DENDRITIC CELLS Vo1. 6, 1996

Metachronous occurrence of gastric T-cell rich B-cell lymphoma and nodal T-cell lymphoma in a HTLV-1 carrier

Kazuhisa Hasui[1], IIiroshi Shirahama[1], Kazunobu Sueyoshi[1], Eiichi Sato[1] and Mitsuru lmakuma[2]
[1]Second Department of Pathology, Kagoshima University Faculty of Medicine, Kagoshima, [2]Kimotsuki-Gun Medical Association's Hospital, Oonezime-Cho, Kagoshima Prefecture, Japan

Correspondence to: Kazuhisa Hasui, Second Department of Pathology, Kagoshima University Faculty of Medicine, Sakuragaoka 8-35-1, 890 Kagoshima, Japan

ln a HTLV-1 carrier Japanese man gastric T cell-rich B cell lymphoma occurred at 67 years of age and nodal T cell malignant lymphoma appeared at 72 years of age. The gastric T-cell rich B-cell Iymphoma formed an ulcerated tumor in the posterior wall of the body, involving up to the serosa and regional lymph nodes. A few lymphoma cells had cytoplasmic monoclonal κ and λ type immunoglobulin. Signals of HTLV-l pX Tax DNA sequence and stains of three antibodies against HTLV-1-encoded proteins (gp46env, p40tax and p19gag) in some lymphoma cells suggested that this gastric lymphoma was a HTLV-1-related case. Six years later, large cell type T-cell malignant lymphoma occurred in left inguinal lymph node. Many S100+ dendritic cells and lysozyme+ cells were intermingling among the lymphoma cells. Some epithelioid cells among the lymphoma cells showed weak stains of the three antibodies against HTLV-l-encoded proteins. The PCR analysis showed oligoclonal rearrangement bands of TCR β gene in the agar-gel electrophoresis. After three months' follow-up without chemotherapy, recurrent swelling of left inguinal lymph node and splenomegaly appeared. After chemotherapy the biopsied left inguinal lymph node showed necrosis and residual T cell malignant lymphoma. The uncommon features such as many T cells in the gastric lymphoma and many S100+ cells, many lysozyme+ cells and oligoclonal rearrangement of TCR β gene in the nodal T-cell lymphoma might be induced by HTLV-1 infection. The patient has survived without any complaints. The HTLV-l-related lymphoid malignancies other than adult T-cell leukemia/lymphoma would be not-aggressive and might be the diseases in the microenvironment under the immunocompromised state in HTLV-1 carrier. (Dendritic Cells 6: 14-21, 1996)
Key words: HTLV-1, malignant lymphoma, pathology, histochemistry, polymerase chain reaction

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) infection induces adult T-cell leukemia/1ymphoma (ATLL) and the other non-neoplastic HTLV-1-related diseases (Takatsuki et a1, 1992: Osame et a1, 1992). We have been trying to detect HTLV-1 in lymphoma cells histochemically and found that there were T-cell and B-cell malignant lymphomas of which lymphoma cells show signals of HTLV-1 pX Tax region (Hasui et a1, 1994: Hasui and Sato, 1994). We experienced gastric B-cell and followed nodal T-cell malignant lymphomas in an aged HTLV-1 carrier. Examining these lymphomas by means of polymerase chain reaction (PCR) detecting clonality of lymphoma cells and HTLV-1 infection, and trying paraffin-immunohistochemistry of three antibodies against HTLV-1-encoded proteins, we report these lymphomas and discuss the relation of HTLV-1 infection and the pathogenesis of these lymphomas.

Case

The patient is a 67 year-old Japanese man. He had no particular history of illness. He complained of epigastralgia in June 1989. Gastroendoscopic examination showed an ulcerated tumor of malignant lymphoma in the posterior wall of the gastric body. Preoperative laboratory examination indicated no abnormal data; WBC: 6,500/mm^3 (Stab: 3%, Seg.II: 25%, III: 31%, IV: 7%, V: 2%, Ly.: 24%, Mono.: 3%, eosino.: 4%, no atypical 1y.), RBC: 4,110,000/mm^3 , Hb: 12.0g/d1, Ht.: 39%, total protein: 6.8g/d1 (A1.: 54.2 %, α1-g1.: 2.9%, α2-g1.: 9.5%, β-g1.:11.9%, γ-g1.: 21.4%), total bilirubine: 0.69mg/d1, LDH:268W-U, GOT: 16KaU, GPT: 14KaU, Na.. 143mEq/L C1:103mEq/L, K: 3.8mEq/L and Lymphocytes in peripheral blood (OKT4: 48.8%, OKT8: 18.5%, OKIa: 26.0%, κ: 3%, λ: 2%, γ:

1%, α : 1%, μ : 5%). And he was a HTLV-1 carrier. Gastrectomy was performed in August 25, 1989. The ulcerated tumor of malignant lymphoma was 4x4 cm large. The gastric malignant lymphoma involved regional lymph nodes of stomach.

After the gastrectomy he did not received any chemotherapy. In 6 years he was free from any diseases except lumbar intervertebral disc tissue displacement manifesting feeling of numbness in right leg and pain in lower back in July 1993. In his hospital days suffering from the disc tissue displacement, any abnormal data were not seen in peripheral blood and laboratory examinations; WBC: 6,100/mm^3 (stab: 6%, Seg.II: 30%, III: 31%, IV: 2%, Ly.: 26%, Mono.: 4%, atypical 1y.: less than 1%), RBC: 4,260,000/mm^3, Hb: 13.8g/d1, Ht: 40%, Platelet.. 190,000/mm^3, total protein: 7.2g/dl, GOT: 23KaU, GPT: 9KaU, CHE.. 0.78 Δ pH, γ -GTP: 12.4mu/m1, ALP: 5.8KAU, LDH: 269W-U, Na: 141 mEq/L, C1: 102 mEq/L, K: 4.1 mEq/L, and Ca: 5.0 mEq/L.

In March 1995 right axi11ar lymph node swelled and in one month lymph nodes in general body did. He complained of high fever. An increased serum level of CRP without increased number of WBC was indicated: WBC: 5,200/mm^3 (Stab: 4%, SeE.II: 24%, III: 29%, IV: 1%, Ly.: 26%, Mono.: 12%, Atypical 1y.: 3%), RBC: 4,130,000/mm^3, Hb: 14.0 g/d1, Ht: 36%, Platelet: 110,000/mm^3, LDH; 396W-U, GOT: 26KaU, GPT: 14KaU, CHE: 0.72 Δ pH, γ -GTP: 31.7mu/m1, ALP: 10.7KAU, total protein: 7.7g/dl, Na: 140mEq/L, C1: 100mEq/L, K: 4.2mEq/L and CRP: 7.2 ‘S/d1. Serovirological examination indicated high serum level of rubella lg G (Ig G: x32.9, Ig M: below x0.80), EBV-VCA Ig G (-VCA Ig G: x320, -VCA Ig A: x10, -VCA Ig M: x10, -EBNA: x10), anti-Toxplasma antibodies (x640) and anti-adult T-cell leukemia-associating antigens antibodies (ATLA, x1024). The biopsied left inguina1 lymph node was diagnosed as T-cell malignant lymphoma. Because of the laboratory data suggesting an inflammation, careful follow-up without chemotherapy was made.

In July 1995 left inguina1 lymph node swelled. The ultrasound image of abdomen showed splenomegaly (9.6x4.4cm) and no swelling of abdominal deep lymph nodes. Peripheral blood and laboratory examinations indicated no abnormal data; WBC: 6,700/mm^3 (Stab: 20%, Seg.II: 24%, III: 29%, IV: 22%, Ly.: 2%, Mono.: 5%, atypical 1y.: 2%), RBC: 3,880,000/mm^3, Hb: 12.0 g/d1, Ht: 34%, Platelet: 160,000/mm^3, Na: 140mEq/L, C1: 102mEq/L, K: 3.7mEq/L, Ca: 4.9mEq/L and CRP: 0.4’S/d1. After chemotherapy the left inguina1 lymph node was biopsied and diagnosed as necrosis and residual T-cell malignant lymphoma.

The patient is alive and free from the recurrence of the malignant lymphomas without any chemotherapy.

Histochemistry

Paraffin-immunohistochemistry (pIHC)

pIHC was performed to see lymphoma cell phenotype of the gastric lymphoma (removed in Aug. 1989) and the nodal T-ce11 1ymphomas (biopsied in March and July 1995). The dewaxed sections of the lymphomas were processed by such antigen retrieval methods (AR) as the incubation in boiling 4M urea solution by a home electric range for 5 min. or the incubation in a high pressure cookpot for 5 min. (Hasui et a1, 1995) before the incubation of the primary antibodies.nThe primary antibodies employed were MT-1, UCHL-1, DAKO CD3, OPD4, DAKO CD8 and TIA1 for T cells, MB-1, L26, LN-1, -2, -3, anti-immunoglobulin κ and λ type light chains and γ, α and μ type heavy chain antibodies for B cells, anti-S 100 protein antibody and anti-1ysozyme antibody for dendritic cells and histiocytes, and BerH2 and LeuMl for activating antigens. The reacted antibodies were visualized by Elite ABC method and DAB peroxidase reaction for 10 min. at room temperature.

pIHC of monoclonal antibodies (MoAbs) against HTLV-1-encoded proteins

pIHC of MoAbs against HTLV-1-encoded proteins (gp46env, p40tax and p19gag) was performed (Hasui and Sato et a1, 1995). The dewaxed sections of the lymphomas were treated by the AR incubating in 4M urea solution for 5 min. by means of a high pressure cookpot for MoAb Lt-4 against p40tax and in 0.01M citrate solution pH 6.0 for 5 min. for MoAb p19 against p19gag. The incubation in the primary MoAbs; 6C2 against glycoprotein (gp) 46env (Cellular Product lnc.), Lt-4 (given from Dr. Yuetsu TANAKA, Dept. of Biosci., Kitasato Univ.) and p19 (Cellular Product lnc.), and Elite ABC method were performed by means of an immunostain apparatus MicroProbe (Fisher Scientific) controlling incubating temperature. DAB peroxidase reaction was performed for 10 min. at room temperature.

ln-situ hybridization (ISH) of HTL V- 1 mRNA of pX Tax region and Epstein-Barr virus (EBV) small RNA EBER-1

The method of ISH detecting mRNA of HTLV-1 pX Tax region employing a biotinylated concatamer probe synthesized by PCR was reported previously (Hasui et a1, 1994: Hasui and Sato, 1994). The method of ISH detecting EBER-1 was also reported previously by Tokunaga et a1 (1992).

Polymerase chain reaction (PCR)

The PCR analysis detecting clonality of lymphoma cells and HTLV-1 proviral DNA was performed, by extracting DNA from the 10 μm thick paraffin section of the lymphomas. The dewaxed section was digested in 100 μml proteinase K solution overnight at 37℃. After destroying the proteinase K activity in boiling water for 10 min., 5 μml of the supernatant of the proteinase K solution was employed for the template DNA solution for the PCR of a pair of primers lgJH1 and lgJH2 detecting rearrangement of immunoglobulin heavy chain (IgH) gene (McCarthy et a1, 1990), of 6 pairs of primers TCR βV, D1, D2, Jl and J2 detecting rearrangement of T-cell receptor (TCR) β gene (McCarthy et a1, 1991: 1992) and of a pair of primers SK43 and SK44 detecting HTLV-1 proviral DNA pX Tax region (Hasui et a1, 1992). The PCR protocol was following; pre-PCR denaturing at 94.C for 5 min., 30 cycles of denaturing at 94℃ for 30s, annealing at 45℃ for 30s and extension at 72℃ for 30s, and final extension at 72℃ for 5 min. The PCR products were examined by 4% agar gel electrophoresis in order to see bands of amplified DNA at the expected length.

Pathology

ln the gastric tumor of lymphoma the ulcer was surrounded by the mucosa associating reactive lymphoid hyperplasia with T-zone hyperplasia (Fig. 1a and b). In the T-zone hyperplasia S100+ dendritic cells scattered. A mixed proliferation of large lymphoma cells, some immunoblast-like cells and many small to medium-sized cells having irregular-shaped nuclei was seen in the ulcer base (Fig. 1c), involving up to the serosa. The ratio of the large lymphoma cells to the small to medium-sized lymphocytes varied from area to area. The large lymphoma cells were MB-1+/- L26+/- LN-2+ LN-3+ UCHL-1+ CD3+ (cytoplasmic) OPD4+/- CD8+/- TiA1- CD25- BerH2- LeuM1- cells. Some of immunoblast-like cells and large lymphoma cells had κ and λ type monoclonal cytoplasmic immunoglobulin (Fig.1e and f). The many small to medium-sized cells were CD3+ OPD4+ DAKO CD8+ and TIA1- T cells (Fig. 1d). Some of large lymphoma cells and the T cells having irregular-shaped nuclei showed positive stains of the three MoAbs; 6C2, Lt-4 and p19 (Fig. 2). In ISH detecting mRNA of HTLV-1 pX Tax region some lymphoma cells showed signals. In ISH detecting EBER-1 only a few small lymphocytes showed weak signals. Several trials of PCR analysis detecting rearrangement of lgH gene, TCR β gene and HTLV-1 proviral DNA pX Tax region failed to show any bands of amplified DNA in the agar gel electrophoresis of the PCR products. The gastric malignant lymphoma was diagnosed as HTLV-1-related T-cell rich B-cell malignant lymphoma.

The left inguina1 lymph node biopsied in March 1995 showed diffuse proliferation of large lymphoma cells having vesicular nuclei with thin nuclear membrane (Fig. 3a and b). Some epithelioid cells existed among the lymphoma cells (Fig. 3b). The lymphoma cells were CD3+ UCHL-1+ CD43+ MT-1+ OPD4+ TIA1- CD25- BerH2- LeuM1- T- cells (Fig. 3c). Some B cells were intermingling among the lymphoma cells but showed no mitoses (Fig. 3d). S100+ dendritic cells increased and tended to aggregate (Fig. 3e). Lysozyme+ cells also increased (Fig. 3f). Only a few epithelioid cells and dendritic cells showed weak stains of the three MoAbs; 6C2, Lt-4 and p19 (Fig. 4). PCR analysis showed oligobands of amplified DNA in a smear of TCR β gene and a weak band of amplified DNA of lgH gene (Fig. 5). A band of amplified DNA of HTLV-I proviral DNA pX Tax region was seen at the expected length in the agar-gel electrophoresis (Fig. 5). The nodal 1ymphoma was diagnosed as CD4+ T-cell large cell malignant lymphoma with many S100+ dendritic cells and lysozyme+ cells, some of which were HTLV-1-infected cells.

The left inguina1 lymph node biopsied in July 1995 after chemotherapy showed necrosis and residual T-cell 1ymphoma revealing a mixted proliferation of the large lymphoma cells, epithelioid cells and the other cells. But several trials of the PCR detecting clonality of the lymphoma cells and HTLV-1 infection failed.

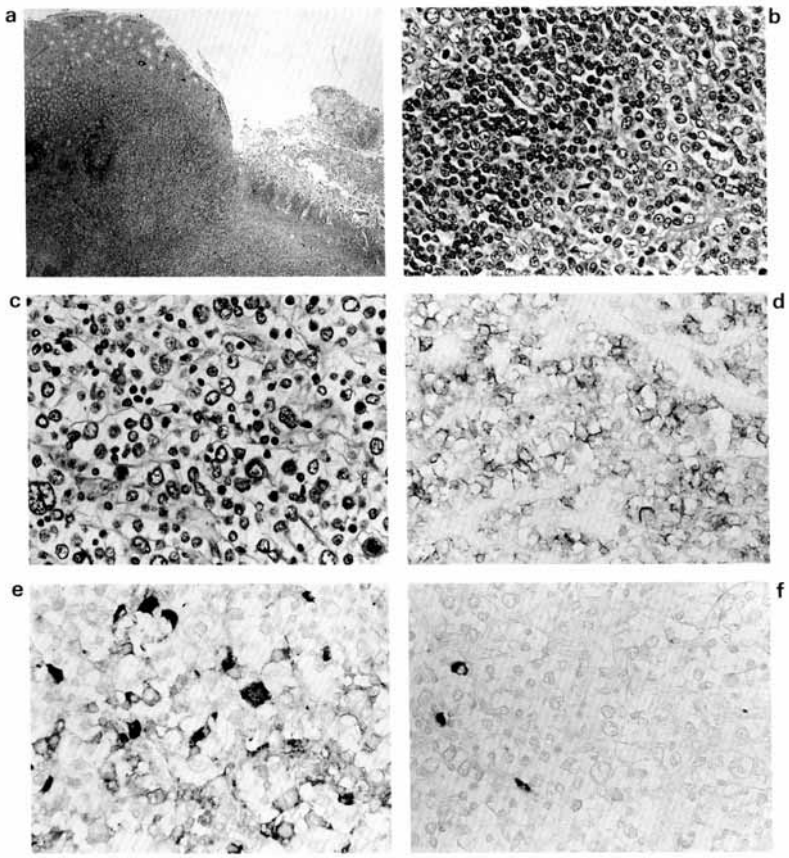


FIGURE 1. The gastric lymphoma in the aged HTLV-l carrier. a) H.E. stain, 2x10. Marginal area around ulceration comprised the residual mucosa with reactive lymphoid hyperplasia and enlarged T-zone area. b) H.E. stain, 20x10. ln the T-zone area medium-sized T-cell's proliferated with some S100+ dendritic cells. c) H.E. stain, 20x10. The gastric lymphoma showed a mixed proliferation of large lymphoid cells and medium-sized lymphocytes. The ratio of the large lymphoid cells to the medium-sized lymphocytes varied area to area. Some of the medium-sized lymphocytes had irregular-shaped nuclei. d) Antigen-retrieval paraffin-immunohistochemistry (AR-plHC) of monoclonal antibody OPD4 for CD4, 20x10. The medium-sized lymphocytes and some large lymphoid cells were positive for OPD4 (CD4). e and f) AR-pIHC, 20x10. Some large lymphoid cells had monoclonaI κ type immunoglobulin light chain (e) and only some plasma cells had λ type immunoglobulin light chain (f).

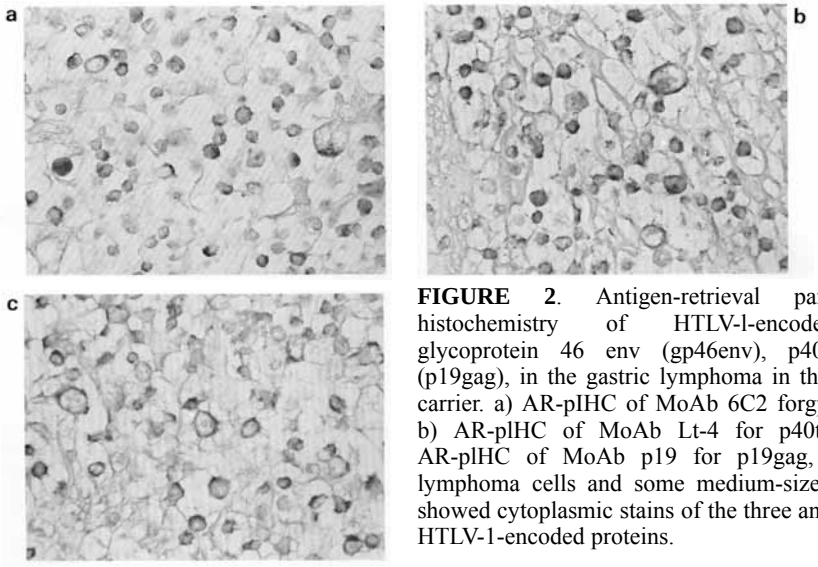
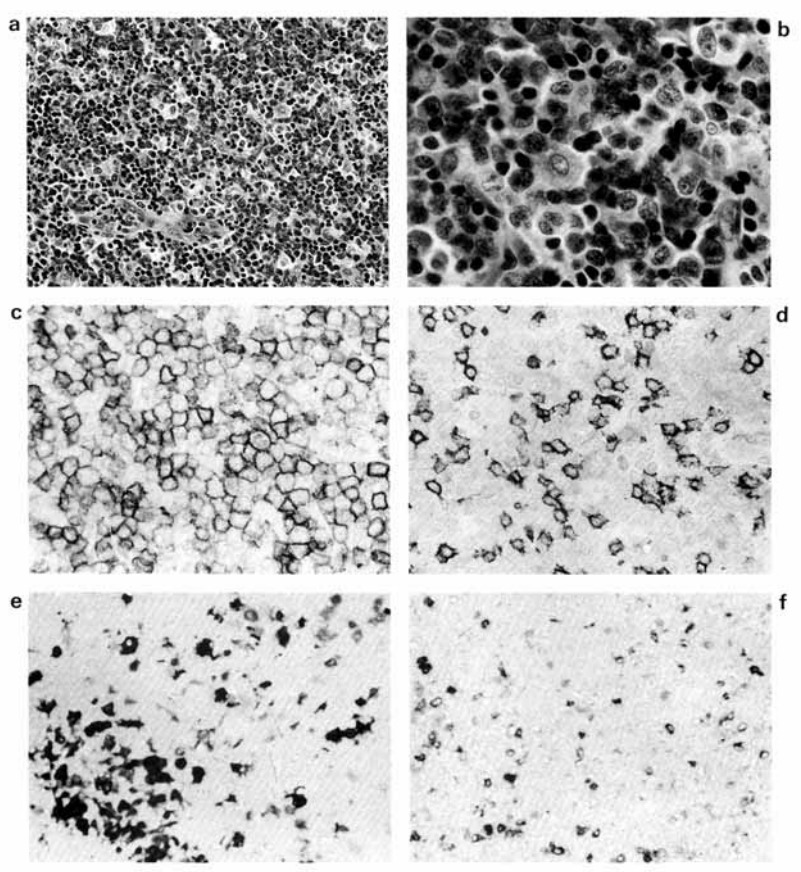


FIGURE 2. Antigen-retrieval paraffin-Immuno-histochemistry of HTLV-l-encoded proteins; glycoprotein 46 env (gp46env), p40tax and p19 (p19gag), in the gastric lymphoma in the aged HTLV-l carrier. a) AR-pIHC of MoAb 6C2 forgp46env, 40x10. b) AR-plHC of MoAb Lt-4 for p40tax, 40x10. c) AR-plHC of MoAb p19 for p19gag, 40x10. Large lymphoma cells and some medium-sized lymphocytes showed cytoplasmic stains of the three antibodies for the HTLV-1-encoded proteins.

FIGURE 3. The nodal T-ce11 malignant lymphoma in the aged HTLV-1 carrier.

a) H.E. stain, 4x10. Diffuse proliferation of lymphoma cells.
b) H.E. stain, 20x10. Large lymphoma cells showed several mitoses and irregular-shaped and vesicular nuclei with thin nuclear membrane. There were small lymphocytes and some epithelioid cells among the lymphoma cells.
c)AR-pIHC of OPD4 for CD 4, 20x10. Most of the large lymphoma cells were positive.
d) AR-pIHC of L26 for pan B-ceIIs. Some lymphocytes were positive but no mitotic cells were seen in them.
e) plHC of anti-S100 protein antibody, 10x10. Many S100+ dendritic cells tended to aggregate.
f) plHC of anti-Iysozyme antibody, 10x10. Relatively many lysozyme+ cells were seen among the lymphoma cells.



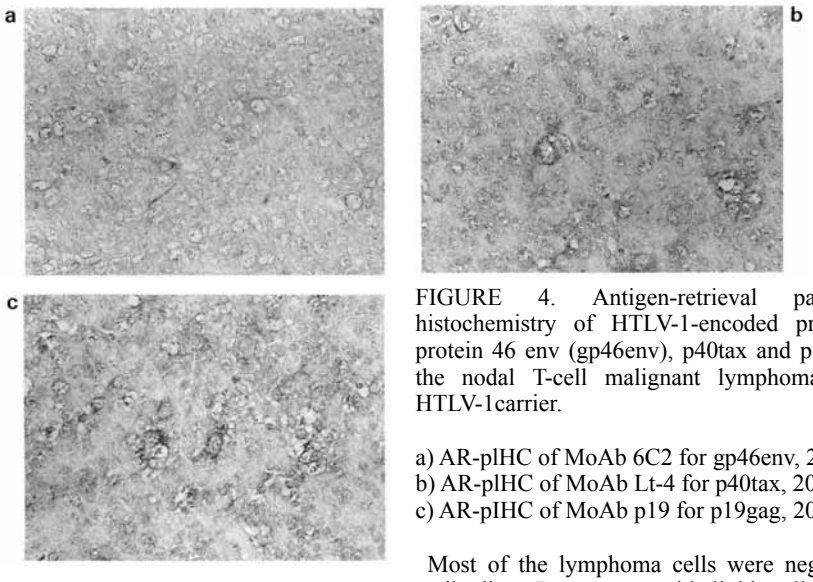


FIGURE 4. Antigen-retrieval paraffin-immuno-histochemistry of HTLV-1-encoded proteins; glycol-protein 46 env (gp46env), p40tax and p19 (p19gag), in the nodal T-cell malignant lymphoma in the aged HTLV-1carrier.

a) AR-plHC of MoAb 6C2 for gp46env, 20x10.
b) AR-plHC of MoAb Lt-4 for p40tax, 20x10.
c) AR-pIHC of MoAb p19 for p19gag, 20x10.

Most of the lymphoma cells were negative for these antibodies. But some epithelioid cells showed faint cytoplasmic stains of these three antibodies and especially the stain of 6C2 was weaker than the stains of Lt-4 and p19.

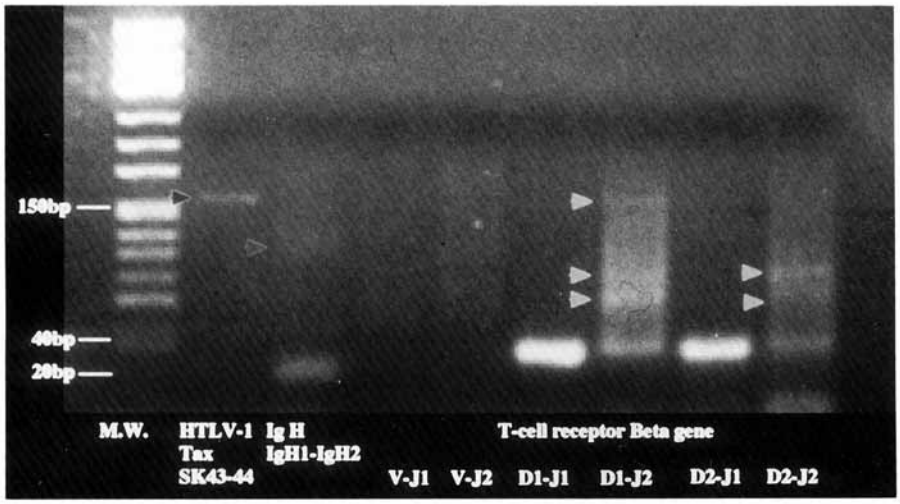


FIGURE 5. Agar-gel electrophoresis of PCR products detecting HTLV-l infection and rearrangement of immunoglobulin heavy chain gene and T-cell receptor B chain gene in the followed nodal T-cell malignant lymphoma in the aged HTLV-1 carrier. A band of PCR of the primer pair of SK43 and SK44 was seen at the expected length (159bp over a molecular weight marker band at 150bp). A weak and obscure band of PCR of the primer pair of lgJHl and lgJH2 was seen at the length of about 110bp. Oligobands of PCR ofthe primer pairs of D1-J2 and D2-Jl were seen in a smear from 40 bp upto over 150bp.

Discussion

HTLV-1 induces specific diseases such as ATLL and HAM. On the other hand, it was reported by Osame and Sonoda et a1 (1992) that HTLV- 1 carrier people suffering from HTLV-1-related diseases, at least HAM, were characterized by their HLA phenotype. And ATLL cells would be neoplastic autologous T-cells. Therefore, pathogenesis of HTLV-1-related diseases must be analyzed from viewpoints of HTLV-1 infected cells and immunological and immunogenetic aspects.

This case was an aged HTLV-1 carrier Japanese man suffering from metachronous gastric T-cell rich B-cell and nodal T-cell 1ymphomas. And the two lymphomai had peculiar features which could not be categorized according to the entities in the classification of malignant lymphomas (Lermert and Fe11er, 1992).

In the gastric lymphoma, at least, monoclonal cytoplasmic immunoglobulin in some lymphoma cells suggested an existence of neoplastic B cells. Because B-cell lymphomas having monoclonal cytoplasmic immunoglobulin indicated a low rate of MB-1- and/or L26- positive cases (Hasui K, 1991), the gastric lymphoma was such a MB-1- and L26-almost negative lymphoma. Cytoplasmic CD3 stain dose not mean its genotype expression.. A few large cells reacted by OPD4 and DAKO CD8 might be the blasts of the many T-cells having irregular-shaped nuclei. Several trials to see clonality of the intermingling atypical T cells in the gastric lymphoma failed. On the other hand, cross and nonspecific reaction of these antibodies in the pIHC with AR must be considered. This gastric lymphoma was at least T-cell rich B-cell malignant lymphoma.

In the nodal T-cell 1ymphoma, histopathological features of low and high grade T-cell 1ymphomas were recognized. A diffuse and large cell type T-cell 1ymphoma is categorized as high grade malignant and a mixed cellular composition of some B-cells and many S100+ dendritic cells is one of the histopathological features of low-grade T-cell malignant lymphomas (Lennert and Fe11er, 1992). Intermingling of many lysozyme+ cells is not often seen in usual T-cell malignant lymphomas.

These uncommon features in the two lymphomas might be understood to be induced by HTLV-1 infection. Increase of S100+ dendritic cells was recognized by immunohistoquantitative method in ATLL (Hasui and Sato et a1, 1992). Histochemical methods detecting HTLV-1 infection has not yet been established. The ISH detecting HTLV-1 proviral DNA and its mRNA, and the pIHC of MoAbs against HTLV-1-encoded proteins needed the developing highly sensitive detection methods such as lmmunoMax (Merz et a1, 1995) to improve their detection sensitivity. In the gastric lymphoma, at least, signals of

HTLV-1 pX Tax region and positive stains of MoAbs against gp46env, p40tax and p19gag in lymphoma cells indicated HTLV-1 infection. The many OPD4+ DAKO CD8+ TIA1- T cells might be induced by the HTLV-1 infection. In the nodal T-cell 1ymphoma, weak stains of the three MoAbs against HTLV-1-encoded proteins in some of epithelioid and dendritic cells and the band of PCR-amplified DNA sequence of HTLV-1 proviral DNA pX Tax region suggested that HTLV-1 infection in these cells modified the histogenesis of the nodal T-cell malignant lymphoma. It must be explained by highly sensitive pIHC whether the T-ce11 1ymphoma cells were infected by HTLV-1 or not, because our trial of lmmunoMax method in pIHC of MoAbs against p40tax succeeded to show a quite small amount of p40tax in ATLL cells.

The PCR detecting clonality of the nodal T-cell lymphoma cells showed oligobands in a smear of PCR products for TCR β gene and a weak band in the PCR product for immunoglobulin heavy chain (IgH) gene. An aberrant rearrangement of lgH is sometimes seen in non-lymphoid hematopoietic malignancies but there was possibility that some B cells among the lymphoma cells had rearranged lgH gene but did not proliferate among the neoplastic T cells. The oligobands in a smear of the PCR products for TCR β gene indicated an appearance of oligoclonal proliferation in polyclonal proliferation of T-cells, although immunohistopathologically the nodal lymphoma was obvious T-cell malignant lymphoma. The nodal T-cell 1ymphoma may be in a quite early phase of T-cell 1ymphoma or T-cell 1ymphoma occurring in the microenvironment of the immunity with disturbance in T-cell function. We reported that lymph follicle-1ysis often found in acquired immunodeficiency syndrome was found in non-neoplastic swollen lymph nodes of HTLV-1 carriers (Hasui et a1, 1992). HTLV-1 infection would induce such immunocompromised microenvironment in HTLV-1 carrier. The uncommon clinicopathological features of the nodal T-cell 1ymphoma such as laboratory data suggesting an inflammation and high serum level of rubella lgG, EBV-VCA IgG and anti-toxoplasma antibodies may be the reflection of such immunocompromised state in HTLV-1 carrier.

At last, the gastric T-cell rich B-cell malignant lymphoma and the nodal T-cell lymphoma were surely HTLV-1-related lymphomas other than ATLL. The survival of this aged HTLV-1 carrier man suffering from metachronous occurrence of the HTLV-1-related lymphomas meant that the HTLV-1-related neoplastic lymphoid malignancies other than ATLL would be not-aggressive and might occur in the microenvironment under the immunocompromised state in a HTLV- 1 carrier.

This case report was supported in part by a Grant-in-Aid from the Ministry of Education (NO. 05670176).

References

Hasui K: Paraffin-immunohistochemical analysis of 226 non-Hodgkin's malignant lymphomas in the endemic area of human T-cell leukemia virus type I. Acta Pathol Jpn 41: 350-362, 1991

Hasui K, Sueyoshi K, Kitajima S, Sato E: HTLV-1-associated non-neoplastic lymphadenopathy - Atypical follicular lesions of lymph nodes found in anti-human T-cell leukemia virus type 1 (HTLV-1) antibodies-positive subjects without neoplastic disorders. In: Lymphoreticular cells, eds. Takahashi K, Rim SH. Lymphoreticular Ceu Foundation, Kumamoto, Japan. pp239-251, 1992

Hasui K, Sato E, Sakae K, Goto M, Tokunaga M: Immunohistological quantitative analysis of S100 protein-positive cells in T-cell malignant lymphomas, especially in adult T-cell leukemia/1ymphomas. Path Res Pract 188: 484-489, 1992

Hasui K, Sato E, Iwamoto H, et al.: Malignant lymphomas of young people under 20 years of age in an endemic area of human T-cell leukemia virus type I. In: Lymphoreticular cells and diseases, eds. Kim S.H. and Takahashi K. Hematopoietic-Lymphoreticular Study Group, the Korean Society of Pathologists, Seoul, Korea, pp181-198, 1994

Hasui K, Sato E: Human T-cell leukemia virus type I (HTLV-1) in malignant lymphomas in a HTLV-I-endemic area, Kagoshima, Japan: HTLV-I 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. Dendritic Cells 4: 125-137, 1994

Hasui K, Jia Hong Mei, Jia Xin Sham, Nomoto M, Sato E: Antigen retrieval in

paraffin-immunohistochemistry detecting monoclonality of immunoglobulin light chain in B-cell malignant lymphomas: Comparison with in-situ-hybridization. In: Lymphoreticular Cells and Diseases. eds. Hara H, Lee JD, Lymphoreticular Study Group, Department of Pathology, Kochi Medical School, Nankoku, Japan, pp.32l-329, 1995

Hasui K, Sato E, Tanaka Y, Sueyoshi K, Kitajima S: Histochemical detection of HTLV-1 in T-cell malignant lymphomas in a HTLV-I-endemic area, Kagoshima, Japan. Journal of AIDS and Human Retrovirology 10: 236, 1995 (abstract)

Lennert K, Fe11er AC: Histopathology of non-Hodgkin's lymphomas. Berlin: Springer 1992

McCarthy KP, Sloane JP, Wiedermann LM. Rapid method for distinguishing clonal from polyclonal B-cell populations in surgical biopsy specimens. J Clin Patho1 43: 429-432, 1990

McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E and Wiedermann LM. The rapid detection of clonal T-cell proliferations in patients with lymphoid disorders. Am J Patho1 138: 82l-828, 1991

McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E and Wiedermann LM. A simplified method of detection of clonal rearrangements of the T-cell receptor- γ Chain gene. Diagnostic Molecular Pathology 1: 173-179, 1992

MerE H, Malisius R, Mannweiler S, et al.: Methods in laboratory investigation: ImmunoMax, a maximized immunohistochemical method for the retrieval and enhancement of hidden antigens. Lab Invest 73: 149- 156, 1995

0same M, Kumamoto I, Izumo S et al.: HTLV-I-associated myelopathy (HAM): Epidemiology, clinical features, and pathomechanism. In: Gann Monograph on Cancer Research No.39, Advances in adult T-cell leukemia and HTLV-1 research. eds. Takatsuki K, Hinuma Y, Yoshida M. Japan Scientific Societies Press Tokyo, CRC Press, Boca Raton Ann Arbor London Tokyo, pp57i68, 1992

Sonoda S, Yashiki S, Fujiyoshi T, et al.: Immunogenetic factors involved in the pathogenesis of adult T-cell leukemia and HTLV-I-associated myelopathy. In: Gann Monograph on Cancer Research No.39, Advances in adult T-cell leukemia and HTLV-1 research. eds. Takatsuki K, Hinuma Y, Yoshida M. Japan Scientific Societies Press Tokyo, CRC Press, Boca Raton Ann Arbor London Tokyo, pp81-93, 1992

Takatsuki K, Yamaguchi K, Watanabe T, et al.: Adult T-ceu leukemia and HTLV-1 related diseases. In: Gann Monograph on Cancer Research No.39, Advances in adult T-cell leukemia and HTLV-I research. eds. Takatsuki K, Hinuma Y, Yoshida M. Japan Scientific Societies Press Tokyo, CRC Press, Boca Raton Ann Arbor London Tokyo, pp1-15, 1992

Tokunaga M, Nagasato H, Oyamada S et a1.:Epstein-Barr virus in Hodgkin's disease - An analysis using EBER-1 in situ hybridization. Byori to Rinsho 10:116l-l167, 1992 (in Japanese)