RAPID COMMUNICATION Jpn. J. Cancer Res. 84, 1–3, January 1993

Southernmost Carriers of HTLV-I/II in the World

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To clarify the real distribution of HTLV-I and -II carriers among indigenous people in central and South America, blood samples collected from indigenous people in isolated regions of Southern Chile were examined. Among 199 inhabitants from Chiloe Island and Pitrufquen town, three cases (1.5%) showed positive anti-HTLV-I antibodies. Two out of the three (82-year-old male and 58-year-old female) reacted to HTLV-II-specific Gag and/or Env proteins but not to HTLV-I-specific ones. The latter case was confirmed as an HTLV-II carrier by polymerase chain reaction test.

Key words: HTLV-I — HTLV-II — American Indians

Human T-cell leukemia virus type I (HTLV-I) is the etiological agent for adult T-cell lymphoma/leukemia (ATL) and HTLV-I associated myelopathy (HAM)/tropical spastic paraparesis (TSP). On the other hand, a second type of HTLV (HTLV-II) was initially isolated from a patient with hairy cell leukemia.[1)] Recent epidemiologic surveys have suggested that the majority of intravenous drug users with HTLV-reactive antibodies are infected with HTLV-II rather than HTLV-I,[2)] which suggested that some carriers of HTLV-II are present among the general population. There has been some difficulty in distinguishing HTLV-II carriers from HTLV-I carriers, because of substantial cross-reactivity owing to the high sequence homology between the two types of HTLV. Recently, however, it has become possible to identify the HTLV-I- and HTLV-II-specific provirus DNA in lymphocytes and the antibodies to specific regions of Gag and Env proteins in sera from individuals infected with HTLV-I and HTLV-II, respectively.[3)]

Worldwide epidemiological studies indicate that HTLV-I carriers are mainly distributed among the Japanese in Japan, Hawaii and Brazil and blacks in Africa, the Caribbean basin, the Pacific coast of Colombia and the United States. Besides the Japanese in Asia, several percent of Melanesians in Papua New Guinea and Australia are carrying HTLV-I. Recent epidemiological studies detected HTLV-I carriers among the indigenous people in South America, who might be the descendants of Asiatic immigrants of old, having passed through Beringia and/or the Pacific Ocean[4)] as migration routes. In Japan, it is estimated that there are more than one million carriers of HTLV-I but specific antibodies to HTLV-II have not yet been detected.[5)] Recently, it was reported that HTLV-II-positive cases tested by polymerase chain reaction (PCR) were detected among patients with HTLV-I-associated myelopathy in the southern part of Japan,[6)] but further examination is desirable by using specific antibodies to HTLV-II. Among blacks in Africa and Melanesians near Australia, no confirmed HTLV-II carriers have been found. Recently, however, several reports revealed that HTLV-II carriers are distributed among indigenous people in central America, i.e. in the Guami people in Panama.[7)] Then, it was hypothesized that HTLV-II may have originated from indigenous people in central America.

To establish the real distribution of HTLV-II among indigenous people in central and south America, it was important to clarify the existence of HTLV-II carriers among indigenous people in the southernmost part of South America. In exploratory studies on HTLV-I-associated diseases in South American Indians, it was revealed that patients with TSP/HAM were distributed from the northern part of Colombia to the southern part of Chile. However, there was still no specific evidence that HTLV-II exists among indigenous people in South America.

To explore the clustering of HTLV-II carriers among native people in Chile, we conducted an international collaborative study on HTLV-I/II in Japan, Colombia and Chile. For the present study, we focused on small

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islands and isolated villages in South Chile about 1,000 km south of Santiago, the capital city of Chile, where many purely indigenous people still live in a situation of considerable isolation. Finally, we selected four areas; the Chiloe Islands, Santa Juan de la Costa, Pitrufken and Icalma, and studied two Indian groups, Huilliches and Mapuche, including in part Mestizo Indians of mixed Caucasian ancestry. During January 1991, we collected blood samples only from inhabitants recognized to be pure Indians and Mestizos; 70 in Chiloe, 38 in Santa Juan de la Costa, 129 in Pitrufquen and 10 in Icalma (Fig. 1).

We screened for antibodies to HTLV-I in sera by the gelatin particle agglutination test (PA test: Serodia HTLV-1 Fujirebio, Tokyo). Sera positive in the PA test (titer >16) were re-tested for immuno-fluorescence on MT-1 cells (IF test) and antibody specificity was confirmed by western immuno-blotting analysis (WB test: Fujirebio). Furthermore, positives in the PA and IF tests were tested by enzyme-linked immunosorbent assays (ELISA) for specific antibodies to Gag and Env associated proteins of HTLV-I and -II by using synthetic peptides as antigens.[3] Confirmation was obtained by using the syncytium inhibition assay[8] to distinguish between HTLV-I and HTLV-II infections. To detect the provirus DNA of HTLV-I and II in peripheral blood, PCR was performed as described.[9] The primer set [upstream primer: 5′AAAAGCGTGGAGACAGTTCAGGAGG3′ (350–374) and downstream primer: 3′AACCCCCGAGCAGGCCCTATGCT5′ (779–801)] amplifies the R and U5 regions of the LTR of HTLV-I (452 bp) and -II (498 bp). The nucleotide number coincides with the number of ATK[10] in the GenBank data base. For southern blotting, the ECL system (Amersham) was

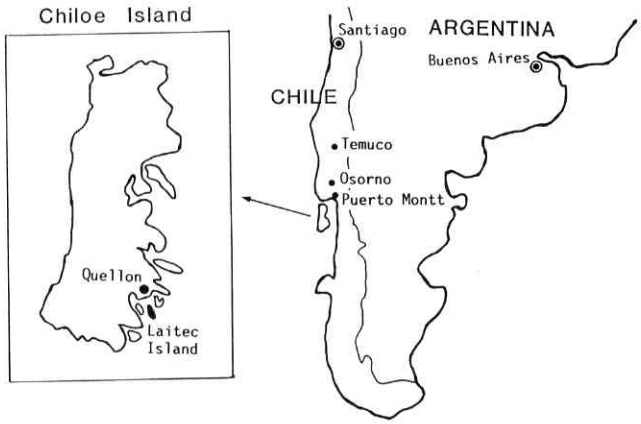

Fig. 1. Map of South Chile showing Chiloe Island.

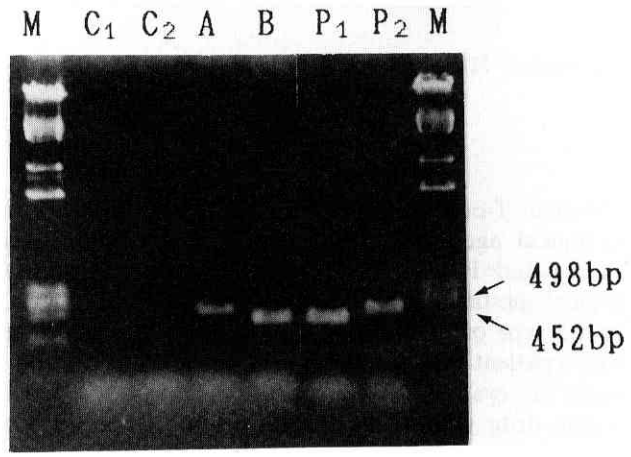

Fig. 2. DNA amplification of HTLV-I and HTLV-II with common PCR primer. Columns: M, marker (lamda DNA digested by *Pvu*II); C_1, negative control (buffer alone); C_2, negative control (normal peripheral blood); A, a positive case of HTLV-II (MPO in Table I); B, a positive case of HTLV-I (RHC in Table I); P_1, positive control of HTLV-I; P_2, positive control of HTLV-II.

Table I. Reactivity of Synthetic Peptides Derived from HTLV-I and -II by ELISA and Expression of Provirus DNA by PCR among Three Anti-HTLV Antibodies Positives Confirmed by IF and WB Tests in 247 Indigenous People in South Chile (1990)

Subject (Number)	Age (Sex)	Anti-HTLV antibody				Decrease of absorbance[a]				Type of provirus DNA	Final judgement
		IF	WB (IgG)			HTLV-I		HTLV-II			
			p19	p24	gp46	Gag	Env	Gag	Env		
PLP (14)	82(M)	×10	−	+	+	6.3	−9.0	89.8	89.2	ND[b]	HTLV-II
RHC (26)	38(F)	×40	+	+	+	39.3	17.1	15.2	2.0	HTLV-I	HTLV-I
MPO(135)	58(F)	×320	+	+	−	−3.6	−1.5	27.0	56.1	HTLV-II	HTLV-II

IF: Indirect immunofluorescence test by using MT-1 cells. WB: Western blotting test (Fujirebio; −, +: no or weak, clear band). ELISA: Enzyme-linked immunosorbent assay. PCR: Polymerase chain reaction.

a) Percent inhibition by ELISA.

b) Not determined but confirmed by syncytium inhibition test.

used. Purified plasmid DNAs of HTLV-I and -II were used as probes.

Three out of 70 (4.3%) persons examined in Chiloe Island and one out of 129 (0.8%) in Pitrufquen showed positive for HTLV-I antibodies in the PA test and finally three out of four showed positive in the confirmation test (Table I). Among them, two cases (14 and 135 in Table I) were reactive to HTLV-II-specific Gag and/or Env proteins but not to HTLV-I-specific ones. The former (82-year-old man from Chiloe Island) was confirmed to be anti-HTLV-II antibody-positive by syncytium inhibition assay. In PCR assay, a positive band to ethidium bromide staining which strongly hybridized to HTLV-I or -II probe was detected after a 2-month co-culture with normal cord blood lymphocytes in cases 26 (RHC) and 135 (MPO), respectively (Fig. 2). Finally, case 26 (RHC) was confirmed to be an HTLV-I carrier. These carriers of HTLV-I and -II are the first to be reported from these southernmost areas of the world.

The present study has identified one or possibly two HTLV-II carriers among indigenous people on an isolated island, Chiloe, in Southern Chile. It is not likely that these people could have had contact with indigenous HTLV-II carriers in central America. From the anthropological viewpoint, it is very interesting that HTLV-II carriers are distributed even in the southernmost areas of South America. It can be hypothesized that HTLV-II has been endemic among indigenous people not only in Central but also South America. The next step will be to confirm the existence of antibody positives to HTLV-II in other areas of South America by the new method that allows discrimination between HTLV-I and HTLV-II antibodies.

This study was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

(Received September 7, 1992/Accepted October 12, 1992)

REFERENCES

1) Kalyanaraman, V. S., Sarngadharan, M. G., Robert-Guroff, M., Miyoshi, I., Blayney, D., Golde, D. and Gallo, R. C. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science,* **218,** 571–573 (1982).
2) Lee, H., Swanson, P., Shorty, V. S., Zack, J. A., Rosenblatt, J. D. and Chen, I. S. High rate of HTLV-II infection in seropositive IV drug abusers in New Orleans. *Science,* **224,** 471–475 (1989).
3) Washitani, Y., Kuroda, N., Shiraki, H., Nishimura, Y., Yamaguchi, K., Takatsuki, K., Fernando, L. P., Fang, C. T., Kiyokawa, H. and Maeda, Y. Serological distribution between HTLV-I and HTLV-II antibodies by ELISA using synthetic peptides as antigens. *Int. J. Cancer,* **49,** 173–177 (1991).
4) Zamora, T., Zaninovic, V., Kajiwara, M., Komoda, H., Hayami, M. and Tajima, K. Antibody to HTLV-I in indigenous inhabitants of the Andes and Amazon regions in Colombia. *Jpn. J. Cancer Res.,* **81,** 715–719 (1990).
5) Kiyokawa, T., Yamaguchi, K., Nishimura, Y., Yoshiki, K. and Takatsuki, K. Lack of anti-HTLV-II seropositivity in HTLV-I-associated myelopathy and adult T-cell leukaemia. *Lancet,* **338,** 451–452 (1991).
6) Kira, J., Koyanagi, Y., Hamakado, T., Itoyama, Y., Yamamoto, N. and Goto, I. HTLV-II in patients with HTLV-I-associated myelopathy. *Lancet,* **338,** 25 (1991).
7) Lairmore, M. D., Jacobson, S., Gracia, F., De, B. K., Castillo, L., Larreategui, M., Roberts, B. D., Levine, P. H., Blattner, W. A. and Kaplan, J. E. Isolation of human T-cell lymphotropic virus type 2 from Guami Indians in Panama. *Proc. Natl. Acad. Sci. USA,* **87,** 8840–8844 (1990).
8) Miyamoto, K., Kamiya, T., Minowada, J., Tomita, N. and Kitajima, K. Transformation of CD8+ T-cells producing a strong cytopathic effect on CD4+ T-cells through syncytium formation by HTLV-II. *Jpn. J. Cancer Res.,* **82,** 1178–1183 (1991).
9) Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science,* **239,** 487–491 (1988).
10) Seiki, M., Hattori, H., Hirayama, Y. and Yoshida, M. Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA,* **80,** 3618–3622 (1983).