CONCISE COMMUNICATIONS

Demographic, Ethnic, and Geographic Differences between Human T Cell Lymphotropic Virus (HTLV) Type I–Seropositive Carriers and Persons with HTLV-I Gag–Indeterminate Western Blots in Central Africa

Philippe Mauclère, Jean-Yves Le Hesran, Renaud Mahieux, Roger Salla, Jermie Mfoupouendoun, Emmanuel Tina Abada, Jacques Millan, Guy de Thé, and Antoine Gessain

Centre Pasteur du Cameroun, ORSTOM-OCEAC, and AIDS Control Unit, Yaoundé, Cameroon; Unité d'Épidémiologie des Virus Oncogènes, Institut Pasteur, Paris, France

Using stringent Western blot (WB) criteria, human T cell lymphotropic virus (HTLV) type I seroprevalence among 3783 persons from representative rural populations of Cameroon averaged 1.1% and was higher in females (1.5%) and in Pygmies (2.0%), increasing with age. Furthermore, an HTLV-I Gag–indeterminate WB profile (HGIP), exhibiting strong reactivities to p19, p26, p28, p32, p36, and pr 53 but lacking both p24 and env reactivity, was observed in 1.6% of the same populations. The prevalence of the HGIP was similar between males and females, did not increase with age, and appeared to cluster in tropical forests of southern Cameroon, especially among Pygmies (reaching 4%). These contrasting epidemiologic features, together with the lack of detection by polymerase chain reaction of HTLV-I sequences in the peripheral blood mononuclear cells of the persons with HGIP, strongly suggest that such a WB profile does not appear to reflect an HTLV-I–related viral infection but possibly an environmental (viral or parasitic) factor endemic in tropical rain forest areas.

Human T cell leukemia virus (HTLV) type I is the etiologic agent of adult T cell leukemia (ATL) and of tropical spastic paraparesis/HTLV-I–associated myelopathy (TSP/HAM). This virus is endemic in restricted areas of southern Japan, the Caribbean, and parts of South America and tropical Africa. In these regions, 10–15 million inhabitants are infected by HTLV-I, the majority being healthy carriers of the virus. The uneven geographic distribution and higher prevalence in older and female persons represent two major epidemiologic features of HTLV-I.

An important problem in determining the HTLV-I seroprevalence in a given community is caused by the interpretation of the Western blot (WB) pattern according to different seropositivity criteria. While less troublesome in Japan and the Caribbean, this is especially important in tropical Africa and in Melanesia, where a high percentage of WBs exhibit an indeterminate pattern with seroreactivity not only directed against the *gag*–encoded proteins p19, and/or p24, and/or pr 53 but also against p26, p28, p32, and p36, of unclear origin [1–8]. While some groups have investigated the biologic characteristics of such seroindeterminate reactions [2, 9–14], there is, to our knowledge, no epidemiologic study of the characteristics of these HTLV-I–like seroindeterminate patterns.

Previous reports have suggested that central Africa, especially Cameroon, was a highly HTLV-I–endemic area, with a possible north-south gradient of viral seropositivity [6–8]. However, several of the initial studies in these regions used nonstringent WB criteria, and a high percentage of serum and plasma samples had a pattern now considered to be indeterminate [1, 3, 5–8]. The main goals of the present work were to revisit the situation of HTLV-I seroprevalence in different representative geographic areas and ethnic groups in Cameroon by using stringent serologic WB criteria and then to compare the epidemiologic determinants of HTLV-I prevalence with those of the HTLV-I–indeterminate WB patterns.

Materials and Methods

Area and study population. Cameroon occupies 475,000 km^2 and is located in the Gulf of Guinea above the equator (2° to 12° north latitude). Tropical forest covers the main part of the southern region, and the northern area is mostly savanna and hills. The Cameroonian population of 12 million comprises >200 ethnic groups divided into three main cultural and linguistic groups. The largest group includes the Bantou and related populations semi-Bantous (Bamileké, Bamouns) living mainly in the southern and

CHAD

150 km

NIGERIA

Poli
980
(18/4)

Touboro
540
(0/2)

CENTRAL AFRICAN
REPUBLIC'

Nditam
490
(0/15)

Ngat
354
(2/6)

Yokadouma
683
(14/19)

Bipindi
70
(3/6)

Mekas
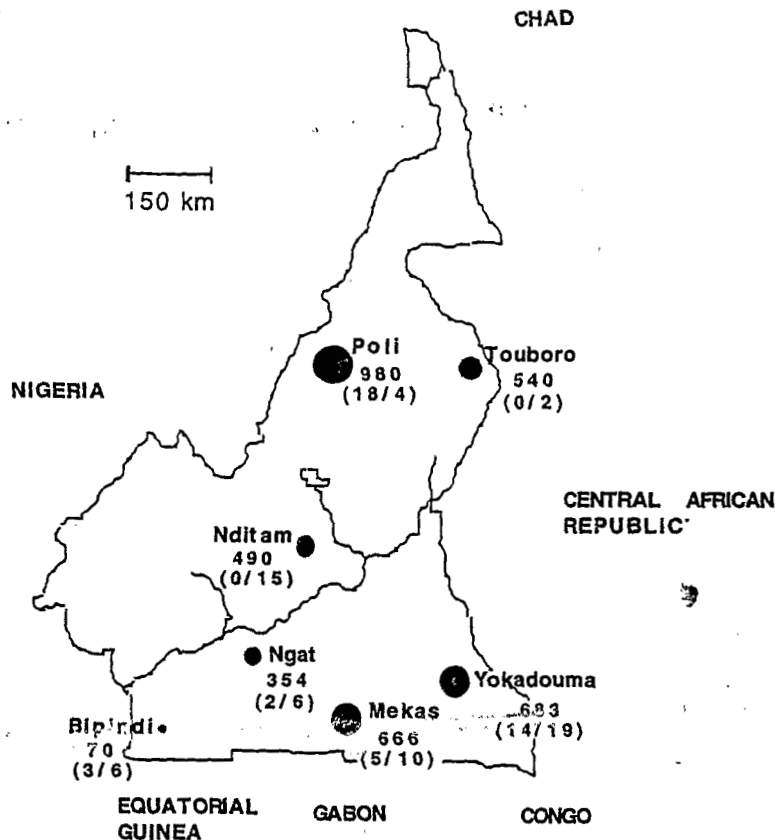666
(5/10)

EQUATORIAL   GABON   CONGO
GUINEA

Figure 1. Map of Cameroon showing different study locations. For each location are given no. of samples studied and no. of HTLV-I—seronegative persons and of HGIP-seroreactive individuals.

in the western parts of the country; most are christians or animists. In the southern area, the Pygmy group represents ~25,000 persons. They are considered as the most ancient inhabitants and have lived mostly in the southern rain forest region from the Atlantic shore in the west to the border with the Central African Republic to the east. The population of the northern part of the country is quite homogenous and comprises mainly Foulbé muslims, agriculturists, and cattle breeders.

Collection of biologic specimens. Serum samples from 3783 persons were collected in order to conduct seroepidemiologic population studies, between 1992 and 1994, in representative surveys of seven groups of villages (figure 1): Poli and Touboro in the north, Nditam in the center, and Ngat, Yokadouma, Mékas, and Bipindi in the southern forest area. Blood specimens were collected from the majority (all >5 years old) of the village inhabitants. The groups were comparable in sex ratio and mean age. None of the persons had an overt HTLV-I—associated disease (ATL or TSP/HAM). For every subject, an aliquot of serum was obtained from a 5-mL venipuncture sample and kept frozen (−20°) until HTLV-I and -II screening.

HTLV-I and -II serologic tests. All sera were screened by an ELISA (PLATELIA HTLV-I NEW; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). This test, which uses viral HTLV-I lysate as antigen, detects both IgG and IgM antibodies [15]. All the samples with an optical density absorbance ratio (ODR) $\geqslant 2$ were tested with an IFA using either HTLV-I (MT2)— or HTLV-II— (C19) producing cells as antigens [1]. All IFA-positive or borderline samples were subjected to WB (HTLV 2-3, Diagnostic Biotechnology; Singapore) for confirmation [1]. A serum was con-

sidered HTLV-I—positive when clearly reactive against both recombinant (r) gp21 and MTA-1 as well as against at least both p19 and p24; it was considered HTLV-II—positive when reactive against rgp21, p24, and K55. A serum sample was considered negative when it exhibited no bands and indeterminate when partially reactive.

Statistical analysis. Descriptive analyses were performed using Epi Info 5.01b. Comparison between ethnic groups and sex were carried out using χ^2 test. For analysis of the age effect, the χ^2 test for trend was used.

HTLV-I molecular analysis. High molecular weight DNA was extracted from ex vivo peripheral blood mononuclear cells (PBMC) from 5 HTLV-I—seropositive healthy carriers, 5 HTLV-I—seronegative persons, and 5 persons exhibiting an HTLV-I Gag—indeterminate profile (HGIP). Single-round (35 cycles) polymerase chain reaction (PCR) was performed on 1.5 μg of DNA using three pairs of oligonucleotide primers derived from the *gag, pol,* and *tax* genes of the HTLV-I ATK genome. In order to check for the possibility of a low virus load among persons with HGIP seroreactivity, a seminested PCR was conducted within the *gag* region on the same PBMC DNAs.

Results

Serologic results. Of the 3783 samples screened by ELISA, 2275 (60.1%) were considered negative, having an ODR lower than the cut-off value. Among the 1508 (39.9%) ELISA-positive samples, all specimens with an ODR $\geqslant 2$ (763, 20.2%)

were further tested by IFA. Among the 506 samples considered IFA-positive or borderline (high nonspecific background), 42 (1.1%) were confirmed as HTLV-I–seropositive by WB, none as HTLV-II–seropositive, 419 as HTLV-seroindeterminate, and 45 as HTLV-seronegative. All HTLV-I WB–positive specimens were clearly positive by IFA and more reactive on MT2 than on C19 cells.

Careful examination of the 419 indeterminate WB patterns led to the identification of a peculiar pattern exhibiting strong p19, p26, p28, p32, p36, and pr 53 bands, faint or absent p24 reactivity, and no env reactivity (rgp21, gp46, MTA-1 bands) (figure 2A). We tentatively named this WB pattern HGIP. This pattern was chosen because it had the most intense bands, it was the closest related pattern to complete HTLV-I seroreactivity, and it represented the most common WB profile among the indeterminate patterns (62/419, 14.8%). Moreover, such patterns had been scored as HTLV-I–seropositive in a number of previous seroepidemiologic studies in tropical areas [1, 5, 7, 8]. Miscellaneous other seroindeterminate WB profiles were seen, exhibiting mostly isolated and faint p19 or p24 bands. On rare occasions, the WB exhibited an isolated MTA-1 or K55 band, and, among the HTLV-I–seropositive sera, 2 exhibited a complete profile but without reactivity against MTA-1 peptide.

Comparative epidemiologic features of HTLV-I and HGIP. Among the 3783 samples tested, (1977 females and 1806 males; sex ratio, 0.91), the overall HTLV-I seroprevalence averaged 1.11% (42/3783); for HGIP it reached 1.64% (62/3783; $\chi^2 = 3.90$, $P = .048$).

Striking differences between the epidemiologic determinants of the HTLV-I–seropositive persons and those having an HGIP were observed (figure 2B). While HTLV-I seroprevalence showed the usual increase with age (χ^2 trend = 4.49, $P = .004$), the HGIP seroprevalence was stable from 5–60 years old (range, 1.4%–2.1%) (χ^2 trend = 0.11; $P = .74$; figure 2B). Among 538 children and adolescents (5–15 years), there were 10 samples (1.85%) with an HGIP but only 1 (0.18 %) with confirmed HTLV-I infection. While there was a significantly higher HTLV-I seroprevalence in females (1.52%, 30/1977) than in males (0.66%, 12/1806; $P = .01$), the seroprevalence of HGIP was quite similar in males (1.66%, 30/1806) and in females (1.60%, 32/1977) ($\chi^2 = .01$, $P = .91$) (figure 2B).

The HTLV-I seroprevalence in the north (1.18%, 18/1520) was similar to that in the south (1.06%, 24/2263), but there was an obvious geographic gradient of HGIP seroprevalence from the north (0.39%, 6/1520) to the southern forest areas (2.47%, 56/2283) ($\chi^2 = 22.84$, $P = 1.8 \times 10^{-6}$). Furthermore, within the southern forest area, the Pygmies had a significantly higher level of HTLV-I antibodies (2.05%, 17/831) than the neighboring Bantous (0.49%, 7/1432; $P = .0005$) but also a significantly higher seroprevalence of HGIP (3.97% [33/831] vs. 1.61% [23/1432] in the Bantous; $P = .0004$) (figure 2C, D).

PCR results. Although a positive PCR signal was clearly detected (with the three primer pairs involving *gag, pol,* and typical HTLV-I seroreactivity, as in the HTLV-I–positive control DNAs, no signal could be detected in the PBMC DNAs of the 5 persons with an HGIP or in the DNAs of the 5 HTLV I- or II–seronegative persons. When using the *gag* nested PCR, we confirmed that the subjects with HGIPs did not have the HTLV-I genome in their PBMC.

Discussion

A review of the literature concerning the seroindeterminate WB profiles led us to discern two different situations. The first could be observed in very low HTLV-I–endemic areas or populations, such as the blood donors in Europe or the United States, in which the presence of the HTLV-I–seroindeterminate pattern is infrequent and comprises mostly faint reactivities against isolated Gag bands. The HGIP, as described in this report, appears to be very rare among those populations [11–13]. In contrast, in some tropical areas (mostly Africa, Melanesia, and Philippines), the prevalence rate of the indeterminate WB reactivities is high, and the herein-described HGIP frequently represents a large proportion [1, 3, 5–10, 14].

We are confident that all of the HTLV-I–seropositive samples were detected by our algorithm, on the basis of the known high sensitivity of both the ELISA and the IFA used in this study. Furthermore, all 42 HTLV-I–seropositive samples had an ODR >3, with 88% being >4. Regarding the HTLV-I Gag–indeterminate profile, 62 HGIP (92%) seroreactive samples had an ODR >3, and 76% were >4. Thus, we considered that the great majority of the samples with such HGIP reactivity was detected in our study, and that the few missed ones would not have significantly modified the epidemiologic analysis.

This study is the first, to our knowledge, to demonstrate that there exist major epidemiologic differences between the distribution of HTLV-I–seropositive carriers and persons with an HGIP. A previous study [13] has, however, demonstrated that the HTLV-seroindeterminate US blood donors were different from the HTLV-seropositive blood donors and more like HTLV-seronegative controls in their demographic characteristics and the presence of risk factors for HTLV infection. However, in this study, which included only US blood donors from the Atlanta region, all persons who exhibited an HTLV-indeterminate WB profile were included in the analysis, irrespective of their HGIP profile. In Cameroon, the overall seroprevalence of HGIP was higher than that of HTLV-I, with no differences between males and females nor increase with age, both characteristics of HTLV-I infection. Furthermore, we observed a geographic gradient for HGIP but not for HTLV-I infection. Thus, HGIP was rare in the north and more frequent in the southern forest, especially in the Pygmies. Such differential epidemiologic features, together with the lack of detectable HTLV-I sequences in the PBMC of these persons, strongly suggest that such an indeterminate Gag WB pattern is not related to an HTLV-I–related infection. Furthermore, we do not believe that this pattern represents slow seroconversion of HTLV-I because
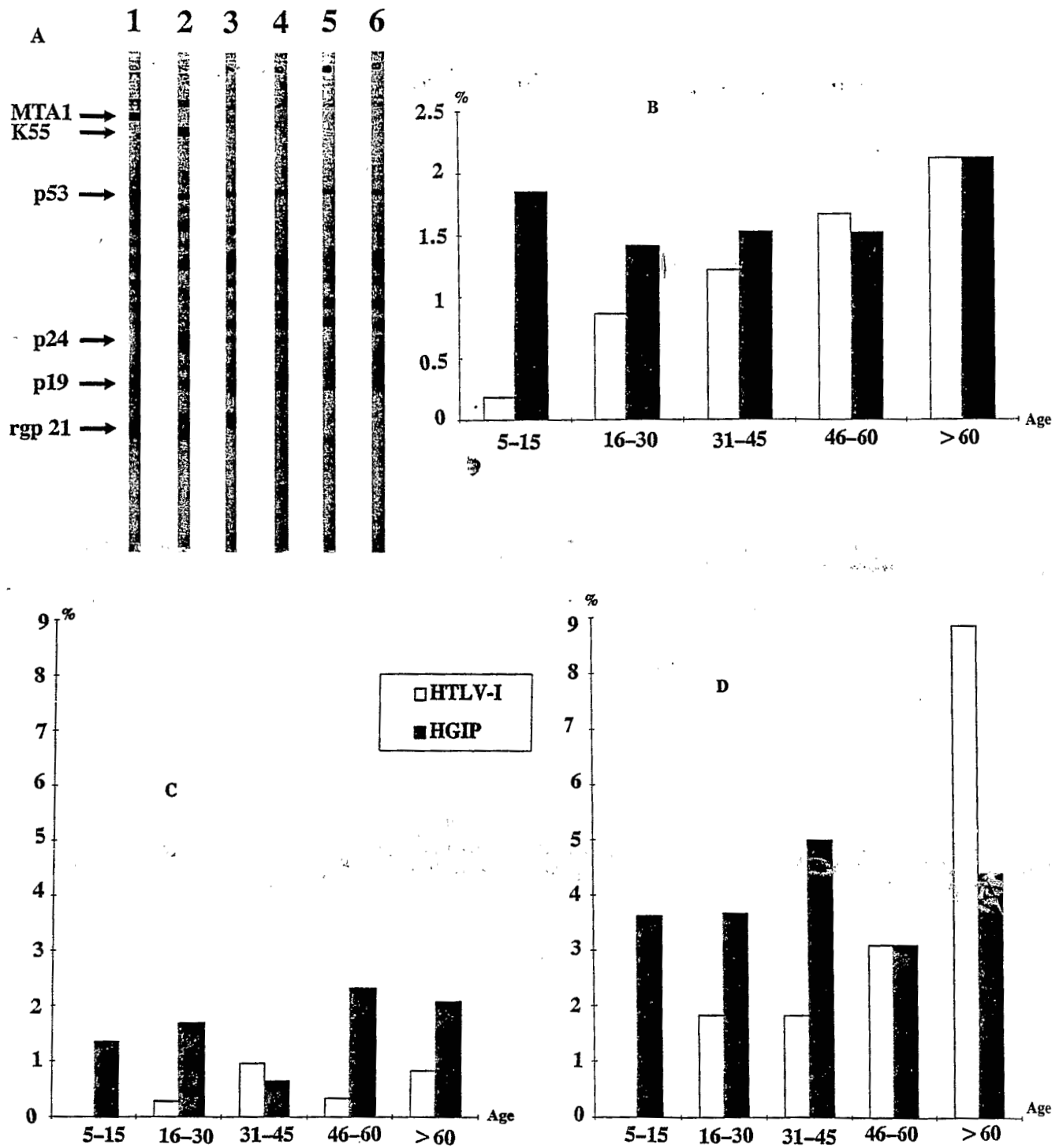
Figure 2. A, Western blot (WB) analysis using WB from Diagnostic Biotechnology (Singapore; HTLV blots 2–3). Lane 1, HTLV-I–positive control; lane 2, HTLV-II–positive control; lanes 3–6, sera from persons with HTLV-I Gag–indeterminate Western blot profile (HGIP). In lane 3, recombinant (r) gp21 band is slightly above control profile for rgp21 band and is not considered as specific rgp21 reactivity. Such band was seen in some HGIP seroreactivity. B, Seroprevalences of HTLV-I and HGIP according to age among 3783 sera from Cameroonian villagers living in representative locations. C, D, Comparison of seroprevalences of HTLV-I and HGIP according to age among 2263 sera from southern Cameroonian Bantous (C) and Pygmies (D).

was tested by Western blot after 2 years, and we did not notice any modification of their HGIP profiles.

Moreover, our data further suggest that environmental factor(s) present in the deep rain forest area of central Africa might be causally associated with the presence of HGIP reactivities in the sera of residents of such a tropical ecosystem. Hyperinfection by *Plasmodium falciparum* has been proposed as one of these possible factors, based both on some preliminary epidemiologic data from the Philippines [9] and also on biologic results using competitive-adsorption serologic assays [10]. These studies have shown partial amino acid homologies between an HTLV-I p19 epitope (PDSDPQI, aa residues 110–116) and a 7-aa sequence of the Exp-1 protein of the *P. falciparum* blood stage parasite [9, 10]. In this connection, the level of malarial infection is much higher in the southern than in the northern part of Cameroon. However, Bantous living in the same environment as the Pygmies much less frequently have the HGIP profile. Another hypothesis to be considered is that the HGIP might represent antibody response to specific epitopes (related to HTLV-I p19 Gag) of endogenous retroviral sequences [16], reactivated by prolonged stimulation by microbial antigens, including *Plasmodium*. Further work is in progress to confirm or disprove these hypotheses using samples from central African populations with varying levels of malarial endemicity (Mahieux et al., unpublished data).

The features regarding the real HTLV-I seroprevalence in Africa (~5–10 million infected inhabitants) with a north to south gradient have evolved over the past few years, mainly due to the changes of WB criteria for HTLV-I seropositivity [1, 3, 5]. The present study indicates that the HTLV-I seroprevalence in rural Cameroon is lower than that previously described for this country [7, 8]. This is due to two factors: The WB seropositivity criteria used here were more stringent than in some of the previous studies [7, 8], and there exist clusters of high HTLV-I prevalence within areas with an overall low seroprevalence. This feature is well illustrated in the southern Pygmy population, which has a 4-fold higher HTLV-I seroprevalence rate (2.05%) than the Bantou population (0.49%) living nearby. Such ethnic and geographic clustering could be related to either a founder effect followed by a persistence of a high transmission rate in a given population or to yet unknown cultural and ethnoenvironmental factors. Finally, the data presented here imply that HTLV-I serologic surveys performed in small, restricted populations may not be representative of the surrounding regions and highlight the difficulty of estimating real HTLV-I seroprevalence in the African continent.

References

1. Gessain A, Mahieux R, de Thé G. HTLV-I "indeterminate" Western blot patterns observed in sera from tropical regions: the situation revisited. J Acquir Immune Defic Syndr Hum Retrovirol 1995;9:316–9.
2. Biggar RJ, Gigase PL, Melbye M, et al. ELISA HTLV retrovirus antibody reactivity associated with malaria and immune complexes in healthy Africans. Lancet 1985;2:520–3.
3. Biggar RJ, Neequaye JE, Neequaye AR, et al. The prevalence of antibodies to the human T lymphotropic virus (HTLV) in Ghana, West Africa. AIDS Res Hum Retroviruses 1993;9:505–11.
4. Biggar RJ, Saxinger C, Gardiner C, et al. Type-I HTLV antibody in urban and rural Ghana, West Africa. Int J Cancer 1984;34:215–9.
5. Bonis J, Preux PM, Nzisabira L, et al. HTLV-I in Burundi (east Africa): lack of reactivity to the HTLV-I immunodominant envelope epitope. J Acquir Immune Defic Syndr 1994;7:1099–100.
6. De Thé G, Gessain A, Gazzolo L, et al. Comparative seroepidemiology of HTLV-I and HTLV-II in the French West Indies and some Africa countries. Cancer Res 1985;45:4633–6.
7. Delaporte E, Peeters M, Durand JP, et al. Seroepidemiological survey of HTLV-I infection among randomized populations of western central African countries. J Acquir Immune Defic Syndr 1989;2:410–3
8. Louis JP, Gardon J, Trebucq A, et al. Particularités épidémiologiques de l'infection rétrovirale à HTLV-I en Afrique Centrale. Bull Soc Pathol Exot 1993;86:163–8.
9. Hayes CG, Burans JP, Oberst RB. Antibodies to human T lymphotropic virus type I in a population from the Philippines: evidence for cross-reactivity with *Plasmodium falciparum*. J Infect Dis 1991; 163:257–62.
10. Lal RB, Rudolph D, Alpers MP, Sulzer AJ, Ya-Ping S, Lal AA. immunologic cross-reactivity between structural proteins of human T-cell lymphotropic virus type I and the blood stage of *Plasmodium falciparum*. Clin Diagn Lab Immunol 1994;1:5–10.
11. Lal RB, Rudolph DL, Coligan JE, Brodine SK, Roberts CR. Failure to detect evidence of human T-lymphotropic virus (HTLV) type I and type II in blood donors with isolated gag antibodies to HTLV-I/II. Blood 1992;80:544–50.
12. Lipka JJ, Young KK, Kwok SY, Reyes GR, Sninsky JJ, Foung SK. Significance of human T-lymphotropic virus type I indeterminant serological findings among healthy individuals. Vox Sang 1991;61:171–6.
13. Khabbaz R, Heinene W, Grindon A, Hartley TM, Shulman G, Kaplan J. Indeterminate HTLV serologic results in US blood donors: are they due to HTLV-I or HTLV-II? J Acquir Immune Defic Syndr 1992;5:400–4.
14. Nerurkar VR, Miller MA, Leon-Monzon ME, et al. Failure to isolate human T cell lymphotropic virus type I and to detect variant-specific genomic sequences by polymerase chain reaction in Melanesians with indeterminate Western immunoblot. J Gen Virol 1992;73:1805–10.
15. North ML, Lemaire JM, Couroucé AM, et groupe de travail "Rétrovirus" de la Société Française de Transfusion Sanguine, les tests de dépistage des anticorps anti-HTLV en 1995. Spectra Biologie 1995;95:47–53.
16. Banki K, Maceda J, Hurley E, et al. Human T-cell lymphotropic virus (HTLV)-related endogenous sequence, HRES-1, encodes a 28-kDa protein: a possible autoantigen for HTLV-I gag-reactive autoantibodies. Proc Natl Acad Sci USA 1992;89:1939–43.