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Clinical, Hematologic, and Immunologic Cross-Sectional Evaluation of Individuals Exposed to Human Immunodeficiency Virus Type-2 (HIV-2)

RICHARD G. MARLINK,[1,2] DOMINIQUE RICARD,[1,3] SOULEYMANE M'BOUP,[3] PHYLLIS J. KANKI,[1] JEAN-LOUP ROMET-LEMONNE,[1] IBRA N'DOYE,[4] KHADY DIOP,[4] MARY ANN SIMPSON,[2] FRANK GRECO,[2] MIN-JI CHOU,[1] VICTOR DEGRUTTOLA,[5] CHUNG-CHENG HSIEH,[6] CHEIKH BOYE,[3] FRANCIS BARIN,[7] FRANÇOIS DENIS,[8] M.F. McLANE,[1] and M. ESSEX[1]

ABSTRACT

We studied the clinical status and certain hematologic and immunologic parameters in healthy prostitutes from Dakar, Senegal who were seropositive for antibodies to human immunodeficiency virus type-2 (HIV-2). Generalized lymphadenopathy and clinical signs or symptoms similar to those which are seen with human immunodeficiency virus type-1 (HIV-1) infection were not present. Comparison to seronegative prostitutes and minor surgery control patients were made and significant elevations were seen in T8 lymphocytes ($p = .03$), IgG ($p = .0001$), and β_2-microglobulin ($p = .03$). The mean T4 lymphocyte count in seropositive prostitutes was lower than in seronegative prostitutes (757 vs. 1179, $p = .15$), but this difference was not statistically significant and appeared to be correlated with age. No significant differences were noted between the seronegative and seropositive prostitutes in lymphocyte stimulation studies to certain mitogens. Antilymphocyte antibodies above background were not present in either population.

We conclude that HIV-2 is a sexually transmitted agent that produces immunologic alterations consistent with a persistent viral infection. HIV-2 seropositive prostitutes studied to date do not show clinical signs of immune suppression, as has been described with HIV-1 infection. The pathogenic potential of HIV-2 appears to differ from that of HIV-1, the etiologic agent of the AIDS pandemic.

[1]Department of Cancer Biology, Harvard School of Public Health; [2]New England Deaconess Hospital, Harvard Medical School, Boston, MA.
[3]Faculty of Medicine and Pharmacy, University of Dakar; [4]National Center for Sexually Transmitted Diseases, Dakar, Senegal.
[5]Department of Biostatistics; [6]Department of Epidemiology, Harvard School of Public Health, Boston, MA.
[7]Virology Laboratory, CHRU Bretonneau and UER Pharmaceutical Sciences, Tours, France.
[8]Department of Bacteriology and Virology, CHRU Dupuytren, Limoges, France.

INTRODUCTION

A NEW CLASS of human T-lymphotropic retroviruses has recently been described.[1,2] Serologic evidence for this new class of retroviruses was initially found in healthy prostitutes residing in Dakar, Senegal. The first viral isolate identified was designated human T-lymphotropic virus type-4 (HTLV-4) and other isolates from West African individuals have included LAV-2[3] and SBL-6669.[4] All these isolates seem to vary slightly from each other,[4] but are serologically highly cross reactive with the simian T-lymphotropic virus type-3 (STLV-3) and share less cross reactivity with the human immunodeficiency virus (HIV or HIV-1), especially in the envelope proteins of these viruses.[1-5] Since all of these viral isolates have been shown *in vitro* to have similar T4 tropism, morphology, and major viral proteins to the HIV class of viruses,[2] and since several isolates have been from individuals suffering from an immunodeficiency state,[3,4] the name human immunodeficiency virus type-2 (HIV-2) has been proposed for this new class of retroviruses,[6,7] with HIV-1 to be used to describe the various isolates constituting the class of virus causing the worldwide AIDS epidemic. For our studies, we will presently designate the STLV-3 "cross-reactive" human viral isolates as HIV-2, to encompass this proposed terminology.

Since February 1985, we have studied the clinical, epidemiologic, and serologic status of over 300 prostitutes in the Dakar area who attend an outpatient clinic. Approximately 5% (15/289) of these prostitutes initially had serologic evidence for exposure to an STLV-3–like virus.[1] Since the prostitutes attending the clinic appeared to be in the same general state of health, whether seropositive or seronegative to this new virus, we examined various clinical, hematologic, and immunologic parameters in detail in 18 of the seropositive prostitutes available for analysis in March 1986. Comparisons to prostitutes seronegative to HIV-2 and to nonprostitute minor surgery patients from Dakar were performed at the same time. Our findings are outlined herein and provide a baseline for the continued analysis of the health status of HIV-2 infected individuals in West Africa.

MATERIALS AND METHODS

All of the 289 female prostitutes attending an outpatient clinic in Dakar, Senegal in February 1985 provided serum samples. With Western blot analysis of the HIV-1 ELISA positive samples, 14 sera were found to have antibodies directed to HIV-2 alone. One prostitute was shown to have antibodies consistent with exposure to both HIV-2 and HIV-1 and one had antibodies only to HIV-1. All of the 15 HIV-2 seropositive prostitutes from the initial serum collection were available for this cross-sectional survey of clinical and hematologic parameters in March 1986. With serologic analysis of the HIV-1 ELISA negative samples in the original sampling, three more HIV-2 seropositive prostitutes were identified through December 1986. These additional seropositive prostitutes are included in the epidemiologic and clinical evaluation of the population since they have also attended the clinic since February 1985; but they are excluded from the immunologic determinations since their serologic status was identified after the hematologic evaluation. A control group of 30 seronegative prostitutes who have also been attending the clinic since February 1985 were selected at random and 14 were willing to participate in March 1986. Older nonprostitute seronegative patients, hospitalized for minor surgical procedures, were also studied in March 1986 for hematologic and immunologic comparisons.

Epidemiological and medical evaluation

All prostitutes studied have been registered in a general medicine and gynecology clinic in Dakar which maintains ongoing clinical records for each patient. Each seropositive prostitute entered in the present evaluation has been followed with clinical examinations every 3 to 6 months. Baseline information obtained has included past and present medical problems, previous infectious diseases, place of origin and residence, travel history, family history, information concerning past immunizations, scarifications, medical injections, blood transfusions, and hospitalizations. A history of sexually transmitted diseases (STDs) was gathered from clinic records, since by law the prostitutes are required to see a clinic nurse once a month to receive a cervical smear and treatment for any active STD. A sexual practice history was obtained

which included the number of years practicing prostitution, the number of sexual partners and frequency of sexual contact, as well as the type of sexual practices. A review of systems and complete physical examination was performed on each prostitute by two medical doctors on our research team. Ultrasonographic examination of the retroperitoneal area, the pelvic cavity, the liver, and spleen was performed on all patients. Complete blood counts and baseline serum studies were obtained, in addition to heparinized blood for white blood cell subtyping and functional assays in March 1986.

Serologic evaluation

All serum samples were evaluated for the presence of antibodies to HIV-2, HIV-1, and human T-lymphotropic virus type I (HTLV-I) by Western blot and/or radioimmunoprecipitation and sodium dodecyl sulfate polyacrylamide gel electrophoresis (RIP/SDS-PAGE) techniques. These previously described techniques[1,2] utilized either an HTLV-3B infected Molt-3 cell line or an HIV-2 infected Hut-78 cell line (HTLV-4-PK289) for HIV-1 antigen or HIV-2 antigen, respectively. All seropositive sera were also evaluated by Western blot to other HIV-2 isolates, LAV-2 and SBL-6669. Western blot techniques or RIP/SDS-PAGE procedures[8] were performed on the sera in each cohort utilizing an HTLV-I-infected cell line, C5/MJ, as antigen for determination of exposure to HTLV-I.

In addition, sera from both the seropositive prostitutes as well as the control subjects were evaluated for antibodies to various endemic and/or sexually transmitted infectious agents. These agents included: Epstein-Barr virus (EBV) using the viral capsid antigen with antibody titers determined by indirect fluorescence of serial dilutions of serum (Electro-Nucleonics, Inc., Columbia, MD); cytomegalovirus (CMV) using the enzyme-linked immunosorbant assay (EIA); and hepatitis-B virus (HBV) using anti-HBc (hepatitis-B core antibody) and anti-HBs (hepatitis-B surface antibody) evaluations with serial serum dilutions in the EIA system (Abbott Diagnostics, North Chicago, IL). Syphilis serology including both a Rapid Plasma Reagin (RPR) and a fluorescent treponemal antibody absorption assay (FTA-Abs.) was performed according to the standard method of the Centers for Disease Control. Evidence for exposure to herpes simplex virus-type 1 and 2 (HSV-1 and HSV-2) was evaluated with an immunodot assay using the glycoprotein gg-2 from HSV-1 or HSV-2[9] (assay kindly performed by Drs. F. Lee and A.J. Nahmias, Emory University).

Immunologic evaluation

The ability to develop delayed type hypersensitivity reactions was assessed by intradermal skin testing with a panel of four microbial antigens which included candida, mumps, trychophyton, and tuberculin (purified protein derivative). A positive reaction was judged to be an area of definite induration greater than 10 mm at 48 h after intradermal injection. Minimal positive reactivity was defined as an area of definite induration less than 10 mm but greater than 5 mm at 48 h.

Analysis of T-lymphocyte subpopulations was accomplished by separating the mononuclear cells from a simultaneous sampling of heparinized venous blood via centrifugation on a Ficoll-Hypaque gradient.[10] Within 24 h of the sampling, the T-lymphocyte phenotype was determined by indirect immunofluorescence with fluorescence-activated cell-sorter (FACS) analysis, using commercially available monoclonal antibodies; Leu-4 for T-cells, Leu-3a for T4-helper/inducer T-cells, Leu-2a for T8-suppressor/cytotoxic T-cells, and Leu-16 for B-cells (Beckton-Dickinson, Mountian View, CA).

Serum immunoglobulin levels for IgG, IgA, and IgM were determined by a nephelometric method[11] on a Multistat III (Instrumentation Laboratory, Spokane, WA). Clonality of immunoglobulin determinations was assessed by serum protein electrophoresis (SPEP) carried out in an agarose gel system (Corning Medical, Pal Alto, CA). Antinuclear antibody screens were performed on human epithelial cells (HEp-2) (Kallestad, Austin, TX). Serum β_2-microglobulin determinations were made by the Phadebas radioimmunoassay method (Pharmacia Diagnostics, Inc. Piscataway, NJ). Circulating immune complexes were determined by the C1q assay method as previously described[12] on freshly frozen serum.

Complement-dependent lymphocytotoxicity was assessed by incubating 10 μl of patient serum with approximately 20,000 peripheral blood lymphocytes from a panel of normal donors of known HLA-type for 60 min at 4°C. The cells were then incubated with rabbit serum complement (Calbiochem, San Diego,

CA) at 25°C and cell viability assessed at 30 min by trypan blue exclusion. Background and HLA-dependent cytotoxicity was determined with known reactivity of various reference human sera with rabbit complement.

Lymphocyte activation *in vitro* was studied measuring the individual's lymphocyte proliferative response by incorporation of [^{3}H]-tridium in response to mitogen stimulation as previously described.[13] Simultaneous cultures were done in triplicate for all samples and mitogens included phytohemagglutinin (PHA) (Difco Labs, Detroit, MI) and Concanavalin A (ConA) (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) in dilutions of 5, 10, and 15 μg/ml. Results of the proliferation assays were evaluated at the dilution which achieved the maximum cpm value and recorded as the mean of the triplicate values in cpm at that dilution. Proliferative responses were then "normalized"[14] by expressing the values as percentages of the mean responses of lymphocytes from the surgical control donors from Dakar.

Statistical analysis

Statistical tests of significance for historical and epidemiologic parameters utilized the χ^2 or Student's *t*-test. Multiple linear regression analysis was used to examine the effect of age, seropositivity status, history of prostitution, and number of sexual partners on the various hematologic measurements. Age was included in the multivariate regression model both as continuous and as a categorical variable. In the latter case two categories of age in years were considered: <45 and ≥ 45. In order to improve the fit of regression model for T-cell subsets, the analysis was performed on the logarithm of the count.

RESULTS

Several of the epidemiological and historical parameters of the study populations are shown in Table 1. The one HIV-2 seropositive prostitute who also displayed a classical serologic profile to HIV-1, as well as the one HIV-2 seronegative prostitute who was seropositive for HIV-1 alone, were subsequently excluded from the statistical analysis (displayed in Table 3).

Clinical evaluation

Certain clinical signs and symptoms indicative of immune suppression have been extensively outlined in relation to HIV-1 infection in Africa.[15,16] These signs and symptoms were the focus of our data gathering in

TABLE 1. HISTORICAL PARAMETERS

	HIV-2 seropositive prostitutes (n = 18)	*HIV-2 seronegative prostitutes (n = 14)*
Mean Age	45	34
Resided last 10 years in Senegal	16/18	8/14
Estimated lifetime sexual contacts*	6002 (p = .0001)	2332
mean number of years practicing prostitution	6.8	4.2
mean number of partners per week	19.8	15.7
History of:		
Immunizations, incl. BCG	14/18	13/14
Numerous medical injections	15/18	9/12
Scarification practices	13/18	11/14
Transfusions	1/18	1/14
Previous surgeries	2/18	2/14

*Estimated frequency of sexual contacts per week × total number of weeks practicing prostitution during prostitute's lifetime; mean value for group.

the clinic setting. Both HIV-2 seropositive and seronegative prostitutes gave no history of neoplasms or unusual infections, no history of chronic constitutional symptoms or significant weight loss, and no history of chronic diarrhea or fevers. By physical examination, both groups had only minimal inguinal adenopathy (defined as lymph nodes ≤1 cm in diameter) with no generalized lymphadenopathy and had no unusual or unexplained skin lesions. Ultrasonographic examination of each prostitute has confirmed the above mentioned determination of the lack of abdominal organomegaly and the absence of retroperitoneal lymphadenopathy. One seropositive prostitute with a 4 cm firm axillary lymph node had resolution of this node upon subsequent examination without further lymphadenopathy seen. Another seropositive prostitute had a mild case of *Condylomata vaginalis*.

Serologic profile

All HIV-2 seropositive prostitutes had serologic profiles matching the original description[1,2] when tested with Western blot and RIP/SDS-PAGE (Fig. 1). This profile includes the transmembrane glycoprotein gp32 by Western blot and the membrane glycoproteins gp160/120 by RIP/SDS-PAGE distinct for HIV-2. These sera also reacted by Western blot to LAV-2 and SBL-6669 antigen (not shown). When tested against HIV-1 antigen, one HIV-2 seropositive individual and one HIV-2 seronegative individual showed evidence

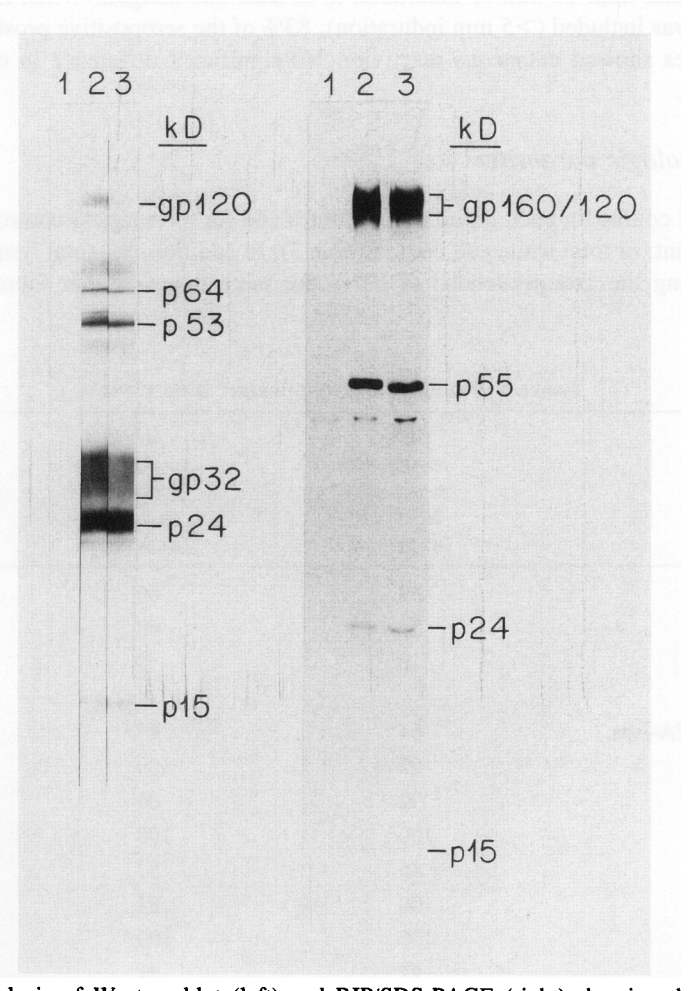

FIG. 1. Serologic analysis of Western blot (left) and RIP/SDS-PAGE (right) showing the typical immunogenic protein profile for HIV-2. The transmembrane glycoprotein of HIV-2, gp32, is best seen by the Western blotting technique, whereas the external membrane glycoprotein gp120 and its closely migrating precursor gp160 are best demonstrated by RIP/SDS-PAGE. Lane 1: uninfected control sera; Lanes 2,3: representative HIV-2 seropositive sera.

of exposure to HIV-1. As stated, for all but serologic and epidemiologic consideration, these two HIV-1 seropositive individuals were excluded from our analysis. Four of the HIV-2 seropositive prostitutes, 3 of the HIV-2 seronegative prostitutes, and 3 of the HIV-2 seronegative surgical controls were found to have antibodies by Western blot and RIP/SDS-PAGE consistent with exposure to HTLV-1. These individuals exhibited no clinical differences from the HTLV-I seronegative individuals, but for the statistical correlations of the hematologic and immunologic parameters, they were both included and excluded, to determine their effect on the statistical analysis. No difference in the analysis was noted in relation to HTLV-1 seropositivity.

In conjunction with the clinical examinations and historical follow-up of the prostitutes, a serologic evaluation was performed to evaluate the antibody status to previous or concurrent infections which may be transmitted sexually and which may alter certain immune parameters. These data are summarized in Table 2. A high prevalence of trichomonas infection, gonorrhea, syphilis, and HSV-2 was seen in both of the two prostitute groups, regardless of serologic status to HIV-2. Of note is the high seroprevalences of HBV, CMV, EBV, and HTLV-I in all three groups studied, though these may not reflect true seroprevalences of the general population.

The absence of delayed type hypersensitivity is used as a measure of decreased cellular immunity. To the four microbial antigens used, 67% of the seropositive and approximately 60% of the seronegative prostitutes reacted with greater than 10 mm of induration to at least one antigen. When minimal positive reactivity to the antigens was included (>5 mm induration), 83% of the seropositive prostitutes and 87% of the seronegative prostitutes showed cutaneous reactivity. No significant difference in cutaneous anergy was noted by these results.

Hematologic/immunologic parameters

The complete blood counts for each group of prostitutes and for the surgical controls were similar in red cell count, platelet count, or total white cell count (Table 3). In addition, the total lymphocyte count, which was also similar among the groups, tended to be in the same range as that found in non-African patients.[17,18]

TABLE 2. PREVIOUS OR CONCURRENT INFECTIONS

	HIV-2 seropositive prostitutes (n = 18) (in percent)	*HIV-2 seronegative prostitutes (n = 14) (in percent)*	*HIV-2 seronegative surgical patients (n = 14) (in percent)*
History of trichomonas	57	66	NA*
History of gonorrhea	54	40	NA
Genital ulcers by exam	0	0	NA
Antibodies to:			
Syphilis (RPR and FTA-Abs.)	64	87	0
HSV-1	92	100	100
HSV-2	92	86	20
HBc	100	100	100
HBs	86	57	71
EBV-viral capsid	100	87	56
CMV	100	100	56
HTLV-I	22	21	21
HIV-1	5.6**	7.1**	0

*Not applicable.
**Represents only one individual in category.

TABLE 3. HEMATOLOGIC AND IMMUNOLOGIC PARAMETERS*

	HIV-2 seropositive prostitutes	*HIV-2 seronegative prostitutes*	*HIV-2 surgical controls*
Mean age	43 (±13)**	34 (±9)***	57 (±8)
Hematocrit (%)	40 (±2.8)	38 (±3.7)	41 (±3.8)
White blood cells (per mm^3 × 1000)	5.5 (±1.2)	5.9 (±2.0)	6.2 (±1.8)
Platelet count (per mm^3 × 1000)	262 (±86)	256 (±58)	est. normal
Absolute lymphocyte count (per mm^3 × 1000)	2532 (±882)	2817 (±1010)	2117 (±608)
Total T-cell	1439 (±395)	1879 (±759)	1094 (±306)
T4 cells	757 (±532) $p = .15$	1179 (±588)	746 (±290)
T8 cells	*509 (±227) p = .03*	*387 (±256)*	*279 (±170)*
IgG (mg%)	*2730 (±645) p = .0001*	*1713 (±460)*	*1924 (±423)*
IgA (mg%)	304 (±108)	257 (±110)	327 (±97)
IgM (mg%)	140 (±54)	130 (±57)	151 (±64)
Lymphocyte proliferation (mean % of normalized response ± SD)			
PHA	89% (±78)	118% (±109)	100%
ConA	55% (±42)	72% (±52)	100%
β_2-microglobulin elevation (normal level < 2.7 mg/l)	*8/17 p = .03*	*1/13*	
Immune complexes	6/9	2/6	

No antilymphocyte antibodies seen in either population.

*Mean values (±SD), unless otherwise indicated. p values given for multivariate analysis of variance between seropositives and seronegatives.

**Excludes four seropositive prostitutes as discussed; one being seropositive also to HIV-1 and three being unavailable for the simultaneous blood sampling of the present survey.

***Excludes one seronegative prostitute who was also seropositive to HIV-1.

Unexpectedly, the analysis of the lymphocyte subsets demonstrated that the total T and T4 lymphocyte counts decreased with age (Fig. 2). Multivariate linear regression analysis demonstrated that the most important predictor of the total T and T4 lymphocyte counts was age. There was a 7% decrease in total T-cell count (95% CI, 3.5–10%) and an 11% decrease in T4 cell count (95% CI, 6.4–16.0%) for each decade after 20 years of age. Although there was a definite trend toward lower T4 counts in seropositive prostitutes when compared to seronegative prostitutes, the effect on T4 counts of being seropositive to HIV-2 or of being a prostitute was not significant at the 5% level when controlling for age ($p = .15$). Treating age as a categorical rather than as a continuous variable did not greatly alter these results. There was no significant difference of the effect of age on T-cell counts among groups, although power to detect such differences was low.

In contrast to the findings for T and T4 cell levels, T8 lymphocyte levels were markedly elevated among seropositive prostitutes compared to both seronegative prostitutes and surgical controls ($p = .03$), whereas there was only a small tendency for T8 counts to decrease with age. With age included as a continuous variable in the multivariate regression model, there was a 25% increase (95% CI, 2–55%) in T8 levels among seropositive prostitutes compared to both seronegative prostitutes and surgical controls.

The immunoglobulin levels were not significantly related to age and there was a highly significant elevation of the IgG isotype among seropositive prostitutes compared to both seronegative control groups

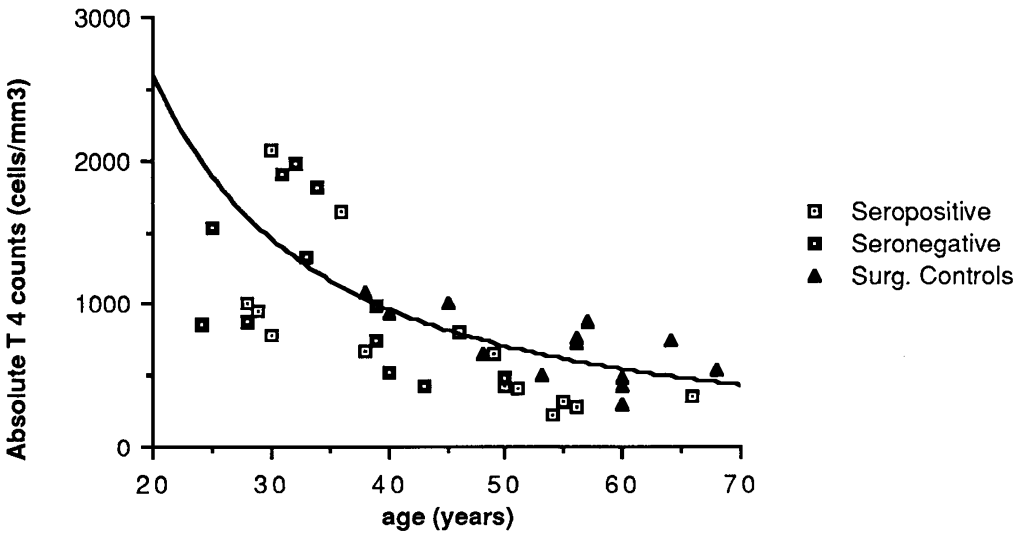

FIG. 2. Plot of T4 lymphocyte determinations by age in seropositive prostitutes (⊡), seronegative prostitutes (■), and surgical controls (▲); with logarithmic line of best fit. All T-cell subset determinations were drawn at one time point for comparison. Three seropositive prostitutes seen in the clinic since 1985 are without values due to serologic testing after March 1986.

(Table 3). No such elevation was observed in the other immunoglobulin subtypes, IgA or IgM. This increased IgG was determined to be polyclonal by SPEP. No significant difference in fluorescence patterns was seen upon testing the two groups of prostitutes for antinuclear antibodies.

The levels of β_2-microglobulin were markedly elevated in the seropositive group compared to seronegative prostitutes, and the effect could not be explained by differences in age. Elevation above the normal cutoff, 2.7 mg/l, was seen in 8 of 17 seropositive prostitutes as compared to only 1 of 13 seronegative prostitutes ($p = .03$). Also, in samples available for determination of circulating immune complexes, 6 of 9 seropositive prostitutes and 2 of 6 seronegative prostitutes demonstrated significant levels.

Lymphocyte proliferation responses to the mitogens PHA and ConA were slightly impaired in the HIV-2 seropositive individuals when compared to the seronegative prostitutes, but this did not reach statistical significance. Also, responses did not significantly correlate to T4 count or age in the analysis. The range of responses was wide within each group tested, including the surgical controls, but the responses of each individual's lymphocytes to mitogenic stimulation were consistent throughout all runs.

For both the HIV-2 seropositive and seronegative prostitutes, the microcytotoxicity assay for antilymphocyte antibodies revealed no increase in killing of established donor lymphocytes above the normal background killing secondary to HLA incompatibility.

DISCUSSION

This initial survey of individual seropositive to HIV-2 provides insights into the epidemiology of this new class of human retrovirus. In addition, although certain hemtologic and immunologic parameters were found to be altered in the seropositive prostitutes, no clinical differences could be seen between the seronegative and seropositive individuals evaluated. No significant difference in previous or concurrent infections by historical or serologic analysis other than HIV-2 infection could explain our findings in this survey. Data from this study will provide a baseline for future long-term study of the natural history of HIV-2 infection.

The lack of unusual clinical findings in the HIV-2 seropositive prostitutes could be explained by a very recent introduction of this retorvirus into the population. We do know, however, that these seropositive prostitutes have been exposed to HIV-2 prior to the February 1985 sampling. Perhaps more importantly,

our initial seroepidemiology for HIV-2 in Dakar has shown evidence for infection as early as 1976, which means the virus has been present in Dakar for at least the past decade.[2]

Because of these facts, we believe the lack of clinical findings is not solely due to a recent introduction of HIV-2 into the population. Furthermore, in addition to these initial 18 HIV-2 seropositive prostitutes, we have clinically surveyed 44 more HIV-2 seropositive prostitutes (62 total) in different parts of Senegal, and the lack of unusual clinical findings has persisted. The absence of generalized lymphadenopathy and of signs or symptoms indicative of immunosuppression such as severe weight loss, chronic diarrhea, documented chronic fevers or pruritis, is distinct in this population as compared to similar cross-sectional surveys of outpatient prostitutes seen in East or Central Africa and infected with HIV-1 (Table 4).

As can be seen from the mean ages of the seropositive and seronegative prostitute groups (Table 3), the prostitutes exposed to HIV-2 were older than those seronegative to HIV-2. As approximated for the seropositive prostitutes, older prostitutes may have an increased number of lifetime sexual contacts and may simply be more likely to be infected by a sexually transmitted agent. The seropositive and seronegative prostitutes in our survey did not have a significantly different history of exposure to blood products or medical injections, although this certainly does not exclude the probability that HIV-2 is also transmitted via parenteral exposure. In addition, the older mean age of the seropositive prostitutes group did make age matching of the seronegative prostitute unavailable for this survey, since we had extreme difficulty identifying seronegative prostitutes in the fifth and sixth decade who were willing to participate.

Future consideration of the influence of age on T-cell counts in addition to its influence on seropositivity will be required for prospective studies in West Africa involving T-lymphotropic retroviruses. Statistical analysis via an analysis of variance procedure to determine any variation in parameters related to age was completed in both a linear and age-group manner on all results to discover the effect of age on any differences seen between the groups examined. As shown, the parameters which significantly correlated to age were the absolute T-cell count and T4 subset determinations. Other parameters, such as serum immunoglobulin levels and lymphocyte proliferation values, did not significantly alter with age. Although the mean total T-cell and T4 cell values in the seropositive prostitute group were lower than in the seronegative group, we found that these differences were primarily due to age. The significance of the trend toward decreased T4 lymphocytes in the seropositive prostitutes will have to be addressed with repeated prospective sampling of a larger age-matched population.

The effect of age on the total T-cell count and on the T-cell subsets has been studied.[22–24] These studies have involved primarily white individuals of European descent and at times show a decrease or an increase in total T-cell or T4 values in elderly individuals who are healthy, depending on the study. No previous surveys have shown a decrease in T-cells among the age range of our survey population, but extensive studies involving healthy Africans are lacking.

A significant elevation of T8 lymphocytes was found in ther seropositive group of prostitutes and this

TABLE 4. CLINICAL FINDINGS IN HIV-1 OR HIV-2 SEROPOSITIVE PROSTITUTES IN OUTPATIENT SETTINGS IN AFRICA

	East or Central Africa			*West Africa*
	Kenya[19]	*(HIV-1) Rwanda*[20]	*Zaire*[21]	*(HIV-2) Senegal*
Number of seropositive prostitutes surveyed	50	29	101	62*
% with generalized lymphadenopathy	54%	83%	NA**	0%
% with signs or symptoms suggestive of HIV-like disease	0%	38%	29%	2%***

*Includes the 18 seropositive prostitutes in this survey, plus 44 more seropositive prostitutes clinically evaluated to date throughout Senegal.

**Not applicable in quoted study.

***One prostitute subjectively complaining of weight loss which has not been substantiated on subsequent visits.

caused the T4/T8 ratios of this group in general to be lower. In addition, a polyclonal increase in the IgG isotype of immunoglobulin was seen. The serology to various other viral agents (shown in Table 2) was an attempt to correlate seropositivity for HIV-2 to exposure to other STDs, but more importantly, it was an attempt to determine if other concurrent or persistent viral infections could be playing a role in elevating these measurements of "immune activation." Differences in the degree of exposure to other viral diseases, such as HSV, EBV, HBV, or CMV, could not be demonstrated between the seropositive and seronegative prostitutes.

Since the immune parameter did show some alteration indicative of a persistent viral infection,[25] several other parameters previously described to be altered in HIV-1 infection were studied to further elucidate any specific abnormalities. β_2-microglobulin, a low molecular weight protein produced by all nucleated cells, was increased significantly in a majority of the HIV-2 seropositive sera. This parameter is a nonspecific indicator of increased cell turnover and/or destruction and may represent a generalized measure of immune system "activation." Elevation of β_2-microglobulin levels has been seen in HIV infection, but mostly in the symptomatic cases.[26,27] Also, immune complexes were noted above normal background levels in 6 of 9 of the seropositives tested and 2 of 6 seronegative prostitutes. Increased levels of immune complexes, however, have been found in healthy Central Africans who were seronegative for HIV-1 exposure.[28] Immune complex elevations, therefore, may be a nonspecific finding in African populations. As noted, we failed to find antilymphocyte antibodies in HIV-2 positive sera, as have been described often in sera from individuals not only with AIDS[29,30] but also in sera from asymptomatic HIV-1 seropositive individuals.[31] Overall, the elevations of T8 lymphocytes, IgG and β_2-microglobulin levels seen in this cohort of HIV-2 seropositive prostitutes may be somewhat similar to that seen with HIV-1, but their relationship to immunocompetence in either infection is unclear.

Certain STLV-3-like retroviruses, initially termed LAV-2[3,32] and SBL-6669[4] have been isolated from patients in Europe with AIDS or chronic constitutional symptoms who were from countries in West Africa. All the HIV-2 isolates are highly cross reactive serologically with STLV-3, though certain differences in the electrophoretic migration of immunogenic proteins have been described.[4] Regardless of further comparative studies, any of the HIV-2 isolates can be used an an antigen source in serologic assays to determine if an individual has been exposed to this class of retrovirus.

That the class of "STLV-3-like" human retroviruses, now termed HIV-2, may cause some degree of immunosuppression would not be surprising. These STLV-3-like retroviruses shared a very close structural similarity to HIV-1. Furthermore, although STLV-3 naturally infects African green monkeys (*Cercopithecus aethiops*) without causing apparent disease,[33] the virus has also been identified in captive macaques suffering from a severe simian immunodeficiency syndrome.[34] Inoculations with STLV-3 into macaques[35] have provided further evidence of its pathogenic potential for immunodeficiency in this species of primate.

In relation to human retroviruses and immunodeficiency, even human T-lymphotropic virus type I (HTLV-I), a more distantly related T-lymphotropic retrovirus, may cause a degree of immunosuppression[36] and even perhaps sporadic cases of AIDS.[37,38] A high seroprevalence rate for antibodies indicative of exposure to this class of STLV-3-like retorviruses is being seen throughout West Africa,[39,40] yet a widespread AIDS epidemic like that related to HIV in Central Africa has not been reported by West African countries. One can conclude, therefore, that only by conducting prospective population-based studies involving individuals infected with HIV-2 will the exact pathogenic potential of this class of retroviruses be determined.

Presently, we can conclude that HIV-2 is a sexually transmitted retrovirus. The immune parameters able to be measured in healthy prostitutes exposed to this agent are consistent with a persistent viral infection from this class of T-lymphotropic retroviruses. The long-term immune competence of an individual exposed to HIV-2 is unclear at this time, yet when the lack of lymphadenopathy and good state of health seen in these HIV-2 seropositive prostitutes is compared to similar cohorts of prostitutes in Central Africa exposed to HIV-1, a marked difference in clinical presentation is noted. This difference when considered with the scarcity of AIDS in Senegal may represent a reduced pathogenicity of HIV-2 when compared to HIV-1.

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REFERENCES

1. Barin F, M'Boup S, Denis F, Kanki P, Allan JS, Lee TH, and Essex M: Lancet 1985,ii:1387–1390.
2. Kanki PJ, Barin F, M'Boup S, Allan JS, Romet-Lemonne JL, Marlink R, McLane MF, Lee TH, Arbeille B, Denis F, and Essex M: Science 1986;232:238–243.
3. Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C, Rouzious C, Klatzmann D, Champalimaud JL, and Montagnier L: Science 1986;233:343–346.
4. Albert J, Bredberg U, Chiodi F, Bottiger B, Fenyo EM, Norrby E, and Bierfeld G: AIDS Research and Human Retroviruses 1987;3:3–10.
5. Marlink R, Allan J, and Essex M: Lancet 1986;i:1389.
6. Montagnier L: Presented at III International Conference on AIDS, Wash., D.C., June 4, 1987.
7. Essex M, Kanki P, Marlink R, M'Boup S, Barin F, Denis F, *et al.* Presented at III International Conference on AIDS, Wash., D.C., June 4, 1987.
8. Lee TH, Coligan T, Homma T, McLane MF, Tachibana N, and Essex M: Proc Nat Acad Sci USA 1984;81:3856–3860.
9. Lee FK, Coleman RM, Pereira L, Bailey PD, Tatsuno M, and Nahmias AJ: J Clin Micro 1985;22:641–644.
10. Boyum A: Scand J Clin Lab Invest 1968;21(suppl 97):77–89.
11. Killingsworth LM, and Savory J: Clin Chem 1972;18:335.
12. Nydegger UE, Lambert PH, Gerber H, and Miescher PA: J Clin Invest 1974;54:297–309.
13. Gupta S, and Safai B: J Clin Invest 1983;71:296–300.
14. Nicholson JKA, McDougal JS, Spira TJ, *et al:* J Clin Invest 1984;73:191–201.
15. Clumeck N, Sonnet J, Taelman H, *et al:* NEJM 1984;310:492–497.
16. Quinn TC, Mann JM, Curran JW, and Piot P: Science 1986;234:955–963.
17. Zacharski LR, Elvebach LR, and Linman JW: Am J Clin Pathol 1971;56:148–150.
18. Bain BJ, and England JM: Br Med J 1975;1:306–309.
19. Kreiss JK, Koech D, Plummer FA, *et al:* NEJM 1986;314:414–418.
20. Van de Perre P, Carael M, Clumeck N, *et al:* Lancet 1985;ii:524–527.
21. Mann JM, Quinn T, Francis H, Miatudila M, Piot P, and Curran J: International Conference on AIDS, Paris (abstract) 1986;108:105.
22. Nagel JE, Chrest FJ, and Adler WH: J Immunol 1981;127:2086–2088.
23. Leffell MS, Lumsden L, and Steiger WA: J Am Geriatr Soc 1985;33:4–7.
24. Ligthart GJ, Schuit HRE, and Hijmans W: Immunology 1985;85:15–21.
25. Haywood AM: NEJM 1986;315:939–948.
26. Krohn K, Antonen J, Valle S-L, Kazakevicius R, Saxinger C, Gallo RC, and Ranki A: Cancer Res 1985;45(suppl):4612s–4615s.

27. Heering P, and Arning M: Lancet 1987;i:281.
28. Quinn TC, Piot P, McCormick JB, Feinsod FM, Taelman H, Kapita B, Stevens W, and Fauci AS: JAMA 1987;257:2617–2621.
29. Williams RC, Masur H, and Spira T: J Clin Immunol 1984;4:118–123.
30. Kiprov DD, Busch DF, Simpson DM, Morand PR, Tardelli GP, Gullett JH, Lippert R, and Mielke H: In: *Acquired Immune Deficiency Syndrome*. M. Gottlieb and J. Groopman J (Eds.). Alan R. Liss, New York, 1984, pp. 299–308.
31. Kiprov DD, Anderson RE, Morland PR, *et al:* NEJM 1985;312:1517.
32. Brun-Vezinet F, Katlama C, Roulot D, *et al:* Lancet 1987;i:128–132.
33. Kanki PJ, Alroy J, and Essex M: Science 1985;230:951–954.
34. Kanki PJ, McLane MF, King NW Jr, Letvin NL, Hunt RD, Sehgal P, Daniel MD, Desrosiers RC, and Essex M: Science 1985;228:1199–1201.
35. Letvin NL, Daniel MD, Sehgal PK, *et al:* Science 1985;230:71–73.
36. Essex ME, McLane MF, Tachibana N, Francis DP, and Lee TH: In: *Human T-Cell Leukemia Viruses*. R.C. Gallo, M. Essex, and L. Gross (Eds.). Cold Spring Harbor Press, Cold Spring Harbor, NY, 1974, pp. 355–362.
37. Kobayashi M, Yoshimoto S, Fujishita M, *et al:* Lancet 1984;i:1361–1362.
38. Miyoshi I: AIDS-like opportunistic infections in HTLV-I carriers. ARC International Symposium on Viruses and Cancers, Martinique, Jan 8, 1986, abstract.
39. Kanki PJ, M'Boup S, Ricard D, Barin F, Denis F, Boye C, Sangare L, Travers K, Albaum M, Marlink R, Romet-Lemonne JL, and Essex M: Science 1987;236:827–831.
40. Denis F, Gershy-Damet G, Lhuillier M, Mounier M, Leonard G, M'Boup S, Gludeau A, Kanki P, and Essex M: Lancet 1987;i:408–411.

Address reprint requests to
Dr. R.G. Marlink
Department of Cancer Biology
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115