Jpn. J. Cancer Res. 81, 1218–1221, December 1990

Geographical Distribution of Subjects Seropositive for Human T-Cell Leukemia Virus Type 1 in Papua New Guinea

Joko Imai,[1] Shin-ichi Terashi,[2] Tom Talonu,[3] Haruko Komoda,[1] Tukutau Taufa,[3] George T. Nurse,[4] Diro Babona,[4] Koichi Yamaguchi,[5] Hiroaki Nakashima,[5] Koh-ichi Ishikawa,[6] Meiko Kawamura[1] and Masanori Hayami[1]

[1]Research Center for Immunodeficiency Virus, Institute for Virus Research, Kyoto University, Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606, [2]Research Center for the South Pacific, Kagoshima University, Usukicho, Kagoshima 890, [3]Faculty of Medicine, University of Papua New Guinea, P. O. Box 5623, Boroko, [4]Red Cross Blood Transfusion Center, Port Moresby General Hospital, P. O. Box 1174, Boroko, [5]Faculty of Medicine, Kagoshima University, Usukicho, Kagoshima 890, and [6]Yamanashi Institute for Public Health, Kofu, Yamanashi 400

Of 1471 sera collected from 1986 to 1989 in Papua New Guinea (PNG), 2.2% were found to be positive for anti-HTLV-1 antibody by successive particle agglutination and immunofluorescence tests. The seropositive rate varied in different provinces and was higher in the coastal areas of the main island and in neighboring small islands than in the highlands of PNG. The frequency of HTLV-1 infection of children was higher, but the age-dependent increase in antibody positivity, generally observed in other HTLV-1 endemic areas of the world, was not clear in PNG. No difference was observed in antibody prevalence in males and females in this study.

Key words: HTLV-1 — Seroepidemiology — Papua New Guinea

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia/lymphoma (ATLL)[1-6] and may be related to tropical spastic paraparesis (TSP)/HTLV-1 associated myelopathy (HAM).[7-11] The vast majority of antibody bearers are healthy virus carriers, and geographically they are clustered in distinct areas, such as southern Japan,[12,13] the Caribbean basin[14-16] and Africa.[16-18]

Recent reports suggest relatively high densities of HTLV-1 carriers in the northwest region of Papua New Guinea[19,20] and part of Indonesian New Guinea,[21] which are located at the equator in the Southern Pacific Ocean. However, little is known about whether HTLV-1 infection is widespread in these countries.

We therefore studied the epidemiological features of HTLV-1 infection in PNG using more than one thousand serum samples obtained in various places in the country. In this paper, we report results on the geographic distribution of infection with this virus in PNG.

MATERIALS AND METHODS

Sera A total of 1471 serum samples were collected from apparently healthy blood donors and patients with various diseases including malignancies (1050 male, 340 female and 81 unknown) at 9 locations in PNG from 1986 to 1989 (Fig. 1). Known ages ranged from 8 months to 67 years old, and 495 adults who could not specify their ages, were grouped as "unspecified adults" (Table I).

Detection of antibody to HTLV-1 All sera were initially screened for HTLV-1 antibody by means of the gelatin particle agglutination test (PA, Serodia ATLA, Fujirebio, Tokyo).[22] Sera that caused agglutination in the screening test at a final dilution of 1:16 or more were then subjected to the immunofluorescence test (IF) using HTLV-1 antigen-bearing MT-1 cells[1] to confirm the antibody specificity.

RESULTS

Prevalence of seropositivity to HTLV-1 in PNG Sera from 1471 Papua New Guinean subjects were first examined for HTLV-1 antibody by PA. Of these sera, 208 (14.1%) were reactive while two sera displayed equivocal agglutination patterns at 1:16 serum dilution and one showed agglutination both with unsensitized and sensitized particles even after an adsorption procedure using unsensitized particles. All 211 sera that gave a positive or equivocal reaction by PA were then examined by IF. At this step, 33 of the 211 sera gave a positive reaction, but it was uncertain whether 28 sera contained HTLV-1 antibody, because of the high nonspecific reaction. We consequently excluded these sera from the seropositive fraction. Therefore, the figures obtained in the confirmatory test are minimal estimations of seropositivity.

Distribution of HTLV-1 seropositivity by age and sex The correlations of age and sex with HTLV-1 seropositivity are shown in Table I, where data on subjects without personal records are omitted. The percentages of

Table I. Age- and Sex-related Distribution of HTLV-1 Seropositive Subjects in Papua New Guinea

Age (yr)	Positive cases/cases tested (%)		
	Total	Male	Female
<10	2/31 (6.5)	2/17 (11.8)	0/14
10–19	8/270 (3.0)	7/236 (3.0)	1/34 (2.9)
20–29	11/395 (2.8)	9/314 (2.9)	2/81 (2.5)
30–39	3/90 (3.3)	3/78 (3.8)	0/12
40–67	0/29	0/24	0/5
Unspecified adult	8/495 (1.6)	4/321 (1.2)	4/174 (2.3)
Total	32/1310 (2.4)	25/990 (2.5)	7/320 (2.2)

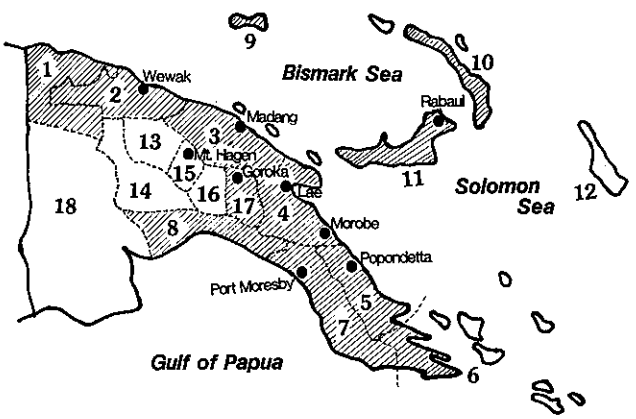

Fig. 1. Geographic distribution of HTLV-1 seropositivity. Numbers correspond to those for names of Provinces listed in Table II. ●, site of sample collection; ▨, province where HTLV-1 seropositive subjects were detected.

seropositive individuals in the different age groups were almost the same (about 3%) except for those in children of under 10 years old (6.5%) and adults of over 40 years old (<3%). The high positive rate (2/31) in children of under 10 years old is not comparable with the figures for other age groups, because most of these samples from children were obtained in only two places (East Sepik and New Britain) that are known to be high prevalence areas (see below). The number of sera of adults of over 40 years old was not sufficient for statistical study, but only 8 of 495 samples (1.6%) in the unspecified adult group were positive for HTLV-1 antibody, indicating lower prevalence than in other groups. Thus, age-dependent increase in the number of HTLV-1 seropositive subjects, which has been observed generally in other HTLV-1 endemic areas of the world,[2, 16] was not seen in PNG.

No significant difference was found in the incidences of HTLV-1 antibody in the two sexes.

Table II. Geographical Distribution of HTLV-1 Antibody-positive Cases according to Birthplace in Papua New Guinea

Province	No. of sera tested	No. of positive cases IF (%)	No. of NS[a] IF (%)
1. West Sepik	18	1 (5.6)	0
2. East Sepik	158	4 (2.5)	2 (1.3)
3. Madang	49	1 (2.0)	3 (6.1)
4. Morobe	94	2 (2.1)	3 (3.2)
5. Northern	71	1 (1.4)	3 (4.2)
6. Milne Bay	35	2 (5.7)	2 (5.7)
7. Central	214	8 (3.7)	3 (1.4)
8. Gulf	78	1 (1.3)	5 (6.4)
9. Munus	28	1 (3.6)	0
10. New Irland	31	2 (6.5)	1 (3.2)
11. New Britain	143	3 (2.1)	1 (0.7)
12. North Solomons	53	0	1 (1.9)
Subtotal	972	26 (2.7)[b]	24 (2.5)
13. Enga	35	0	0
14. Southern Highland	42	0	1 (2.4)
15. Western Highland	50	0	0
16. Chimbu	46	0	0
17. Eastern Highland	70	2 (2.9)	0
18. Western	30	0	0
Subtotal	273	2 (0.7)[b]	1 (0.4)
19. No information on birthplace	226	5 (2.2)	3 (1.3)
Total	1471	33 (2.2)	28 (1.9)

a) Nonspecific reaction.
b) The difference between values for coastal areas (1–12) and highlands (13–18) is significant at $P<0.1$.

Geographic distribution of HTLV-1 seropositivity with respect to birthplace At the time of blood sampling, we also obtained information on the birthplaces of the subjects, which were distributed all over PNG. Table II showed the geographic distribution of HTLV-1 seroposi-

tive subjects. The seropositivity rate of HTLV-1 varied depending on the birthplace of the subjects. The incidences were high in New Ireland, Milne Bay and West Sepik, being 6.5%, 5.7% and 5.6%, respectively. In general, the incidences were high in the coastal regions of the main island and satellite islands (Fig.1). In contrast, none of the subjects from the highlands, except East Highland, possessed HTLV-1 antibody.

DISCUSSION

The average incidence of confirmed seropositivity in all PNG is 2.2%, which is similar to that in West Africa[16, 17] but lower than that reported in the endemic areas of Japan[12, 13] and the Caribbian basin.[14, 15] Kazura *et al.*[19] screened healthy residents in five communities of East Sepik Province for HTLV-1 antibody and reported that the incidence of HTLV-1 seropositivity there was 26%. This value is very different from that of 2.2% in the subjects we examined in the same province. The difference was presumably due to the difference in assay methods employed. We used PA for screening and IF as a confirmatory test, while they used ELISA for antibody detection and competitive ELISA assay with sheep antibody to HTLV-1 for confirmation. Levine *et al.*[16] reported a high test-background in sera obtained from tropical areas such as Ghana and Nigeria. We also observed a high test-background in ELISA of PNG sera (data not shown), so this method probably gives false-positive results. False-positive results are also obtained in PA but PA is highly sensitive, not giving false-negative results, and is generally considered to be a reliable method for primary screening of sera. On the other hand, IF, which we used as a confirmatory test can distinguish clearly between a true positive and a nonspecific reaction. MT-1 cells are a cell line containing 5–10% HTLV-1 antigen-bearing cells, so both HTLV-1 positive and negative cells can be seen simultaneously in the same preparation under the microscope after immunofluorescent staining. However, many of the serum samples in PNG (28/208) were unidentifiable by IF due to a nonspecific reaction (background staining). Some of these, which were excluded from the positive figure in this study, may have had specific antibody. Therefore, the real seropositivity was probably higher than that reported here, although it would not exceed twice our value, even if all the unidentified samples were positive. Weber *et al.*[23] criticized a previous report on seropositivity in PNG and claimed that the seropositive figures reported were not reliable. In fact, some PNG sera gave different reactions depending on the assay methods employed including ELISA and Western blotting (data not shown). But as seropositive persons detected by IF are generally considered to be virus carriers,[24] we believe that HTLV-1 or/and a related viral infection exists in PNG. Virus from apparently seropositive persons must be isolated and analyzed to solve this problem.

The present results clearly showed that HTLV-1 seropositive subjects are present in the coastal regions of the main island and in satellite islands, but not in the highlands. The relation of seropositivity with some specific tribes in the regions should be studied further from the anthropological viewpoint.

The age-related distribution of HTLV-1 antibody positivity in PNG seems to be slightly different from that in other endemic areas of the world, in which the seropositive frequency increases with age. No increase of seropositivity with age was seen in PNG, and so HTLV-1 and/or its related virus infection is probably not age-related in PNG.

The persons from whom the sera were collected manifested no ATLL- or TSP/HAM-like diseases. HTLV-1 is clearly associated with these diseases in HTLV-1 endemic areas, so the significance of HTLV-1 infection in PNG in relation to these diseases should be studied further. The disease caused by HTLV-1 is known to appear after a long latency period, so further longitudinal serologic studies focused on aged persons are required. Moreover, virological analysis, particularly isolation of HTLV-1 or related viruses is necessary.

ACKNOWLEDGMENTS

We are grateful to Dr. J. C. White (Port Moresby General Hospital), Dr. Julius Ngahan, Dr. Barry Mallett, Dr. Henderick Paneor (Angau Memorial Hospital), Dr. S. Seta, Dr. Molly Sanwel, Dr. C. Apa (Mount Hagen General Hospital), Dr. Philip Watt, Dr. Joe Amban (Madang Central Hospital), and Dr. Timothy Iga (Red Cross Blood Transfusion Center, Port Moresby General Hospital) for collecting blood samples in PNG.

(Received May 16, 1990/Accepted September 3, 1990)

REFERENCES

1) Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinosita, K., Shirakawa, S. and Miyoshi, I. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA*, **78**, 6476–6480 (1981).
2) Blattner, W. A., Kalyanaraman, V. S., Robert-Guroff, M., Lister, T. A., Galton, D. A. G., Sain, P. S., Crawford, M. H., Catovlsky, D., Greaves, M. and Gallo, R. C. The

human type-C retrovirus, HTLV, in blacks from the Caribbean region, and relationship to adult T-cell leukemia/lymphoma. *Int. J. Cancer,* **30,** 257–264 (1982).

3) Catovsky, D., Greaves, M. F., Rose, M., Galton, D. A. G., Goolden, A. W. G., McCluskey, D. R., White, J. M., Lampert, I., Bourikas, G., Ireland, R., Brownell, A. I., Bridges, J. M., Blattner, W. A. and Gallo, R. C. Adult T-cell lymphoma-leukaemia in blacks from the West Indies. *Lancet,* **i,** 639–643 (1982).
4) Yoshida, M., Miyoshi, I. and 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. Aci. USA,* **79,** 2031–2035 (1982).
5) Blattner, W. A., Gibbs, W. N., Saxinger, C., Robert-Guroff, M., Clark, J., Lofters, W., Hanchard, B., Campbell, M. and Gallo, R. C. Human T-cell leukaemia/lymphoma virus-associated lympho-reticular neoplasia in Jamaica. *Lancet,* **ii,** 61–64 (1983).
6) Yoshida, M., Seiki, M., Yamaguchi, K. and 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,* **81,** 2534–2537 (1984).
7) Gessain, A., Barin, F., Vernant, J. C., Gout, O., Maurs, L., Calender, A. and de The, G. Antibodies to human T-lymphotropic virus type 1 in patients with tropical spastic paraparesis. *Lancet,* **ii,** 407–410 (1985).
8) Rodgers-Johnson, P., Gajdusek, D. C., Morgan, O. C., Zaninovic, V., Sarin, P. S. and Graham, D. S. HTLV-1 and HTLV-III antibodies and tropical spastic paraparesis. *Lancet,* **ii,** 1247–1248 (1985).
9) Jacobson, S., Raine, C. S., Mingioli, E. S. and McFalin, D. E. Isolation of an HTLV-1-like retrovirus from patients with tropical spastic paraparesis. *Nature,* **331,** 540–543 (1988).
10) Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M. and Tara, M. HTLV-1 associated myelopathy, a new clinical entity. *Lancet,* **i,** 1031–1032 (1986).
11) Yoshida, M., Osame, M., Usuku, K., Matsumoto, M. and Igata, A. Viruses detected in HTLV-1-associated myelopathy and adult T-cell leukemia are identical on DNA blotting. *Lancet,* **i,** 1085–1086 (1987).
12) Hinuma, Y., Komoda, H., Chosa, T., Kondo, T., Kohakura, M., Takenaka, T., Kikuchi, M., Ichimaru, M., Yunoki, K., Sato, I., Matsuo, R., Takiuchi, Y., Uchino, H. and Hanaoka, M. Antibodies to 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,* **29,** 631–635 (1982).
13) Maeda, Y., Furukawa, M., Takehara, Y., Yoshimura, K., Miyamoto, K., Matsuura, T., Morishima, Y., Tajima, K., Okochi, K. and Hinuma, Y. Prevalence of possible adult T-cell leukemia virus-carriers among volunteer blood donors in Japan: a nation-wide study. *Int. J. Cancer,* **33,** 717–720 (1984).
14) Blattner, W. A., Blayney, D. W., Robert-Guroff, M., Sarngadharan, M. G., Kalyanaraman, V. S., Sarin, P. S., Jaffe, E. S. and Gallo, R. C. Epidemiology of human T-cell leukemia/lymphoma virus. *J. Infect. Dis.,* **147,** 406–416 (1983).
15) Schupbach, J., Kalyanaraman, V. S., Sarngadharan, M. G., Blattner, W. A. and Gallo, R. C. Antibodies against three purified proteins of the human type C retrovirus, human T-cell leukemia-lymphoma virus, in adult T-cell leukemia-lymphoma patients and healthy blacks from the Caribbean. *Cancer Res.,* **43,** 886–891 (1983).
16) Levine, P. H., Blattner, W. A., Clark, J., Tarone, R., Maloney, E. M., Murphy, E. M., Gallo, R. C., Robert-Guroff, M. and Saxinger, W. C. Geographic distribution of HTLV-1 and identification of a new high-risk population. *Int. J. Cancer,* **42,** 7–12 (1988).
17) Hunsmann, G., Schneider, J., Schmitt, J. and Yamamoto, N. Detection of serum antibodies to adult T-cell leukemia virus in non-human primates and in people from Africa. *Int. J. Cancer,* **32,** 329–332 (1983).
18) Fleming, A. F., Maharajan, R., Abraham, M., Kulkarni, A. G., Bhusnurmath, S. R., Okpara, R. A., Williams, E., Akinsete, I., Schneider, J., Bayer, H. and Hunsmann, G. Antibodies to HTLV-1 in Nigerian blood-donors, their relatives and patients with leukemias, lymphomas and other diseases. *Int. J. Cancer,* **38,** 809–813 (1986).
19) Kazura, J. W., Saxinger, W. C., Wenger, J., Forsyth, K., Lederman, M. M., Gillespie, J. A., Carpenter, C. J. and Alpers, M. A. Epidemiology of human T cell leukemia virus type 1 infection in East Sepik province, Papua New Guinea. *J. Infect. Dis.,* **155,** 1100–1107 (1987).
20) Brabin, L., Brabin, B. J., Doherty, R. R., Gust, I. D., Alpers, M. P., Fujino, R., Imai, J. and Hinuma, Y. Patterns of migration indicate sexual transmission of HTLV-1 infection in non-pregnant women in Papua New Guinea. *Int. J. Cancer,* **44,** 59–62 (1989).
21) Re, C. M., Tommaseo, M., Furlini, G. and Placa, M. L. High prevalence of serum antibody against human T cell leukemia virus type 1 (HTLV-1) among the Bisman Asmat population (Indonesian New Guinea). *AIDS Res. Hum. Retroviruses,* **5,** 551–554 (1989).
22) Ikeda, M., Fujino, R., Matsui, T., Yoshida, T., Komoda, H. and Imai, J. A new agglutination test for serum antibodies to adult T-cell leukemia virus. *Gann,* **75,** 845–848 (1984).
23) Weber, J. N., Banatvala, N., Clayden, S., McAdam, K. P. W. J., Palmer, S., Moulsdale, H., Tosswill, J., Dilger, P., Thorpe, R. and Amann, S. HTLV-1 infection Papua New Guinea: evidence for serologic false positivity. *J. Inf. Dis.,* **159,** 1025–1028 (1989).
24) Gotoh, Y., Sugamura, K. and Hinuma, Y. Healthy carriers of a human retrovirus, adult T-cell leukemia virus (ATLV): demonstration by clonal culture of ATLV-carrying T cells from peripheral blood. *Proc. Natl. Acad. Sci. USA,* **79,** 4780–4782 (1982).