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Human T-cell leukemia virus type 1(HTLV1) infection in malignant lymphomas in a HTLV1-endemic area, Kagoshima, Japan : HTLV-1 in-situ-hybridization (ISH) analysis employing a highly biotinylated concatamer probe synthesized by polymerase chain reaction (PCR) for HTLV-1 proviral DNA pX Tax region

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Summary
This study aimed to see HTLV-1 infection in 277 cases of malignant lymphoma (ML) in a HTLV-1indemic area by means of ISH employing a highly biotinylated concatamer probe synthesized by a couple of primers SK43 and 44 PCR for HTLV-1 provial DNA pX Tax region. The MLs comprised 115 T-cell MLs, 73 B-cell MLs, 14 Hodgkin's disease (HD) and MLs from the age group less than 20 years old (y.o.). The non-Hodgkin's MLs were categorized according to updated Kiel classification. ISH detected a signal of HTLV-1 proviral DNA including pX Tax region, showing positive stain dominantly in cytoplasm of lymphoma cells and non-neoplastic cells including intermingling small 1ymphocytes, histiocytes and epithelioid cells. ML in which lymphoma cells showed the signal was defined as "HTLV-1-related ML". And ML in which any cells revealed the signal was defined as "HTLV-1-carrier ML". HTLV-1-related MLs were found in all the subtypes of T-cell MLs (69/115, 60%), indicating its high rate (44/45, 97.8%) in T-cell pleomorphic lymphoma with histological features of adult T-cell leukemia/ lymphoma (ATLL). Even in MLs other than the T-cell MLs, HTLV-1ielated MLs were noted in 21 (29%) of B-cell MLs, 8 (57%) of HD and 4 (16%) of MLs from the age group less than 20 y.o.. HTLV-1-carrier MLs were found in 93 (81%) of T-cell MLs, 35 (49%) of B-cell MLs, 8 (57%) of HD, 13 (52%) of MLs from the age group less than 20 y.o.. HTLV-1-related T-cell MLs in which many lymphoma cells showed the signal may be ATLL, but it was unknown whether there were ATLLs in the ISH-negative cases and in HTLV-1-carrier MLs other than HTLV-1ielated MLs. Considering several technical problems in this ISH, the followings were suggested in this study. HTLV-1 may have oncogenetic effect(s) specific for ATLL and non-specific for MLs other than ATLL but ATLL development would need factor(s) other than HTLV-1 infection. There may be HTLV-1-related B-cell MLs and HD. Some of patients with B-cell MLs and in the under 20-year-old ML patients would fail to produce antibodies against HTLV-1, because higher HTLV-1 carrier rates in these patients were recognized in this study than in the serovirological studies.

Key words : Human T-cell leukemia virus type 1(HTLV-1) , Malignant lymphomas, HTLV-1 endemic area, In-situ-hybridization, HTLV-l pX Tax

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is a causative agent for human T-cell leukemia/1ymphoma (ATLL) and the other HTLV-1-associated diseases. But factor(s) other than HTLV-1 infection are thought to concern with the pathogenesis of ATLL, although the almost all patients with ATLL were HTLV-1 carriers.

HTLV-1 proviral DNA sequence in paraffin sections of surgical pathological materials can be detected by polymerase chain reaction (PCR) (Shibata D et a1. 1991). Detection of HTLV-1 proviral DNA sequence in the tissue by means of PCR could show anatomical distribution of HTLV-1-infected cells (Sueyoshi K et a1. 1994). Detection of HTLV-1 proviral DNA sequence in lesions of HTLV-1-associated

diseases by PCR makes it possible to analyze a direct effect of HTLV-1 on pathogenesis of the lesions (Hasui K et a1, 1992). In the nest Stage of study analyzing HTLV-1 in the lesions, in-situ-PCR is expected to show cells having HTLV-1 proviral DNA sequence in the lesions under a microscope. And in-situ-hybridization (ISH) analysis of HTLV-1 in the lesions is expected to show existence of HTLV-1 proviral DNA sequence and its signals in cells comprising the lesions.

This study aimed to see the HTLV-1 infection in malignant lymphoma (ML) in a HTLV-1-endemic area, Kagoshima (Hanada S et a1,1989 I, Sato E et a1,1990), in Japan. Detection of HTLV-1 infection was tried by means of ISH employing a highly biotinylated concatamer probe synthesized by PCR for HTLV-1 proviral DNA pX Tax region (Hasui K et a1, 1994a).

Material and Method

Paraffin sections of 227 cases of MLs, comprising 115 T-cell MLs, 73 B-cell MLs, 14 Hodgkin's disease (HD) and 25 MLs from the age group less than 20- year-old (y.o.) , were employed for this study(Table 1). The non-Hodgkin MLs were categorized according to the updated Kiel classification (Lennert K and Fe11er AC,1992). T-cell pleomorphic lymphoma (T-Pleo) and T-cell anaplastic large cell lymphoma (T-ALC) were classified further into ATLL type, clear cell type and the other (Hasui K,1991). The ATLL type showed peculiar nuclear figures such as stippled heterochromatin distribution (Kikuchi M et a1,1986) and no intermingling of B lymphocytes and plasma cells. The clear cell type showed pale cytoplasm (Lennert K et a1,1985) and intermingling of B lymphocytes and plasma cells. Seventy-five cases of T-Pleo and 9 T-ALC comprised 45 ATLL type, 18 clear cell type and 21 cases of the others (Table 2). In centrocytic lymphoma (CC) of B-cell ML, mantle-zone lymphoma (MzML) revealing marked mantle zone growth pattern was categorized (Hasui K,1991).

Table 1. Malignant lymphomas studied according to updated Kiel classification

		No. of cases examined		
		Total	Adults	Less than 20-yr-old
TOTAL		227	202	25
T-cell malignant lymphoma	T-ML	127	115	12
Low grade malignant T-ML		38	37	1
Chronic lymphocytic leukemia	T-CLL	2	2	-
Pleomorphic lymphoma, small cell type	Pleo-small	9	9	-
T-zone lymphoma	TzML	12	12	-
Lymphoepithelioid cell lymphoma	LeL	4	4	-
AILD type lymphoma	AILD type	11	10	1
High grade malignant T-ML		89	78	11
Lymphoblastic lymphoma	T-LB	9	3	6
Pleomorphic lymphoma other than small cell	Pleo	70	66	4
T-cell anaplastic large cell lymphoma	T-ALC	10	9	1
Immunoblastic lymphoma	T-IB	-	-	-
B-cell malignant lymphoma	B-ML	81	73	8
Low garde malignant lymphomas		38	38	-
Chronic lymphocytic leukemia	B-CLL	4	4	-
Centrocytic lymphoma	CC	14	14	-
Centroblastic/centrocytic lymphoma	CB/CC	4	4	-
Immunocytoma	IC	13	13	-
Plasmacytic lymphoma	PC	1	1	-
Monocytoid B-cell lymphoma	MoBL	1	1	-
Mucosa-associated lymphatic tissue type	MALT	1	1	-
High grade malignant lymphomas		43	35	8
Lymphoblastic lymphoma	B-LB	1	1	-
Centroblastic lymphoma	CB	35	31	4
Immunoblastic lymphoma	B-IB	4	3	1
Burkitt type lymphoma	BL	3	-	3
Hodgkin's disease	HD	19	14	5
Lymphocytic predominance	LP	3	3	-
Mixed cellularity	MC	9	6	3
Lymphocytic depletion	LD	2	2	
Nodular sclerosis	NS	5	3	2

Synthesis of highly biotinylated probe for ETLV-1 pX Tax region

Probe employed in ISH for HTLV-1 pX Tax region was synthesized by 2 times of PCR employing a couple of primers SK43 and 44 for HTLV-1 provial DNA pX Tax region. The detailed procedure of the synthesis of the probe was reported previously (Hasui K et a1, 1994a). DNA extracted from ATL cell line KUT2 was used as a template for the first PCR. Amplified 159bp long DNA product of the first PCR was extracted from agar gel by using sedimentation microtube with filter and was the template DNA for the second PCR. Depending on an amount of the template DNA and a number of PCR cycles, the second PCR with bio-11-dUTP (Dennis LYM et a1, 1990) could produced highly biotinylated 159bp long and concatamer DNA probes (Hasui K et a1, 1994a).

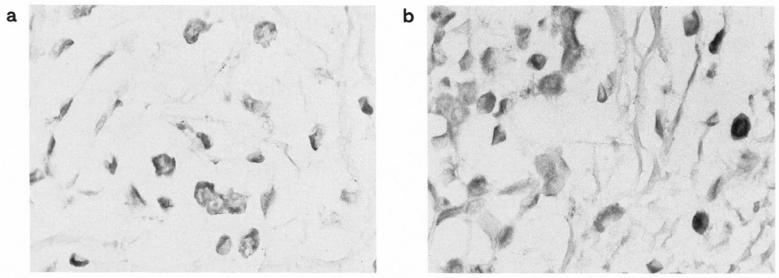

Figure. 1 Comparision of 159bp long and concatamer probes for HTLV-1 pX Tax region in in-situ-hybridization (ISH) designed in this study. A small number of lymphocytes in chronic synovitis in a HTLV-1 carrier showed ISH-positive stronger stain in cytoplasm in the ISH with the concatamer probe (b) than in the ISH with the 159bp long probe (a). No ISH-positive stain was recognized in nuclei and on nuclear membrane of lymphocytes.

In-situ-hybridization (ISH) employing the highly biotinylated probes

The paraffin sections were deparaffinized, treated with 200mg/ml pepsin at pH 2.0 and post-fixed in 4% paraformaldehyde in diethyl pyrocarbonate-buffered saline (PBS). And the sections were denatured in 70% formamide 2x standard saline citrate (SSC) at 70℃ for 5 min, cooled rapidly up to -30℃ in 95% ethanol for 10 min and dried in vacuum chamber. Five to 10 μl biotinylated probe was diluted in 2 ml hybridization solution (0.6M NaC1, 10% dextran sulfate MW : 500000, 50% formamide, 0.1% sodium pyrophosphate, 0.2% polyvinylpyrrolidone MW : 40000, 0.2% ficoll MW : 400000, 5mM Na_2EDTA, 0.05 M Tris/HC1, pH7.5) and was denatured in boiled water for 5 min and cooled and stored in crushed ice. The sections with the probe were covered with glass slide. Hybridization was performed in a moist chamtxr with 70% formamide 2x SSC at 43℃ overnight. The sections were washed at 43℃ 2 times in 2x SSC for 15 min and 2 times in 0.1x SSC for 15 min. The hybridized probe was visualized by DAKO streptoavidine-biotinylated alkaline phosphatase system and DAKO new fucshin system.

As shown in Figure 1, ISH-positive stain in lymphocytes was stronger in ISH employing the concatamer probe than in ISH employing the 159bp long probe (Hasui K et a1, 1994b). The ISH employing the concatamer probe was used in this study.

Result

The ISH showed positive stain dominantly in cytoplasm of lymphoma cells and non-neoplastic cells including intermingling small 1ymphocytes, histiocytes and epithelioid cells.

As shown in ISH (Fig.2) of T-cell MLs, in ATLL type of T-Pleo (Fig.2a and c) and T-ALC relatively many lymphoma cells showed ISH-positive stain in cytoplasm (Fig.2b and d). In a few cases of ATLL type (Fig.2d) a small number of large and giant lymphoma cells showed ISH-positive stain in nucleus. In one case of ATLL type in which lymphoma cells did not show the ISH-positive stain (Table 2), only a small number of intermingling lymphocytes showed ISH-positive weak stain in cytoplasm. In some cases of the others of T-Pleo and T-ALC lymphoma cells showed more or less ISH-positive stain. In some cases of T-Pleo and T-ALC some of non-neoplastic cells including intermingling lymphocytes, histiocytes and epithelioid cells showed ISH-positive weak stain (Fig. 2e and i, 1ymphoepithelioid cell 1ymphoma : LeL). Depending on the ISH-positive figures of T-cell MLs, the ML in which lymphoma cells showed the ISH-positive stain was defined as “HTLV-1-related ML". The ML in which any cells showed the ISH-positive stain was defined as “HTLV-1-carrier ML".

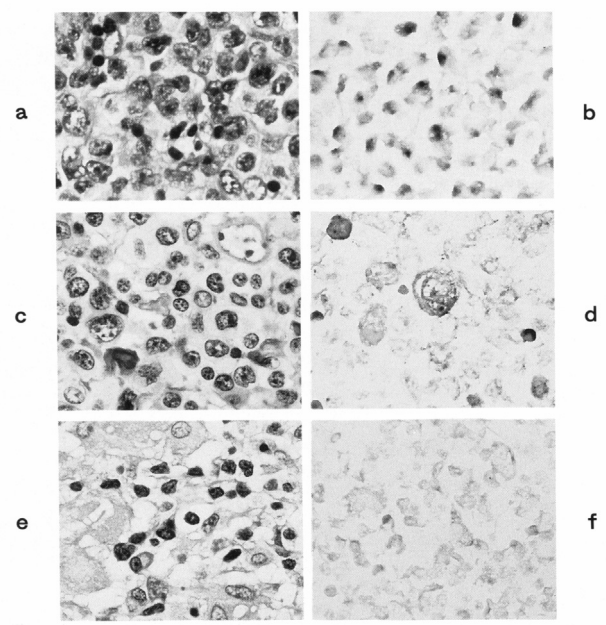

Figure. 2 In-situ-hybridization with the concatamer probe for HTLV-1 pX Tax in ATLL type (a to d) of T-cell pleomorpic lymphoma and T-cell lymphoepithelioid cell 1ymphoma (e and f). Lymphoma cells of ATLL type showed ISH-positive stain dominantly in cytoplasm (b: the ISH of case a). A small number of large lymphoma cells showed ISH-positive rot-like stain in nucleus and a small number of peculiar giant cells showed nuclear and cytoplasmic stain (d:the ISH of case c). In LeL (e) a small number of lymphocytes showed ISH-positive stain (i). Some epithelioid cells show several rot-like ISH-positive weak stain in their cytoplasm (i).

Table 2 indicated that HTLV-1-related MLs were found in 44 (97.8%) cases of ATLL type, none of clear cell type and 8 (38.1%) cases of the others. In small cell type of T-Pleo, one case of HTLV-1-related ML was found in the others. In T-Pleo and T-ALC the rate of HTLV-1-related MLs was extremely higher in ATLL type than those in the clear cell type (p=0.00000000000001) and in the others (p=0. 0000002).

Table 2. In situ hybridization (ISH) with the concatamer probe for HTLV-1 px Tax in the ATLL type, the clear cell type and the others of T-cell pleomorphic and anaplastic large cell lymphomas

	No. of cases	ISH with the concatamer probe for HTLV-1 (HTLV-1-related MLs/examined) ATLL type	Clear cell type	Others
T-cell Pleomorphic lymphoma	75	39/40	0/18	7/17
small cell type	9	-	0/2	1/7
medium-size cell type	25	17/18	0/4	2/3
large cell type	13	8/8	0/1	2/4
medium-sized and large cell type	28	14/14	0/11	2/3
T-cell anaplastic large cell lymphoma	9	5/5	-	1/4

In all the types of T-cell MLs both of HTLV-1-related and -carrier MLs were found (Table 3). But the rates of HTLV-1-related and -carrier MLs were low in small cell type of T-Pleo and AILD (angioimmunoblastic lymphadenopathy with dysproteinemia) type of low grade malignant and in T-cell 1ymphoblastic of high grade malignant (Table 3). And the rates of HTLV-1-related and -carrier MLs were higher in high grade malignant T-cell MLs than in low grade malignant T-cell MLs.

Table 3. ISH with the concatamer probe for HTLV-1 pX Tax in T-cell malignant lymphomas

	HTLV-1-carrier MLs /examined	HTLV-1-related MLs /examined
Low grade malignant	27/37 (73%)	17/37 (46%)
T-CLL	1/2 (50%)	1/2 (50%)
Pleo-small	5/9 (56%)	1/9 (11%)
TzML	11/12 (92%)	9/12 (75%)
LeL	4/4 (100%)	3/4 (75%)
AILD type	6/10 (60%)	3/10 (30%)
High grade malignant	66/78 (85%)	52/78 (67%)
T-LB	2/3 (66%)	1/3 (33%)
Pleo	55/66 (83%)	45/66 (68%)
medium	22/25 (88%)	19/25 (76%)
large	13/13 (100%)	10/13 (77%)
M&L	20/28 (71%)	16/28 (57%)
T-ALC	9/9 (100%)	6/9 (66%)
Total	93/115 (81%)	69/115 (60%)

There were B-cell MLs in which lymphoma cells shows ISH-positive stain in cytoplasm (Fig.3). Twenty-one (29%) cases out of 73 B-cell MLs were HTLV-1-related MLs and 35 (49%) B-cell MLs were HTLV-1-carrier MLs (Table 4). Ten cases of the HTLV-1-related B-cell MLs, in which relatively many lymphoma cells showed ISH-positive stain, comprised 2 B-cell chronic lymphocytic leukemia, 2 CCs (1 diffuse and 1 fo11icular), 3 immunocytoma and 3 centrocytoid centroblastic lymphoma (CB). CC indicated a high rate (57%) of HTLV-1-related MLs, including 5 of 6 MzML. In MzML some histocytes showed ISH-positive stain. In high grade malignant B-cell MLs HTLV-1-related MLs were found in centrocytoid and polymorphous CB. Both of HTLV-1-related and -carrier MLs were more frequent in low grade malignant B-cell MLs (42%) than in high grade malignant B-cell MLs (14%).

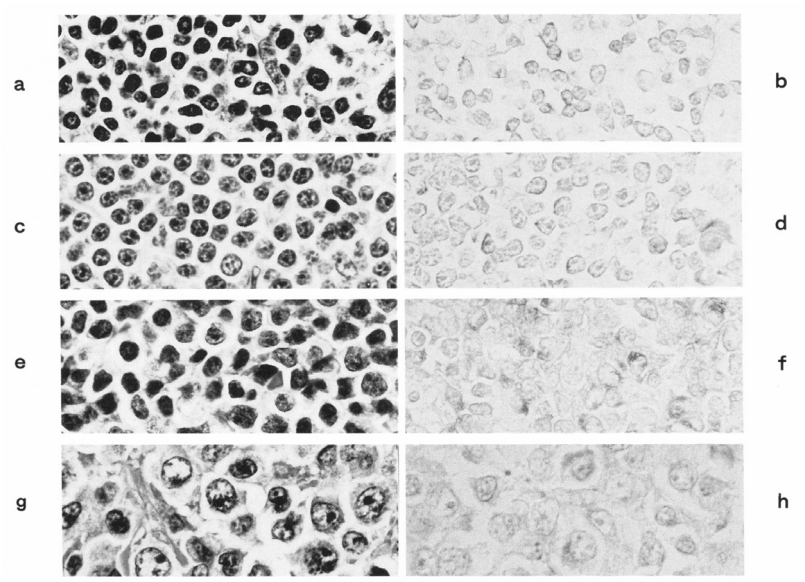

Figure. 3 In-situ- hybrization with the concatamer probe for HTLV-1 pX Tax in B-cell malignant lymphomas.
(a) MzML of centrocytic lymphoma and its ISH (b).
(c) Immunocytoma and its ISH (d).
(e) Centrocytoid centroblastic lymphoma and its ISH (i).
(g) Polymorphous centro-blastic lymphoma and its ISH (h).
In these 4 cases of B-ML some lymphoma cells showed the ISH-positive stain in cytoplasm.

Table 4. ISH with the concatamer probe for HTLV-1 pX Tax in B-cell malignant lymphomas

	HTLV-1-carrer MLs /examined	HTLV-1-related MLs /examined
Low garde malignant	21/38 (55%)	16/38 (42%)
B-CLL	2/4 (50%)	2/4 (50%)
CC	10/14 (71%)	8/14 (57%)
diffuse	3/5 (60%)	2/5 (40%)
MzML	5/6 (83%)	5/6 (83%)
follicular	2/3 (67%)	1/3 (33%)
CB/CC	3/4 (75%)	1/4(25%)
diffuse	1/1 (100%)	1/1 (100%)
follicular	2/3 (67%)	0/3 (0%)
IC	4/13 (31%)	3/13 (23%)
PC	0/1 (0%)	0/1 (0%)
MoBL	1/1 (100%)	1/1 (100%)
MALT	1/1 (100%)	1/1 (100%)
High grade malignant	14/35 (40%)	5/35 (14%)
B-LB	0/1 (0%)	0/1 (0%)
CB	13/31 (42%)	5/31 (16%)
centrocytoid	6/9 (67%)	3/9 (33%)
monomorphous	4/11 (36%)	0/11 (0%)
polymorphous	3/11 (27%)	2/11 (18%)
B-IB	1/3 (33%)	0/3 (0%)
Total	35/73 (49%)	21/73 (29%)

In 8 (57%) of 14 HD (Table 5), a small number of Hodgkin cells, Reed-Sternberg (RS) cells, lacunar cells and lmphocytes showed ISH-positive stain (Fig.4). In mixed cellularity a small number of RS cells showed obvious ISH-positive stain in cytoplasm. In the other subtypes a small number of Hodgkin cells showed ISH-positive weak stain.

In 25 MLs from the age group less than 20 y.o. , there were 13 (52%) HTLV-1-carrier MLs and 7 (28%) HLTV-1-related MLs (Table 6, sited in the reference, Hasui K et a1 1994b).

Table 5. ISH with the concatamer probe for HTLV-1 pX Tax in Hodgkin's disease

	HTLV-1-carrier MLs/examined	HTLV-1-related MLs/examined
Hodgkin's disease	8/14 (57%)	8/14 (57%)
LP	2/3 (66%)	2/3 (66%)
MC	3/6 (50%)	3/6 (50%)
LD	2/2 (100%)	2/2 (100%)
NS	1/3 (33%)	1/3 (33%)

Table 6. In situ hybridization (ISH) with the concatamer probe for HTLV-1 pX Tax in malignant lymphomas in the less than 20-year-old people

	HTLV-1-carrier MLs/examined	HTLV-1-related MLs/examined
T-cell MLs	5/12 (42%)	3/12 (25%)
T-LB	1/6 (17%)	1/6 (17%)
T-Pleo	2/4 (50%)	1/4 (25%)
ALC	1/1(100%)	1/1(100%)
AILD type	1/1(100%)	0/1 (0%)
B-cell MLs	5/8 (63%)	1/8 (13%)
CB	3/4 (75%)	1/4 (25%)
B-IB	0/1 (0%)	0/1 (0%)
Burkitt	2/3 (66%)	0/3 (0%)
Hodgkin's disease	3/5 (60%)	3/5 (60%)
Total	13/25 (52%)	4/25 (16%)

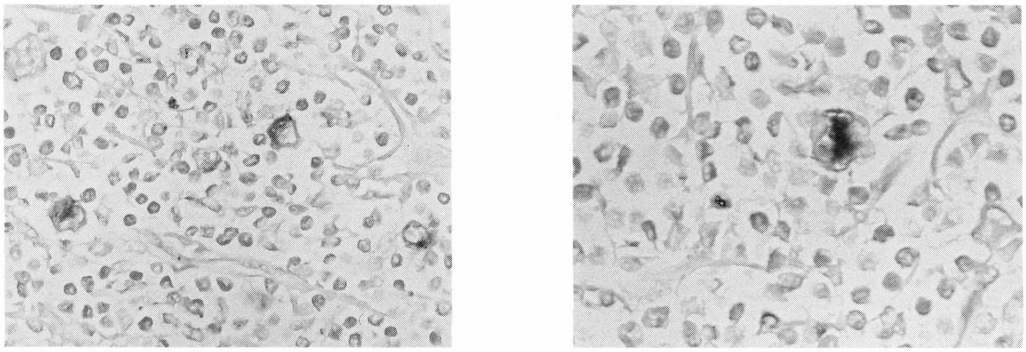

Figure. 4 In-situ-hybridization with the concatamer probe for HTLV-1 pX Tax in Hodgkin's disease. Some of Reed-Sternberg's cells showed the ISH-positive stain in cytoplasm.

Discussion

The ISH in this study was designed to detect both of HTLV-1 proviral DNA sequence and its signal. Although a few large lymphoma cells of ALL type showed the ISH-positive stain in nucleus, in all the ISH-positive MLs the ISH showed its positive stain in cytoplasm, suggesting that this ISH detected a signal of HTLV-1 proviral DNA sequence including pX Tax region and RNA and DNA genome of HTLV-1 in cytoplasm. Because RNA and DNA genome of HTLV-1 may be visualized as granular stain in cytoplasm in MT-2 cells (personal data), the positive figures of this ISH was the signal. As RNAs of HTLV-1 proviral DNA, there are gag-mRNA, env-mRNA, tax/rex mRNA, and variant mRNAs. Rex-protein regulates the gene expression of HTLV-1 proviral DNA (Yoshida M et a1, 1992). Therefore, producing highly biotinylated probe for these mRNAs, this ISH will be able to visualize these mRNAs in lymphoma cells and the other cells of HTLV-1-related and -carrier MLs, probably informing a mode of HTLV-1 proviral DNA expression in these MLs. In order to detect HTLV-1 proviral DNA itself by ISH further studies must be performed.

This study employed a highly biotinylated concatamer probe for a part of HTLV-1 pX Tax region. A DNA product of PCR has no stop code in its sequence, inducing a concatamer formation in the PCR with an excess of template DNA (Sardelli AD, 1991). The concatamer formation in PCR was observed, depending on the amount of template DNA and number of PCR cycles (Hasui K et a1, 1994a). There might be limitation in the usage of the ISH with the concatamer probe, although much stronger stain could be obtained in the ISH with the concatamer probe than in the ISH with 159bp probe as shown in Fig.1(Hasui K et a1, 1994b).

Lymphoma cells showed the ISH-positive stain in 60% (69/115) cases of T-cell ML (Table 3) and in 25% (3/12) cases of T-cell MLs in the age group less than 20 y.o.(Table 6, sited in the reference, Hasui K

et a1, 1994a). Depending on the ISH-positive figures in T-cell MLs, this paper defined HTLV-1ielated T-ML and HTLV-1-carrier MLS. The HTLV-1-related T-MLs in which many lymphoma cells showed the ISH-positive stain may be ATLL. There were ATLL in HTLV-1-carrier T-cell MLs other than HTLV-1-related T-cell MLs and in the ISH-negative T-cell MLs (personal data). In ATLL there would be two types of cases of lymphoma cells with and without expression of HTLV-1 proviral DNA pX Tax region. As shown in T-Pleo and ALC in table 2, the rate of HTLV-1-related MLs was extremely high in ATLL type with peculiar histological features for ATLL. Although several clinical features of ATLL could be explained by an trans-activation of HTLV-1 pX Tax (Yoshida M et a1, 1992), an excess of the signal of HTLV-1 pX Tax in ATLL type, recognized by this ISH, suggested an oncogenetic effect of HTLV-1 proviral DNA pX Tax region. HTLV-1-related MLs in all the types of T-cell MLs suggested oncogenetic factor(s) other an activation of HTLV-1 proviral DNA pX Tax region in the ATLL development, as suggested by analyzing age of ATLL patients according to multistep carcinogenesis hypothesis (Hasui K et a1, 1990).

It has not yet.clarified enough what kinds of cells other than T lymphocyte are infected by HTLV-1 in vivo. This paper showed that HTLV-1 infected histiocytes in low-grade T-MLs, epithelioid cells in LeL (Fig.2e and f), neoplastic B cells in B-cell MLs (Fig.3) and some RS cells of HD (Fig.4). When the unexpected cells showed the ISH-positive stain, at least procedure of the ISH, homology of the probe employed to the other DNA and RNA sequences and a side-effect of the concatamer probe employed in this study must be examined. Because ISH with after-hybridization wash in 2xSSC at 45℃ and 50℃ showed also positive stain in their cytoplasm, the ISH-positive stain in them would be not an artifact of the ISH. And homology of HTLV-1 proviral DNA pX sequence to human DNA sequences was reported not to be recognized, although DNA sequences of interleukin 2 (IL2) gene and IL2 receptor gene were once thought to resemble that of HTLV-1 proviral DNA pX Tax region. Both of the ISH with the concatamer probe and the 159bp long probe for HTLV-1 proviral DNA pX Tax region yielded the same result.

Table 7. In situ hybridization (ISH) for Epstein-Barr virus (EBV)-EBER-1 in B-cell malignant lymphomas in a HTLV-1 endemic area

	EBV-EBER-1-ISH No. of cases examined	EBV-realted B-cell ML
Low garde MLs	40	18
B-CLL	4	0
CC	14	8
CB/CC	4	2
IC	13	6
PC	3	0
MoBL	1	1
MALT	1	1
High grade MLs	35	20
B-LB	1	0
CB	30	17
B-IB	4	3
Total	75	38(51%)

EBV-related B-cell ML: B-cell ML of which lymphoma cells showed the ISH-positive stain dominantly in nuccleus in EBV-EBER-1 ISH

Table 8. Comparision of the ISH of HTLV-1 pX Tax region and EBER-1

	EBV-non-related B-cell ML	EBV-related B-cell ML
HTLV-1-related B-ML	13	9
HTLV-1-carrier B-ML other than -related B-ML	4	8
B-MLs other than -carrier B-MLs	19	15

But the ISH-positive histiocytes and epithelioid cells in low grade malignant T-cell MLs should be studied further from the view point of their phenotype, because some transformed or neoplastic T cells can show figures resembling them (Hasui K et a1. 1994b).

Relatively many lymphoma cells in 10 cases of 21 HTLV-1-Telated B-cell MLs showed the ISH-positive stain. Dominancy of HTLV-1-related MLs was in low grade malignant cases in B-cell MLs (Table 4) and in high grade malignant case in T-cell MLs (Table 3), suggesting different roles of HTLV-1 infection in the pathogenesis of B-cell and T-cell MLs. Many HTLV-1-related B-cell MLs were monoclonal antibody KiMlp (Radzun HJ et a1,1991)-positive B-cell MLs (Hasui K et a1,1992). Furthermore, HTLV-1-related B-cell ML was different from “Epstein-Barr virus (EBV) related B-cell MLs". Studying 75 B-cell MLs by means of the ISH employing EBV encoded small RNA-1 (EBER-1) probe (Tokunaga M et a1,1993) , 38 (51%) B-cell MLs showing the ISH-positive stain dominantly in nucleus were EBV-related MLs (Table 7). EBV-related B-cell MLs comprised many high grade malignant cases as well as HTLV-1-related T-cell MLs. In 68 cases of B-cell MLs studied by the both ISH with the HTLV-1 probe and with the EBER-1 probe, HTLV-1-related B-cell MLs other than HTLV-1-carrier B-cell MLs included smaller number of EBV-related B-cell MLs than HTLV-1-carrier B-cell MLs other than -related B-cell MLs (Table 8), suggesting that HTLV-1-carrier B-cell MLs other than -related B-cell MLs might be easy to be infected by EBV as B-cell MLs in acquired immunodeficiency syndrome. Then, HTLV-1 effected on the oncogenesis of some B-cell MLs in HTLV-1 endemic areas. Features of HTLV-1-related B-cell MLs and its oncogenesis with HTLV-l infection must be studied further.

In 8 of 14 cases of HD some RS cells and a small number of lymphocytes showed the ISH-positive stain in cytoplasm (Table 6, Fig.4). Because there were no cases of HD of which manly RS cells showed the ISH-positive stain, RS cells may be infected by HTLV-1 and show an early phase of the activation of HTLV-1 proviral DNA (Yoshida M et a1.1992). Therefore, the activation of HTLV-1 proviral DNA pX Tax region in lymphoma cells, recognized as the ISH-positive stain in this study, must be analyzed further from a stand point of life cycle of HTLV-1.

On the other hand, rates of HTLV-1-carriers in B-cell MLs and in MLs from the age group less than 20 y.o. were higher than in the previous serovirological studies (Hanada S et a1,1989). As for the sensitivity to detect HTLV-1 infection in the tissue, the ISH may be superior to the fm method and to the serovirogicatl method (Hasui K et a1, 1994a). Some patients of HTLV-1-carrier B-cell MLs and in the under 20-year-old ML patients might fail to produce antibodies against HTLV-1, because the rates of HTLV-1-carrier cases in T-MLs and HD were almost the same as the rates reported by the serovirological methods.

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