Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *85* (1): 29-33, jan./mar. 1990

PREVALENCE OF HUMAN T CELL LEUKEMIA VIRUS-I (HTLV-I) ANTIBODY AMONG POPULATIONS LIVING IN THE AMAZON REGION OF BRAZIL (PRELIMINARY REPORT)

C. M. NAKAUCHI; A. C. LINHARES/+; K. MARUYAMA*; L. I. KANZAKI**; J. E. MACEDO***; V. N. AZEVEDO** & J. S. R. CASSEB****

Instituto Evandro Chagas, Seção de Vírus, Fundação Serviços de Saúde Pública, Av. Almirante Barroso, 492, 66050 Belém, PA, Brasil * Department of Pathology, Chiba Cancer Center Research Institute, Chiba, Japan ** Universidade Federal do Pará *** Instituto Offir Loyola, Belém, PA, Brasil ****Faculdade Estadual de Medicina, Belém, PA, Brasil

Forty-three (31.4%) out of 137 serum samples obtained from two Indian communities living in the Amazon region were found to be positive for HTLV-I antibody, as tested by enzyme-linked immunosorbent assay (ELISA). Eighty-two sera were collected from Mekranoiti Indians, yielding 39% of positivity, whereas 11 (20.0%) of the 55 Tiriyo serum samples had antibody to HTLV-I. In addition, positive results occurred in 10 (23.2%) out of 43 sera obtained from patients living in the Belém area, who were suffering from cancer affecting different organs. Five (16.7%) out of 30 ELISA positive specimens were also shown to be positive by either Western blot analysis (WB) or indirect immunogold electron microscopy (IIG-EM).

Key words: HTLV-I – antibody -- Amazon region

Since the pioneer findings establishing the aetiological relationship between HTLV-I and virulent leukemia affecting humans (Poiesz et al., 1980), data is cummulating to assess the importance of this viral agent as a human pathogen. Apart from being widely recognized as a causative agent of adult T cell leukemia (ATL), HTLV-I has been implicated in the aetiology of other human diseases (Mattock & Parker, 1985; Katayama et al., 1987; Brew & Price, 1988; Maruyama et al., 1989).

Seroepidemiological studies conducted in several countries have demonstrated that many people are infected with HTLV-I, particularly in Japan and the Caribbean region, where prevalence rates yield 15.0% and 14.0%, respectively (Asher et al., 1988). In the United States of America and Europe, HTLV-I infection has been found to be more frequent among intra-venous drug abusers (Hinuma et al., 1981; Tedder et al., 1984; Gallo, 1986). In a more recent study carried out in metropolitan Panama (Reeves et al., 1988), HTLV-I antibody was detected in 5% of the population.

In Brazil there have been few studies, to date, regarding the ocurrence of HTLV-I infection among both urban and isolated populations (Andrada-Serpa et al., 1988, in press; Cortes et al., 1989). Our preliminary study was therefore carried out in order to determine the prevalence of antibodies to HTLV-I in two Indian communities living in the Pará state, Amazon region, as well as in cancer patients.

MATERIALS AND METHODS

Specimens and populations – Sera were collected by venopuncture from 82 Mekranoiti and 55 Tiriyo Indians, living in Pará state, Brazil (Fig. 1). Both Indian tribes are relatively isolated communities with only sporadic contacts with urban men. Most of the specimens were obtained from previous studies (particularly on arboviral diseases) conducted by our staff and kept frozen at -20^{o}C. Serum samples were also obtained from cancer patients attending a local public hospital. These individuals were diagnosed as suffering from neoplastic diseases at different sites (Nakauchi et al., in press).

+ Reprints requests.

Received July 4, 1989.
Accepted January 30, 1990.

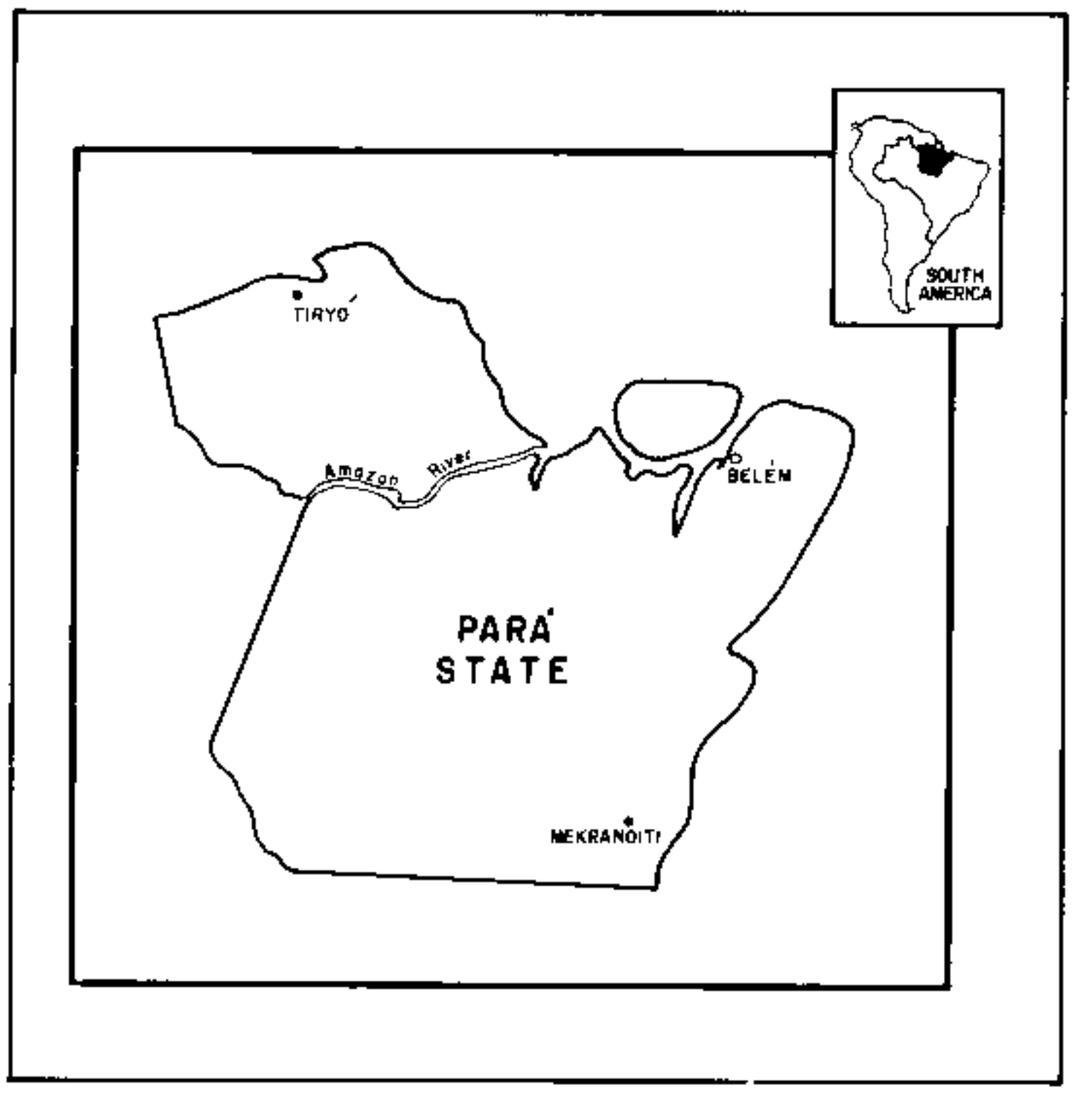

Fig. 1 : map of Pará state, Brazil, showing the situation of study communities.

Specimens from all age groups were obtained from both Indians and cancer patients. In the former group 51 (37.2%) were from children aged 0 to 14 years, 62 (45.2%) from young adults (15 to 49 years old), 6 (4.4%) from those aged 50 years or more and 18 (13.2%) from individuals with unknown ages; in the latter, 6 (13.9%) serum samples were drawn from patients 0 to 14 years of age, 22 (51.2%) from young adults (15 to 49 years of age) and 15 (34.9%) from those aged 50 years or more.

Laboratory methods – All laboratory tests were performed at the Department of Pathology, Chiba Cancer Center Research Institute, Chiba, Japan. ELISA was carried out as essentially described by Burrells & Dawson (1982) and virus preparation was obtained from culture fluid harvested from Lma-66 cell line (Maruyama et al., 1982), followed by partial purification through sucrose density gradient centrifugation. Briefly, a 96-well microtiter plate was sensitized with the virus preparation at an optimal dilution (300 – 800 ng per well) in 0.05 M carbonate buffer pH 9.6, for 60 min at room temperature (RT), and fixed with 3% glutaraldehyde for 15 min at 4°C. Plates were then thoroughly washed with phosphate buffered saline (PBS) containing 0.05% Tween 20 pH 7.2 (PT) and blocked with 1% bovine serum albumin (BSA) in PBS. Following another washing with PT, serum samples diluted 1:100 in PT containing 0.1% BSA (PTB) were added to the wells and incubated for 60 min at RT. After washing again, rabbit anti-human IgG conjugated with peroxidase (Miles Scientific, Naperville, IL) was added and plates incubated for 60 min at RT. The enzyme substrate [2-2' -Azino-di (3-ethylbenzathiazoline sulfonic acid) diammonium salt] was then added. After incubation for 10 min at RT, the reaction was stopped with 0.1 M hydrofluoric acid, pH 3.3. Values of absorbance were determined by using a 410 nm filter. All sera yielding values of optical density equal or greater than 0.3 were regarded as positive (Maruyama et al., 1989). All ELISA tests regularly included both positive and negative controls. In each set of tests 3 positive control sera were used, as follows: monoclonal antibody (MoAb) to p 19 (obtained from CPI, Buffalo, NY), MoAb to p 24 (obtained from Du Pont, Billerica, MA) and serum sample from an ATL patient; in addition, a serum sample from a normal person was routinely used as a negative control.

Thirty ELISA positive specimens were further examined by either Western blot analysis (WB) (Katayama et al., 1987) and immunogold electron microscopy (IIG-EM) (Maruyama et al., 1989). In the WB MoAbs to HTLV-I p 19 (obtained from CPI, Buffalo, N. Y) and p 24 (obtained from Du Pont, Billerica, MA) were routinely used.

RESULTS

Fifty-three sera (29.4%) of the 180 tested for the presence of HTLV-I antibody were shown to be positive, as shown in Table I. Among Mekranoiti and Tiriyo Indians, percentages of seropositivity were respectively 39.0% and 20.0%. The frequency of HTLV-I antibody among patients with neoplastic diseases was 23.2%; 5 (50.0%) out of the 10 seropositive patients were females suffering from cervix cancer. Thirty ELISA-positive serum samples (19 from Indians and 11 from cancer patients) were also tested by either WB or IIG-EM; of these, 5 (16.7%) were found to be positive by the latter two techniques: 4 from cancer patients and 1 from an Indian. In Table II the 5 confirmed ELISA-positive sera are compared with results obtained from WB (p 15, p 19 and p 24 proteins); one of them ("C") was found to be positive for all proteins as well as by IIG-EM.

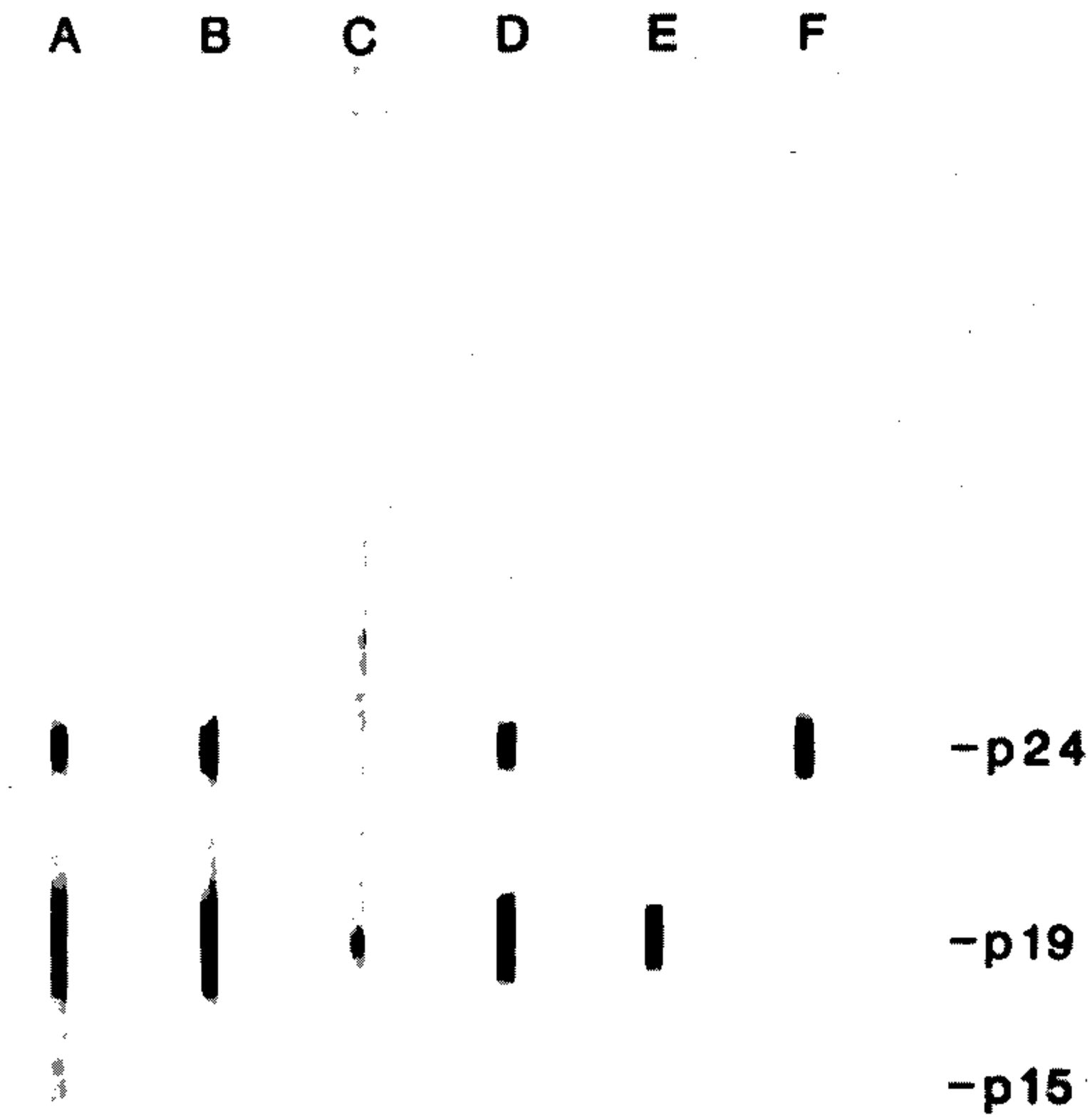

Fig. 2: Western blots of HTLV-I with different sera – A: serum from a Brazilian patient with cancer of uterine cervix (# 97, M. P. R., Belém, Pará) tested at 1:20 dilution. B: serum from a Brazilian patient with cancer of uterine cervix (# 226, A. A. M. C., Benfica, Pará) tested at 1:20 dilution. C: serum from a Brazilian patient with osteosarcoma of rib (# 186, G. C. C., Icoaraci, Pará) tested at 1:20 dilution. D: serum from a Japanese patient with ATL tested at 1:20 dilution. E: mouse monoclonal antibody to HTLV-I p 19. F: mouse monoclonal antibody to HTLV-I p 24.

TABLE I

Frequency of HTLV-I antibody in serum samples collected from Amazonian populations, as determined by ELISA

Population group	No. positive	No. tested	%
Mekranoiti Indians	32	82	39.0
Tiriyo Indians	11	55	20.0
Cancer patients	10	43	23.2
Total	53	180	29.4

TABLE II

Correlation of ELISA positivity with Western blot (WB) and indirect immunogold electron microscopy (IIG-EM) results in five sera

ELISA-positive sera (O. D.)	WB			IIG-EM
	p 15	p 19	p 24	
A (0.59)	–	+	–	+
B (0.39)	+	–	–	–
C (1.21)	+	+	+	+
D (0.38)	–	+	–	–
E (1.01)	–	+	+	+

O. D. = optical density.

Among sera collected from Indians there were no significant differences in comparing positivity for HTLV-I antibody and age-groups. On the other hand, there were no positive results when sera from cancer patients aged less than 15 years were tested.

Figure 2 shows the WB patterns obtained by testing serum samples from 3 cancer patients. Reactivities with viral peptides "p 24" and "p 19" were observed in all tested sera, however, only one specimen produced a clear band against "p 15" viral peptide.

DISCUSSION

Although preliminary, our results strongly suggest that HTLV-I infection is frequent among both urban and isolated populations living in the Amazon region.

Serum samples collected from both children and adults of two relatively isolated Indian communities, yielded percentages of seropositivity – as tested by ELISA – which may indicate endemicity of HTLV-I. Cases of either ATL or lymphoma among these populations have not been recorded by health authorities in charge of their medical assistance. This leads us to justify the seropositivity based on two hypotheses. First, HTLV-I would be responsible for asymptomatic infections among Indians; previous studies (Varmus, 1988) have demonstrated that "although the number of cases of HTLV – associated leukemia and lymphoma is relatively small, many people are infected, especially in Japan and Caribbean and among intravenous drug users in U. S. and Europe". Second, it could be postulated that a HTLV-I antigenically related retrovirus circulates among these populations, inducing a group-specific humoral immune response which would be detected by using the conventional ELISA for HTLV-I antibody. Activation of immune polyclonal system by diseases such as malaria – a highly prevalent parasitic disease in the study area – might also be regarded as a possible source of non-specific ELISA results. This possibility can however be ruled out in those specimens whose specificity was assessed by either WB or IIG-EM. Our results showing that one (5.3%) out of the 19 ELISA-positive Indian sera was positive by either WB or IIG-EM diverge from those of Andrada-Serpa et al. (in press), who found only 0.84% of positive sera among 119 tested for the presence of anti HTLV-I antibody.

The rate of seropositivity among cancer patients was 23.2% and 4 (36.4%) out of the 11 ELISA-positive specimens tested by either WB or IIG-EM were shown to be positive by these two latter techniques. If compared with the results obtained by Andrada-Serpa et al. (in press), our data show a much higher prevalence of anti-HTLV-I antibody among local patients. None of the patients whose sera gave positive results was suffering from ATL. Neoplastic tumours were recorded at several sites, cervix cancer being the most frequent.

On the basis of ELISA results, the percentage of seropositivity among cancer patients was shown to be slightly lower if compared with the rate obtained from the whole Indian population. This difference should be further evaluated through a study involving larger sample sizes of both urban and Indian populations. In this context it should be mentioned that previous investigations (Maruyama et al., 1989) have suggested that rates of HTLV-I antibody are more than 5 times prevalent among cancer patients than in normal individuals.

Our preliminary findings also raised the possibility that local strains are not homologous to those prevalent in Japan. The WB performed with some of our sera demonstrated that, unlike the Japanese ones, local sera reacted with viral peptide "p 15". This may indicate that antigenic composition of HTLV-I local strains differ slightly from that of viruses which circulate in Japan.

Further and broader studies on the occurrence of HTLV-I infection in our region are necessary, in order to assess the real magnitude of this viral agent as a human pathogen.

ACKNOWLEGMENTS

To Mr Francisco Teles Pantaleão and Mr Airton J. da Graça Teixeira for helping us in the collection of serum samples. To members of Department of Pathology, Chiba Cancer Center Research Institute, Chiba, Japan, for their excelent technical assistance.

REFERENCES

ANDRADA-SERPA, M. J.; DOBBIN, J. A.; GOMES, P.; LINHARES, D.; AZEVEDO, J. G.; HENDRIKS, J.; CLAYDEN, S. A.; RUMJANEK, V. M.; TED-

DER & TEDDER, R. S., 1988. Incidence of retroviruses in some Brazilian groups. *Immunology Letters, 18:* 15-18.

ANDRADA-SERPA, M. J.; TOSSWILL, J.; SCHOR, D.; LINHARES, D.; DOBBIN, D. & PEREIRA, M. S. Seroepidemiologic survey for antibodies to human retroviruses in human and non-human primates in Brazil. *Int. J. Cancer.* In press.

ASHER, D. M.; GOUDSMITH, J.; POMEROY, K. L.; GARRUTO, R. M.; BAKKER, M.; ONO, S. G.; ELLIOT, N.; HARRIS, K.; ASKINS, H.; ELDADAH, Z.; GOLDSTEIN, A. D. & GAJDUSEK, D. C., 1988. Antibodies to HTLV-I in populations of the Southwestern Pacific. *J. Med. Virol., 26:* 339-351.

BREW, B. J. & PRICE, R. W., 1988. Another retroviral disease of the nervous system: chronic progressive myelopathy due to HTLV-I. *N. Engl. J. Med., 318:* 1195-1197.

BURRELLS, C. & DAWSON, Mc L., 1982. ELISA methodology: Variations in technical procedures, p. 1-9. In R. C. Wardley & J. R. Crowther. *The ELISA: Enzyme-linked immunosorbent assay in veterinary research and diagnosis.* The Hague/Boston/London Martinus Nijhoff Publ.

CORTES, E.; DETELS, R. ; ABOULAFIA, D.; LI, X. L.; MOUDGIL, T.; ALAM. M.; BONECKER, C.; GONZAGA, A.; OYAFUSO, L.; TONDO, M.; BOITE, C.; HAMMERSHLAK, N.; CAPITANI, C.; SLAMON, D. J. & HO, D. D., 1989. HIV-I, HIV-II and HTLV-I infection in high-risk groups in Brazil. *N. Engl. J. Med., 320:* 953-958.

GALLO, R. C., 1986. The first human retrovirus. *Sci. Am., 255:* 88-89.

HINUMA, Y.; NAGATA, K.; ANAOKA, M.; NAKAI, M.; MATSUMOTO, T.; KINOSHITA, K.; SHIRAKAWA, S. & MIYOSHI, I., 1981. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibody to the antigen in human sera. *Proc. Natl. Acad. Sci. USA, 78:* 6476-6560.

KATAYAMA, I.; MARUYAMA, K.; FUKUSHIMA, T.; NISHIBE, Y. & KAYANO, H., 1987. Cross-reacting antibodies to human T cell leukemia virus – I and II Japanese patients with hairy cell leukemia. *Leukemia, 1:* 401-404.

MARUYAMA, K.; KAWAMURA, K.; MIYAUCHI, M.; MOCHIZUKI, S.; FUKUSHIMA, T.; TAKAGI, T. & OGURO, M., 1982. Establishment of cell lines producing both type C virus and herpes-type virus and derived from an adult T-cell leukemia (Abstract). *Proc. Jpn. Cancer Assoc., 41:* 342.

MARUYAMA, K.; MIYAUCHI, M.; FUKUSHIMA, T.; MOCHIZUKI, S.; KOSHIKAWA, N.; KAWAMURA, K.; TAMAYAMA, C.; NAKANO, M.; ODO, A. & IHA, S., 1989. Antigenic variations of retrovirus in Japanese cancer patients. *Leukemia, 3:* 277-281.

MATTOCK, C. & PARKER, N. E., 1985. HTLV-I infection and schizophrenia. *The Lancet, ii:* 945.

NAKAUCHI, C. M.; LINHARES, A. C.; MACEDO, J. E.; MARUYAMA, K.; KANZAKI, L. I. & AZEVEDO, V. N. Prevalência de anticorpos para o vírus da leucemia humana de células T (HTLV-I) em pacientes cancerosos na região amazônica (nota prévia). *Rev. Bras. Cancerol.* In press.

POIESZ, B. J.; RUSCETTI, F. N.; GAZDAR, A. F.; BUNN, P. A.; MINNA, J. D. & GALLO, R. C., 1980. 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, 77:* 7415-7419.

REEVES, W. C.; SAXINGER, C.; BRENES, M. M.; QUIROZ, E.; CLARK, J. W.; HOH, M-W & BLATTNER, W. A., 1988. Human T-cell lymphotropic virus type I (HTLV-I) seroepidemiology and risk factors in Metropolitan Panama. *Am. J. Epidemiol., 127:* 532-539.

TEDDER, R. S.; SHANSON, D. C.; JEFFRIES, D. J.; CHEING-SONG POPOV, R.; DALGLEISH, A.; CLAPHA, P.; NAGY, K. & WEISS, R. A., 1984. Low prevalence in the UK of HTLV-I and HTLV-II infection in subjects with AIDS, with extended lymphadenopathy, and at risk of AIDS. *Lancet, ii:* 125-128.

VARMUS, H., 1988. Retroviruses. *Science, 240:* 1427-1435.