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# Prevalence of Antibody to Human T Cell Leukemia Virus Type 1 (HTLV-1) in Populations of Ivory Coast, West Africa

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A large cross-sectional serologic survey for human T cell leukemia virus type 1 (HTLV-1) antibody was conducted in 3,177 Ivory Coast residents to evaluate the prevalence of HTLV-1 and to determine possible risk factors and correlates of HTLV-1 infection. Of the 3,177 serum samples, 110 (3.5%) were positive for antibody to HTLV-1 by indirect immunofluorescence assay and Western blot. The prevalence of HTLV-1 antibody in the general adult population was 1.8% and increased significantly with age. No difference between males (1.5%) and females (2%) was found. The highest prevalences were observed in female prostitutes (7.4%), patients with neurologic syndromes (5.8%), and lepers (13.7%). The high prevalence of HTLV-1 infection in prostitutes suggests that heterosexual contact is involved in the transmission of HTLV-1 and that prostitutes could play an important role in the spread of the virus in Africa. The high prevalence of HTLV-1 in patients with neurologic syndromes confirms the association between HTLV-1 and some type of neuropathies, as has been observed in the West Indies and Japan. The high prevalence observed in lepers deserves further investigation to find the cause of the association. Twenty-five individuals, including prostitutes, were coinfected with HTLV-1 and human immunodeficiency virus (HIV). Prospective studies are necessary to evaluate the exact role of HTLV-1 alone or in combination with HIV in inducing specific diseases.

Human T cell leukemia virus type 1 (HTLV-1) is a retrovirus that has been associated with two clinical entities, adult T cell leukemia lymphoma (ATL) and a neuromyelopathy called tropical spastic paraparesis (TSP) or HTLV-1-associated myelopathy (HAM) [1–4]. Both diseases occur in southwestern Japan and the Caribbean basin. Recent studies suggest that HTLV-1 infection may also be prevalent in parts of Africa. The HTLV-1 antibody prevalence has ranged from 0 to 9% in studies of African blood donors and has been as high as 30% in several risk groups in tropical Africa [5–14]. However, most of the preliminary investigations in Africa included heterogeneous populations, and laboratory studies were done on stored blood samples. Positive reactions by screening procedures were not followed by confirmatory assays in most of these studies, which renders the conclusions of limited value. We conducted a seroepidemiologic study of HTLV-1 infection in a country of West Africa, Ivory Coast, using only recently collected serum samples and obtaining a confirmatory assay for every sample that scored positive or equivocal in the screening assay.

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## Subjects and Methods

### *Subjects*

We recruited 3,177 study participants between January 1986 and July 1987. Three different populations were included: groups representative of the general population, groups characterized by sexual promiscuity, and patients with specific clinical diseases or syndromes (table 1).

The adult groups representative of the general population that we enrolled were 513 pregnant women (456 from Abidjan, 35 from area B, and 22 from area C; figure 1), 155 students (125 men, 30 women) from Abidjan consulting at the University medical service for a regular check-up, 99 healthy adults (37

**Table 1.** Prevalences for antibodies to HTLV-1 and HIV in studied population groups.

| Groups | Subgroup area | Mean age, y | Gender ratio, M:F | No. tested | No. (%) positive for antibody to HIV-1 | HIV-2 | HIV-1 and HIV-2 | HTLV-1 | HTLV-1 and HIV |
|---|---|---|---|---|---|---|---|---|---|
| Neonates | Abidjan | 0 | ND | 188 | 3 (1.6) | 4 (2.1) | 0 | 3 (1.6) | 1 (0.5) |
| Children | Areas A and C | 9 | 1.2 | 176 | 1 (0.6) | 0 | 0 | 2 (1.1) | 0 |
| Groups representative of the general population | | | | | | | | | |
| Pregnant women | Areas A–C | 25 | F | 513 | 11 (2.1) | 5 (0.9) | 0 | 10 (1.9) | 1 (0.2) |
| Students | Abidjan | 23 | 4.2 | 155 | 2 (1.3) | 1 (0.6) | 1 (0.6) | 1 (0.6) | 0 |
| Adult populations | Six groups | 32 | 0.9 | 312 | 7 (2.2) | 4 (1.3) | 1 (0.3) | 7 (2.2) | 0 |
| Hotel staff | Area A | 33 | M | 311 | 27 (8.7) | 5 (1.6) | 2 (0.6) | 5 (1.6) | 1 (0.3) |
| Groups characterized by sexual promiscuity | | | | | | | | | |
| Prostitutes | Areas A and B | 26 | F | 390 | 119 (30.5) | 99 (25.4) | 69 (17.7) | 29 (7.4) | 15 (3.8) |
| STD clinic patients | Abidjan | 21 | 9.0 | 50 | 0 | 3 (6.0) | 0 | 1 (2) | 0 |
| Prisoners | Abidjan, area A | 31 | 6.7 | 270 | 16 (5.9) | 33 (12.2) | 1 (0.3) | 9 (3.3) | 4 (1.5) |
| Patient groups | | | | | | | | | |
| Psychiatric | Abidjan | 32 | 2.7 | 96 | 5 (5.2) | 1 (1.0) | 0 | 5 (5.2) | 0 |
| Neurologic | Abidjan | ND | 2.9 | 156 | 11 (7.0) | 7 (4.5) | 4 (2.6) | 9 (5.8) | 3 (1.9) |
| Diabetes | Areas A and B | 50 | 2.5 | 163 | 7 (4.3) | 2 (1.2) | 0 | 3 (1.8) | 0 |
| Sickle cell disease | Abidjan | 17 | 0.9 | 52 | 5 (9.6) | 5 (9.6) | 3 (5.8) | 2 (3.8) | 0 |
| Tuberculosis | Abidjan | 36 | 3.1 | 41 | 4 (9.7) | 2 (4.8) | 0 | 1 (2.4) | 1 (2.4) |
| AIDS-like disease | Abidjan | 38 | 3.0 | 195 | 63 (32.3) | 50 (25.6) | 26 (13.3) | 8 (4.1) | 1 (0.5) |
| Leprosy | Area A | 45 | 1.1 | 109 | 0 | 2 (1.8) | 0 | 15 (13.7) | 0 |
| Total | | | | 3,177 | 281 (8.8) | 223 (7) | 107 (3.4) | 110 (3.5) | 27 (0.8) |

NOTE. HTLV-1 = human T cell leukemia virus type 1, HIV = human immunodeficiency virus, ND = not done. STD = sexually transmitted disease, F = female, M = male. Individuals with combined HIV-1 and -2 infection are included in the numbers for HIV-1 antibody and HIV-2 antibody and in those with combined HTLV-1 and HIV infection.

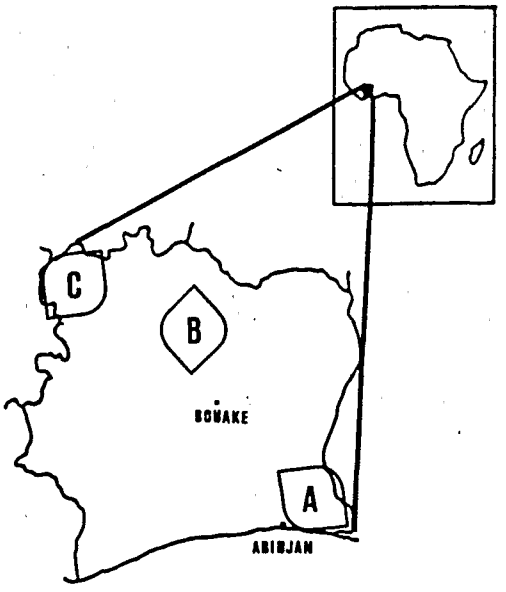


**Figure 1.** Location of Ivory Coast and study areas: Area A, southeast, near Ghana. Area B, north. Area C, northwest, near Guinea.

men, 62 women) recruited from areas A and C for toxoplasma serology, 144 adults (60 men, 84 women) on the staff of the Abidjan prison, 41 adults (26 men, 15 women) on the medical staff of the Abidjan hospitals, and 28 men on the staff of the zoos of Abidjan and Bouake. Two groups of children (126 from area A and 50 from area C; 95 boys, 81 girls) consulting at the scholar medicine service for a regular checkup were also included, as well as 188 neonates from Abidjan. We also took samples from 311 men on the staff of two tourist hotels in area A.

The groups characterized by sexual promiscuity that we enrolled were 390 female prostitutes (105 from Abidjan, 163 from area A, and 122 from area B), 270 prisoners (235 men, 35 women), and 50 patients (45 men, 5 women) presenting with a sexually transmitted disease (STD, mainly syphilis or gonorrhea) at an STD clinic in Abidjan. Among the Abidjan prostitutes, three subgroups were distinguished and were numbered 1–3 with increasing socioeconomic level as determined by the price for sexual intercourse: Subgroup 1 charged each customer the

equivalent of US$0.50; subgroup 2, US$3; and subgroup 3, US$15–$30 [15]. We did not obtain information about the mean number of sexual partners per subgroup. Among the prisoners, 193 were from the prison of Abidjan and 77 were from a prison in area A.

The groups of patients with specific diseases included 52 multitransfused patients with sickle cell anemia from Abidjan, 163 adult diabetics (131 from Abidjan and 32 from area B), 96 psychiatric patients from Abidjan, 41 patients from area A undergoing ambulatory treatment for tuberculosis, 109 patients from area A with leprosy, and 156 patients from Abidjan with neurologic disease (including different neuromyelopathies as well as TSP). In addition, we enrolled 195 inpatients from Abidjan hospitals with an AIDS-like illness, as defined by a severe respiratory infection associated with tuberculosis (87% of cases), weight loss >10% of body weight (80%), profound asthenia (83%), or persistent diarrhea (26%).

## *Methods*

Blood samples were centrifuged to separate the serum. Serum samples were quickly frozen at −20°C and stored until used.

*Serologic assays for antibody to HTLV-I.* For indirect immunofluorescence assay (IFA), HuT 102 clone B2 cells were propagated in RPMI 1640 medium supplemented with 20% inactivated fetal calf serum and containing 1% of an antibiotic-antimicotic mixture (GIBCO/BRL, Grand Island, NY). Medium was changed every 3 d, and the cells were subcultured at a split ratio (1:2).

To prepare IFA smears, the HuT 102 cells were harvested during the exponential growth phase and centrifuged at 400 *g* for 10 min. The pelleted cells were resuspended in phosphate-buffered saline (PBS) to yield a final density of $\sim 2 \times 10^6$ cells/ml. Aliquots (10 µl) of this suspension were loaded in 10-well glass slides (Wiener, Saint-Etienne, France) and air dried. The dried cells were then fixed in acetone at −20°C for 10 min and stored frozen.

For IFA, 20 µl of a 1:10 dilution in PBS of test serum was incubated with the cells for 45 min at 37°C in a humid atmosphere and rinsed twice in PBS. The staining was performed by adding 20 µl of a 1:200 dilution of fluorescein isothiocyanate–conjugated goat antibody to human IgG (Fluoline G; BioMerieux, Marcy l'Etoile, France). After incubation at 37°C for 30 min, the slides were rinsed twice with PBS and examined with an Olympus (BH2) fluorescent microscope. Figure 2 shows the typical pattern observed by IFA.

Serum with a positive or equivocal result by IFA was further analyzed for confirmation by Western blotting. We used a previously described procedure [2] with minor modifications. Briefly, 200 µl of a HuT 102 clone B2 cell lysate (corresponding to $10^7$

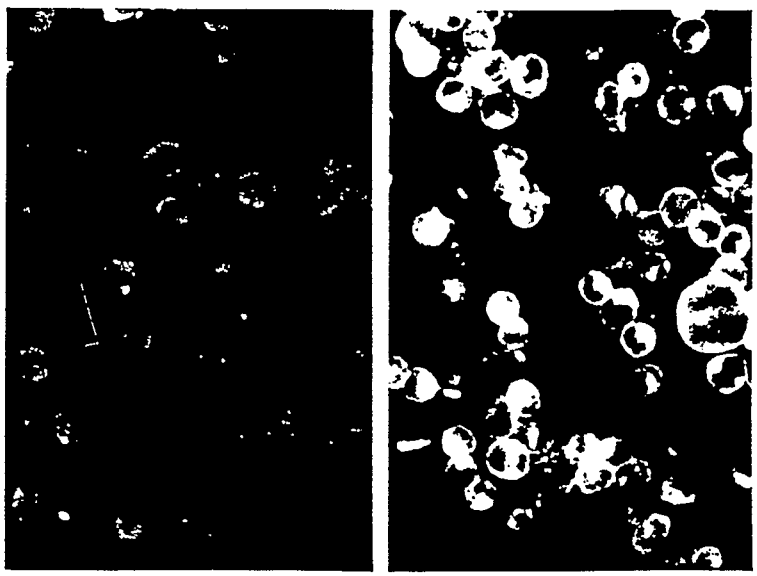

**Figure 2.** Representative negative *(A)* and positive *(B)* patterns of immunofluorescence assay with HuT 102 fixed cells.

cells) or 200 µl of a virus pellet prepared as described [15] from 60 ml of a 24-h supernatant from HuT 102 cells ($3 \times 10^6$ cells/ml) was mixed with 200 µl of twofold-concentrated sample buffer. These mixtures were fractionated by electrophoresis on 10% polyacrylamide slab gel in the presence of sodium dodecyl sulfate according to the procedure of Laemmli [16].

After electrophoresis, the gels were washed for 30 min in three changes of transfer buffer (10 m*M* Tris-HCl [pH 7.0], 2 m*M* EDTA, 50 m*M* NaCl). The proteins in the gels were then passively transferred to nitrocellulose by incubating a nitrocellulose-gel "sandwich" for 36 h at room temperature. The nitrocellulose sheets were incubated at 37°C for 1 h with 10% nonfat dry milk in 10 m*M* sodium phosphate buffer (pH 7.4) containing 150 m*M* NaCl (PBS) and were cut into 0.3-cm strips. After three washings in PBS containing 1% Tween 20 (PBS-T), each strip was incubated overnight at room temperature with 3 ml of a 1:100 dilution of test serum in PBS supplemented with 5% nonfat dry milk in PBS-T. The strips were washed three times with PBS-T.

Affinity-purified and biotin-labeled sheep antibodies to human IgG (dilution 1:400 in PBS-T; Amersham International PLC, Amersham, UK) were added to the strips, and they were incubated at 37°C for 2 h. After the strips were washed three more times with PBS-T, a 1:400 dilution of preformed streptavidin–biotinylated horseradish peroxidase complex (Amersham) in PBS-T was added to the strips, and they were incubated at room temperature for 30 min. After three more washings, the strips' peroxidase activity was detected colorimetrically by the addition of a fresh solution of diaminobenzidine (50 mg in 100 ml of PBS, supplemented with 0.01% hydrogen peroxide).

The main HTLV-1 antigens detected by positive serum samples included the *gag*-encoded proteins p19, p24, and p[illegible] and the *env*-encoded glycoproteins gp61 (for the cell lysate) and gp46 (for the virus pellet). Samples were considered positive for antibody to HTLV-1 if they reacted with at least the envelope protein and one of two major *gag* proteins, p24 and p19. Figure 3 shows representative Western blots using a virus pellet as antigen.

*Other assays.* Every serum sample was also tested for the presence of antibody to human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2) by procedures already described [15]. The prevalence of antibody to these viruses in Ivory Coast has been published [15, 17]. All the samples were also tested for antibody to *Treponema pallidum* by using a hemagglutination assay (SeraTek MHATP; Miles, Fujirebio, Tokyo) and antibody to *Chlamydia trachomatis* by immunofluorescence assay (*C. trachomatis*-spot IF, BioMerieux). Serum was considered positive for antibody to *C. trachomatis* when it reacted at dilutions of 1:16 for men and 1:64 for women [18] and positive for antibody to *T. pallidum* when the antibody titer was ≥1:80 [15].

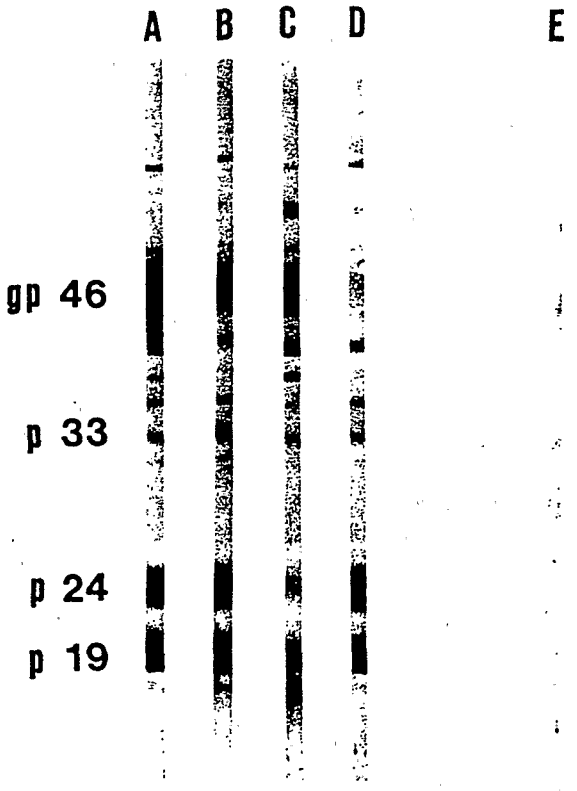


**Figure 3.** Representative Western blots with HTLV-1 antigens. Lanes A–D: positive samples. Lane E: negative sample.

*Statistical tests.* The $\chi^2$ test was used for comparison of proportions between groups. Logistic regression analysis was used to determine the possible correlation between HTLV-1 and STD agents.

## Results

Of the 3,177 samples, 110 (3.5%) were positive for antibody to HTLV-1 (table 1). Among the 1,291 individuals representing the general adult population, 23 (1.8%) were positive for antibody to HTLV-1. There was no significant difference between preva-

Table 2. Prevalences of HTLV-I and sexually transmitted agents in two groups of women.

| Groups | No. tested | Mean age, y | No. (%) positive for antibody to *Treponema pallidum* | *Chlamydia trachomatis* | HIV | HTLV-I |
|---|---|---|---|---|---|---|
| Pregnant women | | | | | | |
| Abidjan | 456 | 25 | 35 (7.7) | 97 (21.3) | 15 (3.3) | 9 (1.9) |
| Area B | 35 | 25 | 2 (5.7) | 7 (20.0) | 1 (2.8) | 0 |
| Area C | 22 | 26 | 2 (9.0) | 2 (9.0) | 0 | 1 (4.5) |
| Total | 513 | 25 | 39 (7.6) | 106 (20.7) | 16 (3.1) | 10 (1.9) |
| Prostitutes | | | | | | |
| Abidjan | | | | | | |
| Subgroup 1 | 42 | 27 | 31 (73.8) | 33 (78.6) | 25 (59.5) | 7 (16.7) |
| Subgroup 2 | 45 | 28 | 11 (24.4) | 22 (49.0) | 11 (24.4) | 4 (8.9) |
| Subgroup 3 | 18 | 23 | 0 | 6 (33.0) | 2 (11.1) | 0 |
| Area A | 163 | 27 | 84 (51.5) | 99 (60.7) | 80 (49.0) | 11 (6.7) |
| Area B | 122 | 26 | 66 (54.0) | 93 (76.2) | 31 (25.4) | 7 (5.7) |
| Total | 390 | 26 | 192 (49.2) | 253 (64.9) | 149 (38.2) | 29 (7.4) |

NOTE. HTLV-I = human T cell leukemia virus type I, HIV = human immunodeficiency virus.

lences observed within the different groups. The rate of seropositivity was similar in pregnant women from Abidjan (1.5%) or from areas B and C (1.7%). In this adult population, the prevalence of antibody to HTLV-I was similar in males (1.5%) and females (2%). Five (1.4%) of 364 children were positive for antibody to HTLV-I.

Nine (3.3%) of 270 prisoners and one (2%) of 50 patients with STD were positive for antibody to HTLV-I. These prevalences were not statistically different from that observed in the general adult population. Antibody to HTLV-I was detected in 29 (7.4%) of 390 prostitutes. This prevalence was significantly higher than that of the general adult population ($P < .001$). Among the prostitutes living in Abidjan, the rate of HTLV-I seropositivity was inversely proportional to socioeconomic level (table 2). The prevalence was 16.7% in subgroup 1, 8.9% in subgroup 2, and 0 in subgroup 3. However, these differences were not statistically significant ($\chi^2$ test). In prostitutes and pregnant women, there was a correlation between the prevalence of antibody to HTLV-I and the prevalence of antibody to the sexually transmitted agents HIV-1, HIV-2, *T. pallidum*, and *C. trachomatis* (table 3).

The rate of HTLV-I seropositivity was significantly higher in patients with certain diseases compared with the general adult population (table 1). Among these patients, the highest HTLV-I seroprevalences were observed with neurologic disease (5.8%, $P < .01$) and leprosy (13.7%, $P < .001$).

The prevalence of antibody to HTLV-I increased with age (figure 4). The HTLV-I seroprevalence was 1.5% at age <20 y, 3.5% at 20–40 y ($P < .05$), and 8.8% at >40 y ($P < .001$). There was no difference in HTLV-I seroprevalence according to gender.

Twenty-seven individuals, mainly prostitutes, prisoners, and neurologic patients (respectively, 15, 4, and 3 individuals) were coinfected with HTLV-I and HIV-1 or -2 (table 1).

## Discussion

This study was performed using only recently collected serum samples in Ivory Coast, so the results are representative of the present HTLV-I infection rate in this country. Every sample that was positive

Table 3. Correlation between prevalences of antibodies to HTLV-I and other sexually transmitted disease (STD) agents.

| | HIV | *Treponema pallidum* | *Chlamydia trachomatis* |
|---|---|---|---|
| HTLV-I | 0.8172 | 0.8854 | 0.9075 |
| | $P < .02$ | $P < .01$ | $P < .01$ |
| HIV | | 0.9193 | 0.7301 |
| | | $P < .01$ | $P < .05$ |
| *T. pallidum* | | | 0.9010 |
| | | | $P < .01$ |

NOTE. Numbers indicate correlation coefficient according to logistic regression analysis. This value is done for STD agents studied in pregnant women and prostitutes. HTLV-I = human T cell leukemia virus type 1, HIV = human immunodeficiency virus.

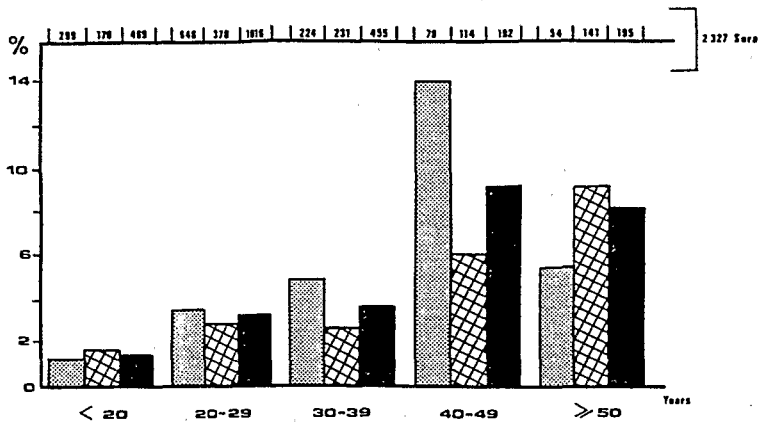


**Figure 4.** HTLV-I seroprevalence according to age for women (▩), men (▨), and both (▨).

or equivocal by immunofluorescence was systematically tested by Western blot using a crude HuT 102 cell lysate or a virus pellet. The criteria for positivity for antibody to HTLV-I by Western blot included a positive reaction with the envelope glycoproteins gp61 or gp46 [19] and at least one of the major *gag* proteins p24 and p19. More recent studies indicate that the samples we considered positive for antibody to HTLV-I were also confirmed by a newly developed enzyme-linked immunosorbent assay and radioimmunoprecipitation assay [20].

This study demonstrated a relatively high prevalence of antibody to HTLV-I in Ivory Coast. Of the adults representing the general population, 1.8% were positive for antibody to HTLV-I. This rate is similar to the seroprevalence observed in comparable African populations when serologic criteria were used in confirmatory assays. For example, in 1983–1984 Hunsmann et al. [10] found HTLV-I antibody rates of 3.9%, 1.7%, 1.6%, 2.6%, 1.2% and 3.2% in Gabon, Kenya, Liberia, Nigeria, Senegal, and Zaire, respectively. Similarly Fleming et al. [9] found a prevalence of antibody to HTLV-I of 2.0% in Nigerian blood donors in 1986.

As was previously observed in endemic areas such as Japan [21] and the West Indies [4], the HTLV-I seropositivity rate increases with age in Ivory Coast. Concerning seroprevalence according to gender, the difference was not significant in the general adult population (1.5% for males, 2% for females). Similar results were found by Biggar et al. [5] in Ghana: 4.1% for males, 3.4% for females. The African results differ from those observed in Japan, where the prevalence is higher in females (18.5%) than in males (12.2%) [21].

Prostitutes had a higher prevalence of antibody to HTLV-I than did the general adult population. This higher seroprevalence in prostitutes and the correlation between exposure to HTLV-I and exposure to sexually transmitted agents (*T. pallidum*, *C. trachomatis*, HIV-1, and HIV-2) suggests that heterosexual contact is involved in the transmission of HTLV-I in Africa. Prostitutes may then be an important factor in spread of HTLV-I in Africa as they are for spread of HIV [15].

Several groups of patients had significantly higher prevalences of antibody to HTLV-I than the adult population. However, it remains to be demonstrated whether this higher HTLV-I prevalence is pathogenically linked to the diseases studied. Since it has been shown that HTLV-I is associated with neurologic disease (TSP or HAM) in the West Indies and Japan, it may be suggested that the high HTLV-I seroprevalence observed in Ivory Coast patients with neuromyelopathy is a causal association. However, another study conducted on tropical neuromyelopathies from Abidjan [22] showed that HTLV-I seemed to play a limited pathogenic role (1 positive case out of 29), suggesting that development of neuromyelopathies in Africa is probably fostered by nutritional and toxicologic problems.

The highest prevalence of antibody to HTLV-I was observed in a group of lepers living in a single village. Since these clustered patients received frequent therapeutic injections, it may be that this high prevalence was due to nosocomial transmission. However, other groups of patients characterized by frequent injections (tuberculosis patients) or transfusions (sickle cell disease patients) had much lower rates of HTLV-I infection. In addition, the leprosy patients had a low rate of HIV infection, in contrast to the tuberculosis and sickle cell disease groups. Prelimi-

nary data from other groups of lepers in Ivory Coast and other African countries, have confirmed this observation of high HTLV-1 prevalence (unpublished data).

Of 3,177 participants, 27 (0.8%) showed serologic evidence for coinfection with HIV-1 or -2 and HTLV-1. Rare cases of dual infection have been previously reported in African patients [23, 24]. It has been recently shown that HIV-1 production from the peripheral blood leukocytes is increased after in vitro HTLV-1-induced mitogenic stimulation [25]. Patients coinfected with both viruses might thus produce more HIV-1 than those not infected with HTLV-1. Bartholomew et al. [26], in Trinidad, described a higher prevalence of AIDS in homosexual men coinfected by HTLV-1 and HIV than in those infected by HIV alone. They suggested that the first virus acquired (probably HTLV-1) enhanced the pathogenicity of the second (HIV).

Prospective studies are now necessary in Ivory Coast to clarify the exact role of HTLV-1 in inducing specific diseases (adult T cell leukemia, non-Hodgkin lymphomas, neuromyelopathies) and the clinical and biologic events occurring in those coinfected with HIV and HTLV-1.

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