Exchange of Dominant Lymphoid Cell Clones in a Patient with Adult T-cell Leukemia/Lymphoma

KEIJI IWATSUKI,[1] FUMIO INOUE,[1] MASAHIRO TAKIGAWA,[1] GAIJIRO IEMOTO,[2] TETSUO NAGATANI,[2] HIROSHI NAKAJIMA[2] and MIZUHO YAMADA[1]

[1]*Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu and* [2]*Department of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan*

We performed a genotypic study on lymphoid cells from a patient with adult T-cell leukemia (ATL). Clonal proliferation of human lymphotropic virus type-1(HTLV-1)-infected helper T-cells was detected in the primary skin tumors, while no clonality of the virus-infected lymphocytes was observed in the peripheral blood. Following intensive chemotherapy, however, we detected the presence of two genotypically different HTLV-1-infected lymphocyte clones in different samples taken from the peripheral blood. These data demonstrated the exchange of the dominant neoplastic clone in the clinical course of ATL, suggesting the possibility that a certain clone among many repertoires of HTLV-1-infected T-cells has a capability of leukemogenesis, instead of the primary tumour cells. ***Key words: Human T-cell lymphotropic virus type 1; Genotypic analysis; Clonality.***

(Accepted June 12, 1989.)

Acta Derm Venereol (Stockh) 1990; 70: 49–52.

K. Iwatsuki, Department of Dermatology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

Adult T-cell leukemia/lymphoma (ATL) is a virus-related lymphoid malignancy associated with human T-cell lymphotropic virus type 1 (HTLV-1) infection. The incidence of patients with ATL is greater in the southwestern part of the Japanese archipelagoes, the Caribbean basin, Africa, the southeastern United States, and South America (1–3). A characteristic hematologic feature of ATL is the presence of atypical lymphocytes with a convoluted nucleus in the peripheral blood (4). Some patiens with ATL, however, have cutaneous tumors and various types of skin lesions without any hematological or internal abnormalities (5, 6). The clinical features of such patients are often indistinguishable from those of HTLV-1-negative cutaneous T-cell lymphomas such as mycosis fungoides and Sézary syndrome (7).

Southern blot analysis has recently been a strong strategy that enables us to differentiate ATL from other T-cell lymphomas of the skin by detection of the clonal integration of HTLV-1 proviral DNA in the neoplastic lymphoid cells (8). Furthermore, a genotypic analysis on the proviral DNA and T-cell antigen receptor (TcR) genes contributes to the detection of a dominant clone of the proliferating T-cells (8–10).

Recently, Maeda et al. (10) have reported that lymphoid cells from ATL patients show exchange of dominant neoplastic clones during culture. This observation suggests that the neoplastic cells are not always derived from a single cell type, but develop among various HTLV-1-infected lymphocytes. This hypothesis may be supported by the fact that polyclonal integration of HTLV-1 proviral DNA was seen in the lymphocytes from the individuals with an intermediate state progressing to ATL (11).

In the present study, we report an ATL patient associated with exchange of the dominant neoplastic cells in vivo during the clinical course of the disease.

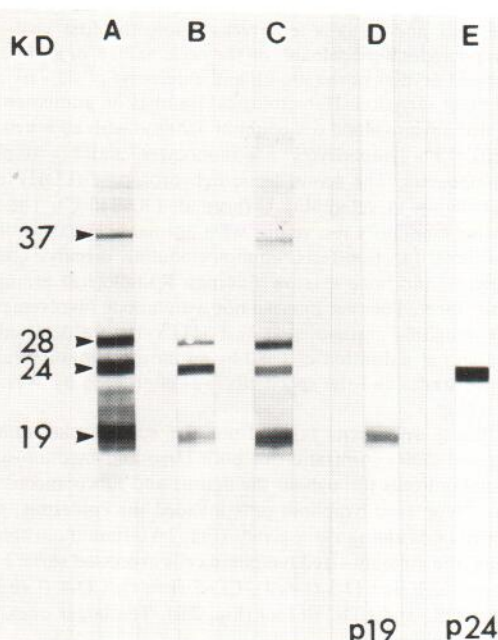

Fig. 1. Western blot analysis of IgG-class anti-HTLV-1 antibodies. *Lane A*: a positive serum from another ATL patient; *B*: our patient's serum; *C*: a serum of our patient's wife; *D*: a mouse monoclonal antibody to HTLV-1 core protein, p19; *E*: a mouse monoclonal antibody to HTLV-1 core protein, p24. The patient's serum contained antibodies against HTLV-1 viral proteins of 19, 24, 28, and 37 kD.

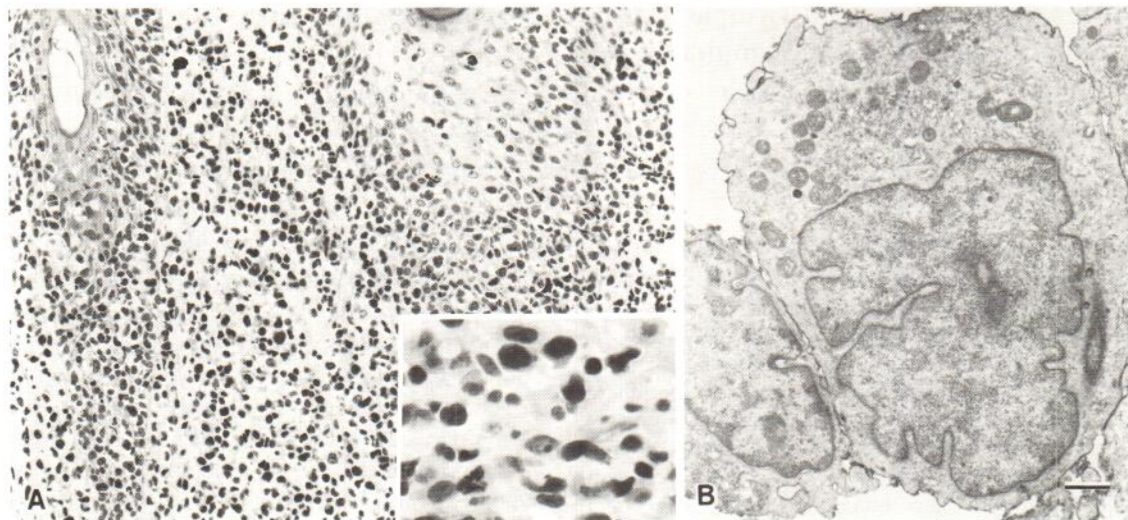

Fig. 2. (*A*) Dense infiltration of atypical large lymphoid cells in the epidermis, dermis, and subcutaneous tissue (×100, inset; ×300). (*B*) An immunoelectron microscopic feature of the CD-4-positive lymphocytes (bar = 1 μm).

CASE REPORT

A 52-year-old fisherman was seen at our clinic because of bilateral subauricular tumours with a 2-month history. Numerous, follicular-pointed, reddish papules had developed on the neck and trunk a few weeks before the first visit. No lymph nodes were palpable on the neck, axillae, or groins. He noticed dryness of the mouth and a decrease of salivary and lacrimal secretion. Hematological findings on admission revealed a white blood cell count of 3,200/μl with 80% neutrophils, 13% lymphocytes, 5% monocytes, and 1% atypical lymphocytes. The serum lactic dehydrogenase (LDH) level was slightly elevated: 495 U (normal: 170–340 U). The following laboratory test results were normal or negative: liver and renal function tests, immunoglobulins, urinalysis, electrolytes, and bone marrow findings. Radiological examinations showed neither internal nor lymph node involvement.

Circulating antibodies against HTLV-1 were detected in the sera at a dilution of 1:80 by an indirect immunoflurescence study, and the specificity was confirmed by Western blot (Fig. 1).

Biopsy specimens taken from the subauricular tumors showed dense infiltration of both large and medium-sized lymphoid cells throughout the dermis and subcutaneous tissue. Neoplastic lymphoid cells invaded the epidermis, skin appendages and salivary glands (Fig. 2A). Immunohistologically, the medium-sized lymphoid cells expressed surface antigens such as CD-3 (Leu 4), CD-5 (Leu 1), CD-4 (Leu 3a), HLA-DR, and CD-25 (Tac) (Fig. 2B). The larger ones lost CD-5 antigens.

Extracted DNA samples from the skin tumors, peripheral blood lymphocytes, and lymphocytes cultured in the presence of recombinant interleukin-2 (IL-2) were analysed by Southern blot using a HTLV-1 proviral gene probe as previously described (10). The results demonstrated that DNA samples from the tumors had two clonal bands showing the presence of the proviral genome at 38 and 13 Kb when digested with Eco R1 (Fig. 3A). Three clonal bands were observed at 11.5, 8.5, and 6.2 Kb in Bam H1-digested DNA fragments. In contrast, no clonal band was observed in DNA extracted from the peripheral blood lymphocytes.

A favourable therapeutic effect was observed by daily administration of 60 mg prednisolone and 100 mg cyclophosphamide. After one year of remission, however, cutaneous nodules and papules recurred, in association with the appearance of atypical lymphocytes in the peripheral blood, even though he received intensive chemotherapy. DNA was extracted from the peripheral blood lymphocytes for Southern blot analysis on two different occasions during the second hospitalization. Both samples, when digested with Eco R 1, showed a clonal band including the proviral genome at 10.5 and 13.8 Kb, respectively (Fig. 3B). In the DNA fragments digested with Pst 1, the internal fragments of proviral genome were detected at 1.3, 1.7, and 2.4 Kb in both materials. Clonal bands including long terminal repeat (LTR) of the provirus were not detected. These results indicated that lymphoid cells composed of the primary skin tumors and two lymphocyte samples obtained during the second admission were different from each other in the genomic site of insertion of HTLV-1 provirus.

Persistent generalized herpes simplex infection was seen during the terminal period of his disease. He died of pneumonia following ATL crisis about 2 years after the onset of the disease.

DISCUSSION

Adult T-cell leukemia/lymphoma (ATL) has recently gained special interest because HTLV-1, or ATLV (adult T-cell leukemia virus) was isolated from the neoplastic cells by two independent groups (13, 14),

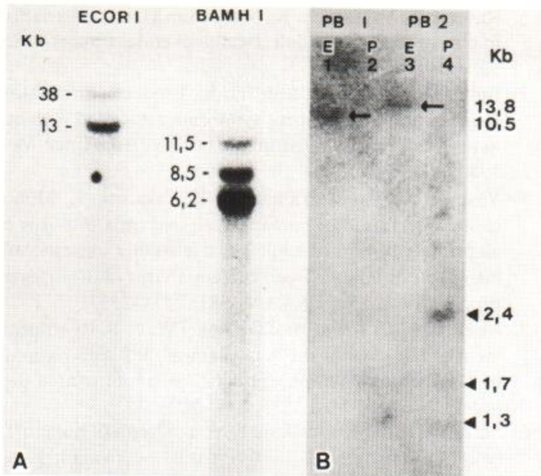

Fig. 3. (*A*) Detection of clonal integration of HTLV-1 proviral DNA in the primary skin tumours. Clonality of HTLV-1-infected lymphocytes was not observed in the peripheral blood at the first hospitalization. (*B*) Detection of clonal integration of HTLV-1 proviral DNA in the peripheral blood lymphocytes obtained during the second admission. Peripheral blood samples were obtained on May 11, 1987 (lanes 1 & 2) and August 24, 1987 (lanes 3 & 4). *E*: EcoR 1-digested DNA; *P*: Pst 1-digested DNA.

and determined to be the same strain of retrovirus. With the accumulation of case reports, ATL is now known to be a HTLV-1-related T-cell malignancy having various clinical manifestations and courses.

Patients with ATL sometimes show clinical and laboratory features similar to those of cutaneous T-cell lymphomas, including mycosis fungoides and Sézary syndrome (6, 7). An accurate diagnosis of ATL, therefore, can no longer be made by phenotypic analysis of the tumor cells alone or by histopathologic findings. Furthermore, the presence of anti-HTLV-1 antibodies, even if the specificity was confirmed by Western blot analysis simply provides evidence for the episode of HTLV-1 infection, but is not a direct clue for virus-related leukemogenesis (8). The most reliable and direct finding is the detection of clonal integration of HTLV-1 proviral DNA in the neoplastic cells by Southern blot analysis. In particular, the genotypic study is an essential method by which to distinguish ATL from other T-cell lymphomas of the skin in the endemic area where about 5–30% of the population have been reported to be healthy carriers of HTLV-1 (2, 3). In our patient, the presence of circulating HTLV-1 antibodies and clonal integration of the proviral DNA in the skin tumour enabled us to make a diagnosis of ATL.

Physical and hematological examinations indicated that our patient belonged to the lymphomatous type of ATL with minimum leukemic findings. Clonal proliferation of HTLV-1-infected cells was detected in the skin tumours, but not in the peripheral blood at the first admission. This suggests that the neoplastic cells primarily affected the skin. Interestingly, the neoplastic cells rather selectively infiltrated salivary and lacrimal glands, and skin appendages. The patient, therefore, showed clinical features of sicca syndrome. A genotypic analysis on a patient with ATL showing primary skin tumors was recently reported by Takahashi et al. (15).

A clonal band including HTLV-1 provirus was noticed in each of the two DNA samples from the peripheral blood lymphocytes during the second admission. The reactive bands, however, differed from each other with respect to molecular size of the DNA fragment, and displayed different autoradiographic patterns compared with the pattern obtained from the primary skin tumors. These findings, therefore, showed the possibility that the T-cell clone proliferating in the skin tumour was different in origin from the leukemic cells appearing later in peripheral blood, and that among the peripheral blood leukocytes the dominant clone changed during the clinical course. Because the peripheral blood leukocytes were obtained during intensive chemotherapy, it is also possible that the chemotherapeutic drugs induced mutation of the neoplastic cell DNA, thereby resulting in different autoradiographic patterns, even though the cells were of the same origin.

Dasgupta & Lilly (16) have shown that a rearrangement pattern within TcR gene of primary murine lymphoma cells did not remain constant in cells of the same tumour during passages *in vivo* and after adaptation to growth in culture. They have proposed two possibilities to interpret the phenomenon: further rearrangements occurred within the same tumor cell line and successive outgrowth of separate lineages from lymphomas that were polyclonal in origin.

Yamaguchi et al. (11) have reported the presence of polyclonal integration of HTLV-1 proviral DNA in the lymphocytes obtained from individuals with intermediate state progressing to ATL. Maeda et al. (10) have clearly shown, by Southern blot analysis, that dominant neoplastic T-cell clones changed during culture, and suggested the possibility that one neoplastic T-cell clone among many repertoires of T-cells randomly infected with HTLV-1 gained a capability of proliferation. These findings may account for an early

leukemogenetic change during healthy HTLV-1 carriers progress to ATL. Our results suggest that a similar phenomenon actually occurs in patients with ATL during the clinical course. Cytotoxic effects of the intensive chemotherapy on the primary neoplastic cells may be one of the driving forces of such exchange of dominant T-cell clones.

ACKNOWLEDGEMENTS

We would like to thank Drs M. Maeda, T. Uchiyama (Kyoto University), M. Miwa (National Cancer Center Institute), T. Yoshida, Y. Koide (Hamamatsu University), and Eisai Co. for technical advise. This work was supported by the following grants: Cancer Research (62-38) from the Ministry of Health and Welfare, and Grant-in-Aid for Encouragement of Young Scientists (01770727) from the Ministry of Education, Japan. Clinical features of the present case have briefly been described in an article by M. Yamada et al. published in Int. J. Dermatol. 28; 107–113, 1989.

REFERENCES

1. Blattner WA, Blayey DW, Robert-Guroff M, et al. Epidemiology of human T-cell leukemia/lymphoma virus. J Infect Dis 1983; 147: 406–416.
2. Hinuma Y, Komoda H, Chosa T, et al. Antibodies on adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: A nation-wide seroepidemiologic study. Int J Cancer 1982; 29: 631–635.
3. The T- and B-cell Malignancy Study Group. Statistical analysis of clinico-pathological, virological and epidemiological data on lymphoid malignancies with special reference to adult T-cell leukemia/lymphoma: The second nation-wide study of Japan. Jpn J Clin Oncol 1985; 517–538.
4. Takatsuki K, Uchiyama T, Sagawa K, Yodoi J. Adult T-cell leukemia in Japan. In: Seno S, Takuku F, Irino S, eds. Topics in Hematology. Amsterdam: Excerpta Medica, 1976: 73–77.
5. Yamaguchi K, Nishimura H, Kohrogi H, et al. A proposal for smoldering adult T-cell leukemia: A clinicopathological study of five cases. Blood 1983; 62: 758–766.
6. Kawano F, Yamaguchi K, Nishimura H, et al. Variation in clinical course of adult T-cell leukemia. Cancer 1985; 55: 851–856.
7. Bunn, Jr. PA. Clinical features. In: Broder S, moderator. T-cell lymphoproliferative syndrome associated with human T-cell leukemia/lymphoma virus. Ann Inter Med 1984; 100: 543–557.
8. Yoshida M, Seki M, Yamaguchi K, Takatsuki K. Monoclonal integration of human T-cell leukemia provirus in all primary tumors of adult T-cell leukemia suggests causative role of human T-cell leukemia virus in the disease. Proc Natl Acad Sci USA 1984; 81: 2534–2537.
9. Aisenberg AC, Krontiris TG, Mak TW, et al. Rearrangement of the gene for the beta chain of the T-cell receptor in T-cell chronic lymphocytic leukemia and related disorders. N Engl J Med 1985; 313: 529–533.
10. Maeda M, Shimizu A, Ikuta K, et al. Origin of human T-lymphotrophic virus 1-positive T cell lines in adult T-cell leukemia. Analysis of T-cell receptor gene rearrangement. J Exp Med 1985; 162: 2169–2174.
11. Yamaguchi K, Kiyokawa T, Nakada K, et al. Polyclonal integration of HTLV-1 proviral DNA in lymphocytes from HTLV-1 seropositive individuals: and intermediate state between the healthy carrier state and smouldering ATL. Br J Haematol 1988; 68: 169–174.
12. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350–4354.
13. Poiesz BJ, Ruscetti FW, Gardar AF, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 1980; 77: 7415–7419.
14. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci USA 1982; 79: 2031–2035.
15. Takahashi K, Tanaka T, Fujita M, et al. Cutaneous-type adult T-cell leukemia/lymphoma. A unique clinical feature with monoclonal T-cell proliferation detected by Southern blot analysis. Arch Dermatol 1988; 124: 399–404.
16. Dasgupta UB, Lilly F. Chemically induced murine T lymphomas: Continued rearrangement within the T-cell receptor gene during serial passage. Proc Natl Acad Sci USA 1988; 85: 3193–3197.