Absence of Human T-Lymphotropic Virus Type I in Japanese Patients With Cutaneous T-Cell Lymphoma

By Arata Kikuchi, Takeji Nishikawa, Yasuo Ikeda, and Kazunari Yamaguchi

Cutaneous T-cell lymphoma (CTCL) is a disease entity characterized by a primary sporadic T-cell proliferation in the skin. Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus that causes adult T-cell leukemia/lymphoma. Recently, several authors have detected the HTLV-1 genome in genomic DNA from patients with CTCL and proposed a causal relation of HTLV-1 to CTCL. However, it remains controversial because these studies contain some problems in materials used to detect HTLV-1. We investigated both fresh and cultured T lymphocytes (128 specimens) derived from 50 Japanese patients with CTCL, where HTLV-1 is endemic, by using polymerase chain reaction with four sets of primers including gag, pol, env, and pX regions of HTLV-1 to elucidate the relationship between HTLV-1 and CTCL in Japan. However, none of the 128 DNA specimens revealed positive for HTLV-1 in contrast to the previous studies. We conclude that CTCL, which does not include HTLV-1, is present although the pathogenesis of CTCL may be different by areas or races.

MATERIALS AND METHODS

Selection of patients. We have selected 50 patients with CTCL, in which clinical features, pathological findings and DNA analyses were all typical for CTCL and serum anti-HTLV-1 antibodies were negative in both particle agglutination and enzyme-linked immunosorbent assay (ELISA) methods. The patients included 28 men and 22 women and the ages varied from 24 to 88 (mean: 55.9). The diagnoses of the patients with CTCL are seronegative for HTLV-1 except CTCL patients in HTLV-1 carrier, and CTCL is considered to be sporadic.

HTLV-1 is a type of retrovirus first detected in human tumors, which causes adult T-cell leukemia/lymphoma (ATLL), a neoplasm of the mature T-lymphocyte. Patients with ATLL are seropositive for HTLV-1 and often develop a rapid dissemination to the internal organs from the early stages. Skin manifestation of ATLL constitutes papules or multiple skin nodules, but sometimes shows indurated erythema or erythroderma as seen in CTCL. The tumor cells, expressing CD4/suppressor phenotype, are heterogenous in size and their nuclei have irregular contours showing flower-like arrangements. The prognosis of ATLL is very poor. Involvement of the skin characterizes both disorders, making it difficult to differentiate between them. Furthermore, several authors have recently shown the HTLV-1 genome in genomic DNA from patients with CTCL and proposed a causal relation between HTLV-1 and CTCL, which causes more confusions in the diagnosis as well as the mean of HTLV-1 in these two disorders. However, in these studies there were some problems in the diagnosis of CTCL as well as in the interpretation of the laboratory data as follows: (1) The diagnoses of CTCL was not always differentiated from chronic or smouldering ATLL. (2) HTLV-1 carriers (seropositive for HTLV-1) were included in some polymerase chain reaction (PCR) studies. (3) Conditions (stringency) of PCR varied. Samples employed in these studies varied including fresh lesional skin, peripheral blood mononuclear cells (PBMC) and cultured cell lines.

In an endemic area of ATLL like Japan diagnoses of CTCL are very critical because the treatment as well as the prognosis of ATLL are quite different from those of CTCL. Thus, in this study, we examined 128 DNA specimens of fresh lesional skin, PBMC, and T-lymphocyte clones derived from 50 Japanese patients with CTCL, who showed typical clinical and histopathological features and had no serum HTLV-1 antibodies to clarify the relationship between HTLV-1 and CTCL in Japan.

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1 genome were not detected (CTCLs in HTLV-1 carrier),
were employed as positive controls.

Cell culture. The fresh skin specimens from the patients with
CTCL were incubated in RPMI medium 1640 containing 20% fetal
bovine serum and interleukin-2 after an initial 72-hour stimulation
with phytomhemagglutinin. When the cultured cells once constantly
were grown in the medium, phenotypic analyses were done by using flow
cytometry with anti-CD4 monoclonal antibody. If the cultured cells
express CD4 on their surface, genomic DNAs were derived for the
subsequent PCR.

DNA preparation. Genomic DNAs were derived from the fresh
lesional skin and PBMC of the patients with CTCL. Briefly, after
phenol chloroform extraction the DNA was precipitated with ethanol
and redissolved in sterile H2O. DNA concentration was estimated
by absorption at 260 nm.

PCR amplification. Amplification of DNA was performed in a total
volume of 50 µL in a reaction mixture containing 225 µmol/
L each of deoxyadenosine triphosphate, deoxyctydine triphosphate,
deoxyguanosine triphosphate, and deoxythymidine triphosphate, 50
to 125 pmol each of primer, 50 mmol/L KCl, 2.5 mmol/L MgCl2,
and 10 mmol/L tris-HCl (pH 8.3), and 2 U of Taq polymerase
(Takara, Shiga, Japan). Solutions were covered with mineral oil
to prevent condensation. Fifty cycles of denaturation 2 minutes at 94°C,
primer annealing for 1 minute at 60°C, and chain elongation for 30
seconds at 72°C were performed, using reagents from Takara Gene
Amp reagents kit and the Perkin Elmer Thermal Cycler (Perkin
Elmer Cetus, Norwalk, CT). The primers used for the amplification
of HTLV-1 are shown in Table 1.

RESULTS

T-cell cultures were established in 4 cases of erythema
stage MF, 5 of plaque stage MF, 5 of tumor stage MF, 3 of
SS, 4 of Ki-1+ CTCL, and 13 of CTCL other than MF, SS,
or Ki-1+ CTCL. Totally, 128 DNA samples were taken from
50 lesional skin, 44 PBMC and 34 cultured T-lymphocyte
clones. The results are summarized in Table 2. No samples
revealed positive for HTLV-1 genome. Two CTCLs in
HTLV-1 carrier revealed positive with all four sets of prim-
ers and a smouldering ATLL revealed strongly positive with
all sets of primers examined (Fig 1).

DISCUSSION

In Japan, where has the highest prevalence of HTLV-1
infection in the world, antibodies against HTLV-1 have
been detected in 1.2 million people (most of them are
Carriers of HTLV-1), which are estimated at approxi-
mately 1.0% of all Japanese population, and newly more
than 700 cases of ATLL are diagnosed each year.5 Thus,
the presence of serum HTLV-1 antibodies is not diagnostic
for ATLL and it is essential to confirm the monoclonal
integration of the HTLV-1 DNA by using Southern blot analysis
or inverse PCR,18 a newly developed method to
recognize the monoclonality of HTLV-1 under the detect-
tion level of Southern blotting. In practice, such borderline
cases as a smouldering ATLL showing indolent MF-like
course (our no. 51), HTLV-1 negative ATLL,19
and CTCLs accompanied by serum anti-HTLV-1 antibodies
(our nos. 52 and 53)19 are present.

In lymphoproliferative disorders except ATLL the sero-
negativity for HTLV-1 is common. However, more sensitive
method such as ELISA has recently enabled to show posi-
itivity for HTLV-1 up to 30% in various leukemia/lymphoma
patients including MF.20,21 In certain regions of the United
States HTLV-1 seroprevalence has been particularly high
in prostitutes, homosexuals, and intravenous drug users.22,23
These tendencies are completely different from our back-
grounds (patients who have serum anti-HTLV-1 antibodies
are exceptional in our series), and they also suggest the
higher detection rates of HTLV-1 genome in their PCR stud-
ies. As shown in Fig 1, it is reasonable that the PCR for
HTLV-1 revealed positive in HTLV-1 carriers (but the posi-
tive bands were weaker than those in smouldering ATLL),
which should not be included into these HTLV-1 detection
studies. Moreover, it is no doubt that patients having mono-
clonal integrations of HTLV-1 should be diagnosed as
ATLL.

In addition to these confusions in the diagnoses of
ATLL and in the interpretation of serum anti-HTLV-1
antibodies, there are several problems in the positive
HTLV-1 results with PCR in CTCL. First, technical er-
rors including contamination can easily occur especially
in PCR studies. Second, many positive bands can be de-
tected if the stringency of PCR is low (data not shown).
These results indicate the presence of endogenous
HTLV-1-like sequences within human genomic DNA
or the detection of other retroviral sequences similar to
HTLV-1 (HTLV-5, etc.). Moreover, the false positive re-
sults for HTLV-1 can be detected unless the complete
sequencings for the PCR products are done.11 Third, de-
leted forms of HTLV-1 genome may exist in the genomic
DNA of CTCL patients.8 If the HTLV-1 genome was
inserted into the host DNA as a deleted form, it is reason-
able that the defective HTLV-1 genome neither induce
the host's antibody production nor the replication of the
ATL cells. Lastly, in the positive studies,7-12 most of
the HTLV-1 DNA were proved not in the fresh specimens
but in the cultured cells including non-T−cell lines.5,16
During the cultivating procedures not only clonal expan-
sions but also modifications of the culturing cells can
occur, thus the DNA derived from those cell lines does
not exactly reflect the conditions in the original CTCL.
Furthermore, HTLV-1 sequences have been detected
even in squamous cell carcinoma of the skin,24 suggesting
that an opportunistic or a coincidental infection of
HTLV-1 should be considered.

Table 1. Primers Used for Amplification of HTLV-1

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer Nucleotide Sequence</th>
<th>Product Size</th>
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<tr>
<td>gag</td>
<td>sense 5′-TATGCAGACCATCGCCGTGAG-3′ (1324-1343)</td>
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<tr>
<td>pol</td>
<td>anti-sense 5′-TGGGGCTGGACACGGAGA3′ (1521-1540)</td>
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<tr>
<td>env</td>
<td>sense 5′-AGGCCTAGCAACCTTGTCC-3′ (2631-2650)</td>
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</tr>
<tr>
<td>pX</td>
<td>anti-sense 5′-GGCTTGGTGCCCTGATGA-3′ (7764-7783)</td>
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</table>

Complete proviral sequences is described elsewhere.25

1530 KIKUCHI ET AL

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### Table 2. Summary of the Clinical Data, Serum Antibodies, and PCR Studies

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Serum Antibodies</th>
<th>Detection of HTLV-1 DNA</th>
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<td></td>
<td></td>
<td>PA</td>
<td>ELISA</td>
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<tr>
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<td>gag</td>
<td>pol</td>
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<tr>
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<tr>
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Abbreviations: PA, particle agglutination; ELISA, enzyme-linked immunosorbent assay (cut off < 0.395); PBMC, peripheral blood mononuclear cells; nd, not done; ne, not established; MF, mycosis fungoides (e, erythema stage; p, plaque stage; t, tumor stage); SS, Sezary syndrome; Ki-1'CTCL, primary cutaneous anaplastic large cell lymphoma of T-cell type expressing Ki-1 (CD30) antigen; CTCL*, cutaneous T-cell lymphoma other than MF, SS, or Ki-1'CTCL; CTCL*, CTCL in HTLV-1 carrier; sATLL smouldering adult T-cell leukemia/lymphoma.
We conclude that CTCL which does not include HTLV-1 is present although the pathogenesis of CTCL may be different by respective areas or races.

REFERENCES

25. The complete nucleotide sequences of the HTLV-1 genome have been submitted to the National Center for Biotechnology Information (NCBI) databases. The accession number is J02029.
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