 illustrates the 15 pedigrees of ATLL patients, and shows the main route of viral transmission from mother to children.
Table 1. Incidence of ATLL in Uwa District

<table>
<thead>
<tr>
<th>Total population</th>
<th>296,919</th>
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</thead>
<tbody>
<tr>
<td>Population over 40 years old</td>
<td>141,913</td>
</tr>
<tr>
<td>Rate of seropositives (&gt;40Y)</td>
<td>126/1,173</td>
</tr>
<tr>
<td>Mean incidence of ATLL per year</td>
<td></td>
</tr>
<tr>
<td>Annual incidence of ATLL per 10^5 population (&gt;40Y)</td>
<td>6.5</td>
</tr>
<tr>
<td>Annual incidence rate of ATLL among seropositive group (&gt;40Y)</td>
<td>1/1,633</td>
</tr>
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Table 2. Analysis of Seropositives among the Children of 26 Pairs of Parents

<table>
<thead>
<tr>
<th>Number of seropositives/number of tested</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Father's anti-ATLA antibody</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>Mother's anti-ATLA antibody</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Total</td>
</tr>
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Table 3. Relationship between Flower cell Carriers and Seropositives

<table>
<thead>
<tr>
<th>Flower cell</th>
<th>-</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-ATLA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>tested</td>
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</tbody>
</table>

% of positive cases

<table>
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<tr>
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<th>27</th>
<th>4</th>
<th>56</th>
<th>79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>82</td>
<td>88</td>
</tr>
<tr>
<td>Control*</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>82</td>
<td>88</td>
</tr>
</tbody>
</table>

* Healthy adults and adult patients with non-lymphoid malignancy in Uwa district

Fig. 1. Flower Cell (upper left) and Mitotic Cell in Peripheral Blood of Patient with ATLL. (H.Y., 65 years of age, Kyoto University Hospital).
Fig. 2. Distribution of Birthplaces of Patients with ATLL in Uwa District. A closed circle indicate the birthplace of a patient.
In blood smear of family members of patients with ATLL, the percentage of flower cells was very low from 0.1 to 1.0%. The percentage of flower cell carriers in family members of ATLL patients was 39%, and this value was higher than that of a control group (16%). Family members of ATLL patients comprised 34% of the seropositive flower cell carriers, the value being 60% among total flower cell carriers surveyed (Table 3). The flower cell carriers and seropositives were positively correlated\(^{16}\). Therefore, for each patient with pre-ATLL or smoldering ATLL\(^ {17}\), there are many healthy, seropositive flower cell carriers.

III. Conclusions

HTLV-I mainly infects the family members, especially, from mothers to children of nursing age. This is supported by the report that ATLA positive
T cells were found in the milk of seropositive mothers. The incidence of ATLL is one patient per 1633 seropositive adults. The seropositive individuals who have a very low percentage of flower cells in the blood constitute at least one of the risk groups that develop pre-ATLL (smoldering ATLL) and ATLL, and impairment of cellular immunity among individuals in this risk group promotes the induction and/or development of ATLL. The risk group of ATLL must be further examined using the FTF-148 monoclonal antibody to identify such flower cells as neoplastic cells.

References


