CLINICAL RESEARCH

Human retroviral infections in The Gambia: prevalence and clinical features

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Abstract

The prevalence of infection with human immunodeficiency virus type 1 (HIV 1) is lower in west Africa than in other parts of Africa. Human immunodeficiency virus type 2 (HIV 2) has been isolated from west African patients and may be transmitted by heterosexual contact. The prevalence of antibodies to HIV 1 and HIV 2 was studied by enzyme linked immunosorbent assay (ELISA) among various groups of subjects in The Gambia, west Africa—namely, prostitutes, blood donors, patients with suspected infection with HIV, patients attending clinics for sexually transmitted diseases, and patients with tuberculosis. Four cases of the acquired immune deficiency syndrome (AIDS) due to infection with HIV 1 were detected, of which three had been acquired abroad. No other subject was found to be positive for antibodies to HIV 1. The prevalence of antibodies to HIV 2 among the patients attending clinics for sexually transmitted diseases was found to have increased from 0/117 in 1984 to 10/185

(5%) in the last six months of 1986. One out of 278 blood donors was positive for antibodies to HIV 2 as were 10 out of 80 patients with suspected AIDS.

HIV 2 seems to be transmitted sexually, and, although it has been present for only a short time, it seems to be endemic in The Gambia and is pathogenic.

Introduction

By comparison with central and east Africa few cases of the acquired immune deficiency syndrome (AIDS) have been reported from west Africa. Infection with human immunodeficiency virus (HIV 1) may be less common in west Africa, but few studies of the prevalence of antibodies to HIV 1 have been reported from this region. A recent study from the Ivory Coast reported a prevalence of 12.5% among prostitutes.¹

Human immunodeficiency virus type 2 (HIV 2) is a recently described human retrovirus, which has been isolated from several west African patients, some of whom had clinical AIDS.²⁻⁴ Infected patients were neither homosexual nor intravenous drug abusers, so this virus is probably transmitted by heterosexual contact; epidemiological studies from west Africa have not, however, been reported. Molecular cloning of this virus has shown it to be more closely related to the simian virus simian T lymphotropic virus type III (STLV III (mac)) than to HIV 1.⁵

Kanki *et al* reported the isolation of another new retrovirus, human T lymphotropic virus type 4 (HTLV 4), from healthy Senegalese prostitutes.⁶ This virus is similar to if not identical with the simian virus STLV III.^{7,8} It was initially said to be non-cytopathogenic to lymphocytes in culture and non-pathogenic in man, but recent reports on patients from Guinea-Bissau, Senegal, and The Gambia indicate that it may cause an illness similar to AIDS.^{9,10} Serological evidence suggests that HTLV 4 may be present in the Ivory Coast with a similar prevalence to HIV 1¹; the relation between HIV 2 and HTLV 4 is unclear.

Serological reactivity suggestive of infection with HTLV 4 has been reported in two Gambian women resident in Sweden, one of whom was suffering from pulmonary infiltration and weight loss.⁹ No cases of human retroviral infection have previously been

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reported in The Gambia. We report the results of serological tests for antibodies to HIV 1 and HIV 2 among various groups in The Gambia and describe the clinical features of those found to be infected.

Patients and methods

Patients studied

Venous blood samples were taken from five groups of patients.

Patients attending clinics for sexually transmitted diseases—We studied 117 patients attending the clinic for sexually transmitted diseases at the Medical Research Council Hospital in Fajara with genital ulceration between September 1983 and April 1984; 107 were men and 10 women. A further 185 patients attending this clinic (n=138) and the clinic at the Royal Victoria Hospital in Banjul (n=47) during 1986 were included; 168 were men and 17 women.

TABLE 1—Serological findings in different study groups before and after May 1986. Figures are numbers of patients (percentages)

Group	No in group	Antibody to HIV 1	Antibody to HIV 2
<i>Before May 1986</i>			
Patients with genital ulcers	117		
Patients with tuberculosis	98		1 (1)*
Prostitutes	31		1 (3)
Patients with lymphomas	5		
Total	251		2 (1)
<i>After May 1986</i>			
Patients with sexually transmitted disease:	185		10 (5)
Genital ulcers	73		6 (8)
Urethral discharge	90		2 (2)
Other	22		2 (9)
Blood donors	278		1 (0.3)
Patients with suspected AIDS	80	4 (5)	10 (12)
Total	543	4 (0.7)	23 (4)

*Case 5 from table II: borderline positive for antibody to HIV 2.

Prostitutes—Thirty one prostitutes resident in the provincial town of Basse (population about 10 000) were examined in February 1985. The prevalence of sexually transmitted diseases was high in this group: *Neisseria gonorrhoeae* was isolated from 10, and 23 showed serological evidence of syphilis. Twenty four of the prostitutes were Senegalese and two were from Guinea; only five were native Gambians.

Patients with suspected infection with HIV—Eighty patients were seen at the Royal Victoria Hospital in Banjul or the Medical Research Council Hospital in Fajara during 1986-7 with conditions suggesting impaired cell mediated immunity. Five patients with lymphomas diagnosed during 1985 were also tested.

Blood donors—We studied 278 consecutive unpaid volunteer blood donors attending the government blood donation centre in Banjul in 1986; 275 were

men and three women. All seemed to be healthy; their ages ranged from 20 to 45. Some two thirds of these donors were volunteers living in Banjul, some of whom were regular donors, and the remainder were relatives of patients requiring transfusion.

Patients with tuberculosis—We studied 98 patients with tuberculosis seen before May 1986.

Laboratory methods

SEROLOGICAL TESTING FOR HIV 1

Serum samples were stored at -20°C before testing. All were tested by the Wellcozyme competitive enzyme linked immunosorbent assay (ELISA) (Wellcome Foundation Ltd, Beckenham, Kent) according to the manufacturer's instructions.

SEROLOGICAL TESTING FOR HIV 2

Antibodies to HIV 2 were detected by a solid phase competitive ELISA analogous to the Wellcozyme ELISA test for HIV 1. Antigen was prepared from a cell pellet of Jurkat cells infected three to five days previously with CEM cells infected with HIV 2. The cell pellet was treated with 0.2% propiolactone for two hours at 4°C and processed as previously described.¹¹

Polystyrene strips of eight by two wells (Nunc Ltd) were coated with 100 µl of an optimum dilution of globulin prepared by ammonium sulphate precipitation from a serum known to contain high titres of antibody to HIV 2. After the wells had been washed and quenched with 0.1% bovine serum albumin 100 µg of HIV 2 antigen diluted in phosphate buffered saline containing 0.1% Tween 20 was added to the wells and incubated for 72 hours. Wells were then washed, dried, and stored desiccated at 4°C before use.

A conjugate of specific HIV 2 IgG was prepared from a serum known to contain high titres of antibody. IgG was prepared by ion exchange chromatography and coupled with horseradish peroxidase.¹² To detect antibody to HIV 2 25 µl of test serum or dilutions of serum in normal human serum was incubated with 75 µl of conjugate for one hour at 45°C in wells coated with antigen. Wells were then washed and the binding of the conjugate with horseradish peroxidase measured by the addition of 100 µl of substrate containing tetramethylbenzidine in citrate and hydrogen peroxide buffer. The colour reaction was stopped after 20 minutes at room temperature with 2M sulphuric acid, and optical densities were measured at 450 nm. Any serum or dilutions of serum causing a greater than 50% reduction in optical density was considered to be positive for antibody to HIV 2. Serum samples found to be reactive were retested and the reactivity expressed as the ratio of the optical density of the test serum to that of a standard control. Serum samples whose ratios were consistently less than 1.0—that is, whose optical densities were consistently less than that of a dilution of a positive sample selected to give a 50% reduction in optical density—were considered to contain appreciable amounts of HIV 2 antibody.

WESTERN BLOTTING

Selected serum samples positive for antibody to HIV 2 by the above method were reacted both with human T lymphotropic virus type III B

TABLE II—Clinical details of and outcome for patients with AIDS or conditions related to AIDS

Case No	Antibody to HIV 1/HIV 2	Age (years)	Sex	Countries visited	Presenting features	Associated conditions	Outcome
1	HIV 1	32	M	United States, Zaire	Cachexia, lymphadenopathy	Oral thrush, pulmonary infiltrates	Died
2	HIV 1	25	M	West Germany	Ataxic neuropathy		Alive
3	HIV 1	24	F	Zaire	Diarrhoea, weight loss, dementia	Oral and vaginal thrush	Died
4	HIV 1	40	M	Senegal	Chronic cough, weight loss	Cryptococcal meningitis	Died
5	HIV 2	50	F	None	Tuberculosis not responding to treatment	Oral thrush, cryptococcal meningitis	Died
6	HIV 2	34	M	West Africa	Mesenteric lymphadenopathy	Dementia, Kaposi's sarcoma, cerebral toxoplasmosis	Died
7	HIV 2	25	M	Senegal	Dementia, weight loss	Pneumonia, cerebral atrophy	Died
8	HIV 2	16	M	Senegal	B Cell lymphoma		Alive
9	HIV 2	60	M	Senegal	Chronic diarrhoea, weight loss	Cryptococcal meningitis	Died
10	HIV 2	40	M	Senegal	Chronic diarrhoea and cough, weight loss		Died
11	HIV 2	27	M	Guinea-Bissau, Senegal	Dementia, chronic diarrhoea, weight loss	Oral thrush, strongyloidiasis	Alive
12	HIV 2	27	M	Ivory Coast	Chronic diarrhoea, weight loss	Tuberculosis	Alive
13	HIV 2	29	M	None	Chronic diarrhoea, weight loss	Oral thrush	Died
14	HIV 2	20	M	None	Pulmonary tuberculosis not responding to treatment	Oral thrush	Died
15	HIV 2	30	M	None	Pulmonary tuberculosis not responding to treatment		Alive

strips and with HIV 2 strips (Dupont Western Blot Kits, Dupont Ltd) according to the manufacturer's instructions. Densitometric scans of the strips were converted to a digital format and stored on disc in a microcomputer. After normalisation data for each serum sample were expressed as a graphical linear representation of reflectance and compared with data for reference serum samples known to be positive for antibody to HIV 1 and HIV 2.

Results

Table I shows the results of serological tests in the various groups tested. Two subjects positive for antibodies to HIV 2 were identified before May 1986. One was a patient with pulmonary tuberculosis who failed to respond to conventional treatment. Her serum showed only borderline reactivity for HIV 2 antibody; nevertheless, she satisfied the World Health Organisation's case definition of AIDS in Africa as she subsequently developed cryptococcal meningitis. The other seropositive subject was a healthy prostitute who remained well 18 months later. After May 1986, 5% of patients attending clinics for sexually transmitted diseases were found to have antibodies to HIV 2. The prevalence was higher among those with genital ulcers than those with urethritis, but the difference did not attain significance ($6/73$ v $2/90$; $p=0.08$, Fisher's exact test). One patient with genital ulcers had been seen a few months previously with shingles. The other patients from the sexually transmitted diseases clinics who were positive for HIV 2 antibody were in good general health; none had oral thrush or extrainguinal lymphadenopathy.

Altogether 160 new patients with pulmonary tuberculosis were seen between May 1986 and February 1987. Seven of these failed to respond to treatment and were tested for antibodies to HIV 1 and HIV 2; two were positive for HIV 2 antibody. These patients are included in the patients with suspected AIDS in table I.

Table II gives the clinical details of and the outcome in seropositive patients with AIDS or conditions related to AIDS. Three of the four patients with infections with HIV 1 had travelled outside west Africa whereas none of those with infections with HIV 2 had done so. One of the four (case 1) admitted to several homosexual encounters in San Francisco; another (case 2) had a girlfriend in West Germany who was reported to be ill in hospital; and the husband of another (case 3) had recently died in Kinshasa, Zaire. The wives of two of the patients (cases 1 and 4) were found to be negative for HIV 1 antibody.

One patient (case 8) was aged only 16; he reported, however, that he had been sexually active since the age of 10. No seropositive patient had received a blood transfusion. At necropsy cerebral toxoplasmosis, oral Kaposi's sarcoma, and oral and bronchial candidiasis were found in case 6 and non-specific bronchopneumonia and mild lymphocytic encephalitis with no necrosis or giant cells in case 7. A specimen taken at operation in case 8 showed a retroperitoneal tumour and a B cell lymphoblastic lymphoma like Burkitt's lymphoma, which expressed CD 30 (cluster of differentiation) reactivity on immunocytochemical testing that was suggestive of transformation by the Epstein-Barr virus.¹³

WESTERN BLOTTING

Serum samples from 13 patients positive for antibodies to HIV 2 were blotted on HIV 1 and HIV 2 strips. On the HIV 2 strips all gave a strong reaction with the major HIV 2 envelope proteins gp120/gp110. They also reacted with p64, and all but one (from a patient with AIDS) reacted with p57 and p51. Reactions with p26 and p16 were more variable (figure). None gave a significant reaction with the major envelope components gp160/gp120.

Discussion

Our study shows clearly that HIV 2 is pathogenic in The Gambia. The pattern of disease associated with this virus does not seem to differ appreciably from that associated with HIV 1, although larger numbers of patients must be studied before this can be stated conclusively. Our results also show that, whereas infection with HIV 1 is not yet endemic in The Gambia, infection with HIV 2 has become so. The increase in the prevalence of infection with HIV 2 among patients attending clinics for sexually transmitted diseases from 1984 to 1986 (0/117 to 10/185) and the scarcity of clinical cases of AIDS before May 1986 suggest that HIV 2 has been introduced recently.

Western blots against HIV 1 of 13 serum samples from patients positive for antibody to HIV 2 by competitive ELISA.

In view of the higher prevalence of antibodies to HIV 2 among patients attending sexually transmitted diseases clinics than among blood donors, HIV 2 seems to be transmitted sexually in The Gambia. The prevalence among patients with genital ulcers was higher than that among patients with urethritis, though this did not attain significance ($p=0.08$, Fisher's exact test). A similar association has been noted between infection with HIV 1 and genital ulceration in Nairobi¹⁴ and has been attributed to an increased likelihood of infection in those with a break in the mucosa. Most

patients with genital ulcers in this study gave a short history, so they must have been infected with HIV 2 before developing their ulcer. Several of them had attended the clinic for sexually transmitted diseases previously, and thus a common behavioural factor probably predisposes to both genital ulceration and infection with HIV 2—for example, recourse to a certain type of prostitute.

The recent discovery of one if not two new pathogenic human retroviruses in west Africa suggests that there may be other as yet undiscovered retroviruses in this region. One of our patients (case 5) may have been infected with a retrovirus that was neither HIV 1 nor HIV 2 because she had clinical AIDS but was negative for antibody to HIV 1 and only borderline positive for antibody to HIV 2.

The prevalence of human retroviral infections in The Gambia is at present low but seems to be increasing. There is an urgent need not only for a public education programme to try to limit the spread of these viruses but also for further research into the epidemiology and course of infection with HIV 2. Before an effective retroviral vaccine can be developed many isolates of the retrovirus from west African subjects must be characterised to establish its antigenic diversity.

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Bronchial hyperreactivity in patients who cough after receiving angiotensin converting enzyme inhibitors

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Abstract

Angiotensin converting enzyme inhibitors cause cough in some patients, but the mechanism of this effect is not known. Six patients in whom these inhibitors had caused cough and a further two patients in whom they were suspected to have caused worsening of bronchial asthma were studied. Nine patients in whom angiotensin converting enzyme inhibitors had not been associated with cough served as controls. In the controls lung function and bronchial reactivity were measured once; for the study patients these and the cough index were measured twice before rechallenge for two weeks with an angiotensin enzyme inhibitor and once afterwards. Rechallenge with drug for two weeks caused a significant decrease in the mean concentration of histamine causing a 35% fall in airways conductance and a significant increase in the cough index. Patients with cough

showed bronchial hyperactivity compared with the controls, which increased after rechallenge with the inhibitors.

Cough associated with converting enzyme inhibitors may be a variant of the cough in asthma.

Introduction

Angiotensin converting enzyme inhibitors are being used increasingly in the treatment of hypertension and heart failure. One of us (PFS) reported the occurrence of cough in several patients treated with drugs of this class,¹ and a series of case reports have described this effect.²⁻⁵ Postmarketing surveillance of captopril and enalapril has confirmed this association⁶ but initially suggested that the incidence was low at around 1%.⁷ This is probably an underestimate because cough is common in the general population and not widely recognised as an effect related to drugs: indeed, a retrospective analysis estimated the incidence to be 14%.⁸ To try to identify the mechanism of this side effect we rechallenged with converting enzyme inhibitor a series of patients who had previously reported cough or wheeze in association with this type of drug.

Patients and methods

We studied six patients with hypertension who had developed cough during treatment with enalapril or captopril, which had resolved when the treatment was stopped; one patient had coughed after both drugs. We also studied two patients with hypertension and bronchial asthma who had reported increased wheeze after treatment with enalapril, but another patient with severe cough after treatment with enalapril declined to take the drug again. The side effects caused by these drugs in two of the patients have

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