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SLIM DISEASE: A NEW DISEASE IN UGANDA AND ITS ASSOCIATION WITH HTLV-III INFECTION

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Summary A new disease has recently been recognised in rural Uganda. Because the major symptoms are weight loss and diarrhoea, it is known locally as slim disease. It is strongly associated with HTLV-III infection (63 out of 71 patients) and affects females nearly as frequently as males. The clinical features are similar to those of enteropathic acquired immunodeficiency syndrome as seen in neighbouring Zaire. However, the syndrome is rarely associated with Kaposi's sarcoma (KS), although KS is endemic in this area of Uganda. Slim disease occurs predominantly in the heterosexually promiscuous population and there is no clear evidence to implicate other possible means of transmission, such as by insect vectors or re-used injection needles. The site and timing of the first reported cases suggest that the disease arose in Tanzania.

Introduction

A NEW disease has been recognised in the Rakai district in South West Uganda (fig 1). The first patients were seen in 1982 and new ones are being seen with increasing frequency. Most patients present with fever, an itchy maculopapular rash, general malaise, prolonged diarrhoea, occasional respiratory symptoms, and oral candidiasis, but the most dominant feature is extreme wasting and weight loss. Hence the syndrome is known locally as slim disease.

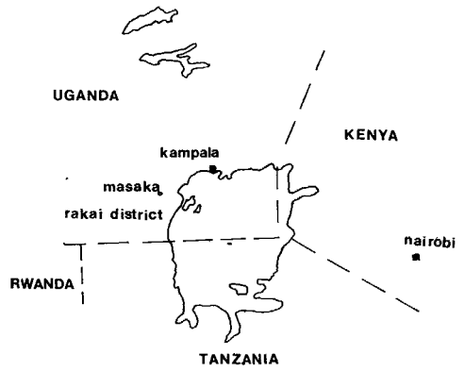

Fig 1—Map of Uganda showing Masaka and the Rakai districts.

Human T-lymphotropic virus type III (LAV,[1] HTLV-III[2]) is endemic in some areas of Uganda and HTLV-III infection is associated with generalised aggressive or atypical Kaposi's sarcoma (KS) but not with endemic KS.[3] An epidemic of the acquired immunodeficiency syndrome (AIDS) is spreading in neighbouring Rwanda and Zaire,[4,5] where HTLV-III infections are now believed to be endemic[6]

(and Biggar RJ, personal communication) and where heterosexual contact has been suggested to be a major route for virus transmission, since the disease is found in an urban, promiscuous heterosexual population and affects both sexes equally. Although slim disease resembles AIDS in many ways, it seems to be a new entity. We describe here the clinical features of this disorder and its association with HTLV-III infection.

Subjects and Methods

Patients

29 patients (of whom 13 were women) aged 17–37 years (mean 26) were identified on a field visit to the Masaka and Rakai districts and were thoroughly investigated. 42 other patients aged 19–60 years (mean 35) were seen in Kampala. Overall, the male:female ratio was 1.2:1. Most of these patients follow rural occupations such as farming and have not been outside the Rakai district, except for a few traders who regularly travel to north-west Tanzania.

Suspected patients and their relatives were screened for evidence of wasting, lymphadenopathy, and oral or pharyngeal candidiasis. Patients with any of these features were asked for a full history and examined fully. Skin test antigens (PPD, mumps, streptokinase, streptodornase, and dermatophytin) were administered intradermally and responses were read after 48 h.

Patients with any of these features were asked for a full history and faecal smears were prepared for both wet-film examination for parasites and for auramine and carbolfuschin staining for cryptosporidia. Sputum was obtained from 8 patients with a productive cough and acid-alcohol fast bacilli (AFB) were sought.

Venous blood was taken for haematological studies and serum was stored for subsequent HTLV-III antibody studies. In a few cases peripheral blood mononuclear cells were prepared for short-term culture, to look for evidence of retrovirus infection.

Controls

Control sera were obtained on the field visit to Masaka from healthy relatives, hospital attendants, and 30 hospital inpatients without evidence of HTLV-III infection, and in Kampala from hospital doctors, nurses, and other hospital workers, as well as from children in the paediatric ward.

Anti-HTLV-III

Anti-HTLV-III was tested for essentially as previously described[7] except that an enzyme-linked rather than radiolabelled anti-HTLV-III-IgG tracer was used in a short-incubation assay. Briefly, a preparation of anti-HTLV-III-IgG from a British patient was coupled with horseradish peroxidase (HRPO) to achieve a substitution ratio of 3 HRPO: 1 IgG. 25 μl of test serum and 75 μl of the optimum dilution of HRPO-anti-HTLV-III were mixed together in wells coated with a crude γ globulin prepared from the high anti-HTLV-III titre serum of a patient. Antigen was then added and the mixture incubated at 45°C for 1 h. After that the wells were washed with saline containing 0·1% tween 20 and aspirated to dryness. 100 μl of tetramethylbenzidine in citrate/acetate buffer pH 6·0 containing 0·003% H_2O_2 were added to each well. After 20 min, the reaction in wells was stopped with 2 mol/l sulphuric acid and the colour was measured spectrophotometrically at 450 nm. Sera or serum dilutions giving significant inhibition of colour formation, usually taken to be 50% or less of the colour generated in wells containing serum negative for all HTLV markers, were considered to contain anti-HTLV-III.

Short-term Culture

Lymphocytes were cryopreserved and thawed when needed. After phytohaemagglutinin (PHA) stimulation, the cells were grown in RPMI supplemented with 20% fetal calf serum and 0·5% recombinant interleukin-2 (Biogen). Evidence of virus expression in fresh peripheral lymphocytes and established cell-lines was sought by testing for reverse transcriptase,[2] indirect membrane antigen immunofluorescence,[7] and syncytium formation.[8]

TABLE I—CLINICAL FEATURES OF 29 PATIENTS WITH SLIM DISEASE

Features	Proportion positive
Signs and symptoms	
Hospital admission required	21/29
Weight loss greater than 20%	18/29
Fevers and sweats	20/29
Oral candida	14/29
Lymphadenopathy	11/29
Persistent diarrhoea	16/29
Genital warts	5/29 (3 male, 2 female)
Respiratory symptoms	12/29
Aaemia	25/29
Laboratory findings	
Acid-fast bacilli in sputum	4/8
Anergy on skin tests	27/29
Maculopapular rash	9/29
Biopsy-proven Kaposi's sarcoma	3/29
Kaposi's sarcoma on rectal biopsy	0/16
Taenia hominis in stool	1/16
Anergy on controls	2/6

Results

A typical history found in reports of the early cases to the Minister of Health for Uganda would be as follows:-

"In the first six months the patient experiences general malaise and intermittent fevers for which he may treat himself or receive aspirin, chloroquine or chloramphenicol. In due course he develops loss of appetite.

"In the next six months intermittent diarrhoea starts. There is gradual weight loss and the patient is pale. Most patients at this point in time rely on traditional healers, as to many the disease is attributed to witchcraft.

"After one year the patient typically develops a maculopapular rash, which is very itchy, all over the body. The skin becomes ugly with hyperpigmented scars. There may be a cough, usually dry but sometimes productive. By this stage, sometimes earlier, the patient is so weak that, if taken to hospital, not much can be done to help him and death follows".

General Characteristics of Masaka and Rakai Patients (Table I)

21 out of 29 patients were bedridden, 18 had intermittent non-bloody diarrhoea, anorexia, and marked change in taste, with weight loss of more than 10 kg body weight; 1 of these had lost half his normal weight. The oral candidiasis was often gross and described by patients as "coating" in the mouth (fig 2).

Lymphadenopathy in more than one extrainguinal site was common but cervival or occipital involvement was rare. Of

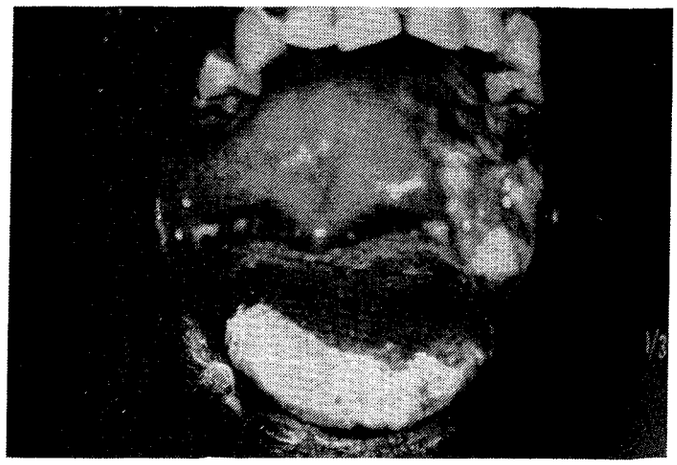

Fig 2—Typical oral lesions.

the 2 women with perineal or vulval warts, 1 had findings suggesting past lymphogranuloma venereum, while another had a high recto-vaginal fistula of recent onset. There was no evidence of Kaposi's sarcoma at proctoscopy, and stool examination revealed abnormalities in only 1 patient (*Taenia hominis*, yeast cells, and occasional *Entamoeba histolytica* cysts). Cryptosporidia were not identified in any of the patients who had diarrhoea. The maculopapular rash affected mainly the limbs but occasionally the trunk. 8 patients had a temperature of 38°C or greater and 9 were hypotensive (lying systolic pressure 100 mm Hg or less).

Patients could be put into one of three major categories, although some overlap did occur.

Gastrointestinal disease.—15 persons had diarrhoea and weight loss of 10 kg or more. All these patients were seropositive for HTLV-III antibodies and all were anergic to skin test antigens.

Pulmonary disease.—12 persons had primarily respiratory symptoms (cough in 12, dyspnoea in 6, haemoptysis in 3), and 8 of these had been admitted to the tuberculosis ward with a provisional diagnosis of pulmonary tuberculosis. Sputum from 4 of them was positive for acid-fast bacilli. All were seropositive for anti-HTLV-III.

Generalised lymphadenopathy.—6 patients had generalised lymphadenopathy and patchy erythema of the palate or fauces as presumptive evidence of the AIDS-related complex (ARC), as defined by current Centers for Disease Control (Atlanta) criteria. They were symptomless and in particular had no wasting or skin rash, no fever and no diarrhoea. 4 of these 6 patients were positive for HTLV-III antibodies and all were anergic on skin testing.

All the 29 patients in table I had a full blood count. 25 had a hypochromic anaemia (Hb<10 g/dl), white-cell counts of $2{\cdot}5$–$4{\cdot}5 \times 10^9$/l (mean $3{\cdot}2 \times 10^9$/l), and absolute lymphocyte counts of 100–1888/μl (mean 664·2/μl). Distribution of T-cell subsets was not determined.

Controls from Masaka and Rakai

5 (17%) of 30 controls were positive for HTLV-III antibodies and 2 out of the 6 persons skin tested were anergic.

Kampala Patients and Controls

34 of 42 slim disease patients seen in Mulago hospital, Kampala (most of whom had come from Masaka or Rakai districts), were HTLV-III-antibody seropositive (table II). Oral candidiasis was present in 34 of 42, and 3 had cryptococcal meningitis. Generalised lymphadenopathy was not a common finding, but 4 out of 34 patients had biopsy-proven lymphadenopathic Kaposi's sarcoma and 1 with oral lesions had disseminated disease. 41 (10%) of 410 healthy medical personnel from Mulago hospital, Kampala, were positive for HTLV-III antibodies.

All the patients with generalised aggressive KS or ARC were seropositive for HTLV-III. Nearly all the ARC patients in this series had diarrhoea and oral candidiasis without marked weight loss and could presumably be early slim disease patients. However, not all those diagnosed as having slim disease had HTLV-III antibodies. We have isolated an HTLV-III-like retrovirus from an HTLV-III-antibody-negative slim disease patient who presented in London (and is therefore not part of this study), and are in the process of detailed characterisation of this virus (Dalgleish AG, Popovic M, Downing B, unpublished).

TABLE II—PREVALENCE OF ANTIBODIES SPECIFIC TO HTLV-III IN STUDY GROUPS

Study group	No positive/ no tested
Slim disease patients in Masaka	29/29
Slim patients not thoroughly investigated (Kampala)	34/42
Patients with generalised aggressive Kaposi's sarcoma (4 of these patients had evidence of slim disease)	11/11
Classical, endemic Kaposi's sarcoma	1/12
AIDS-related complex (ARC)	7/7
Controls (no significant weight loss)	41/410
Controls from paediatric ward in Kampala hospital	0/34
Sexually immature patients (seropositives aged 9, 11, 13, 13)	4/10
Traders from Tanzania	10/15

Some of the controls were related to patients with slim disease. All 5 wives of 5 male slim disease patients were seropositive, although only 1 was ill. 2 of 3 husbands of women with slim disease were seropositive, although both were clinically well.

10 of the control patients were deemed sexually immature; of these, 4 were seropositive (3 males aged 9, 11, and 13 years, and 1 female aged 13 years). Only the 11-year-old boy had clinical symptoms of AIDS-related disease (ARC). The others were symptomless. The youngest person tested was a 2-year-old girl whose father had seropositive slim disease. The infant, like her mother, was well and seronegative.

Short-term Cultures

Lymphocytes from 2 antibody-positive patients with slim disease were grown in short-term culture and co-cultivated with established cell-lines (CEM and HT-H9) positive for CD4 antigen and permissive for HTLV-III replication. Both these cultures showed evidence of reverse transcriptase activity and induced syncytia in the primary peripheral blood monocytes and in established cell-lines. In one of these cultures co-cultivated with HT-H9 cells[5] transient antigen expression was detected by membrane immunofluorescence with high titre HTLV-III-antibody-positive British reference serum on three occasions, although a permanent virus-producing cell-line has not yet been established.

Discussion

"Slim" disease is a new syndrome hitherto unreported in Uganda. It is clearly associated with HTLV-III infection and is not unlike AIDS. It is most unlikely that the disease has not been reported before 1982, since medical records in Uganda are good and go back to 1944. Furthermore, generalised aggressive Kaposi's sarcoma, as distinct from the the classical form, has been described in Uganda since at least 1962, and this could suggest that AIDS has been present since then.

Although many of the features of slim disease satisfy the criteria for AIDS and ARC, it can be distinguished from the other two by the extreme weight loss and diarrhoea. Morover, lymphadenopathy and KS are not as common in slim disease as it is among Western homosexual patients with AIDS, but KS is commoner among slim disease patients than among Western haemophiliacs infected with AIDS virus. Only 4 patients with generalised aggressive KS had the features of slim disease. Although it is impossible to exclude internal KS in these 4 patients, they did not have evidence of KS on oral or rectal biopsy, whereas these biopsies are usually positive if the gastrointestinal tracts is affected by KS. Virtually all slim disease patients had oral lesions, including candidiasis, which

has also been seen in HTLV-III seropositive patients without slim disease. The pulmonary presentation seen in these patients may indicate that tuberculosis (TB) is an opportunistic infection in an area where TB is endemic.

Seronegative slim disease patients may represent an immunologically abnormal subgroup of those with the disease or they may have serum antibodies that recognise a variant antigen not detectable by our ELISA assay which uses reference sera and antigen obtained from British patients, or they may have disease unrelated to HTLV-III infection. Studies designed to elucidate this matter further are in progress.

HTLV-III is now endemic (10%) even among symptomless individuals and it may have been so for at least 10 years, since studies on stored sera have shown that a high proportion of these are positive in the ELISA test for HTLV-III.[9] However, these sera were very sticky and, like other stored sera coming from a region endemic for malaria, may give a false-positive result on direct binding assay systems,[6] or on western blots. However, our survey of HTLV-III seropositivity in Uganda was done by the use of a competitive assay system which is extremely unlikely to be affected by *Plasmodium falciparum* antibodies or other "sticky sera" phenomena. Preliminary evidence indicates that sera taken in 1969 from 300 persons in neighbouring countries are, with one exception, unreactive by competitive ELISA (Desmyter J, Ferns B, personal communication).

If HTLV-III infection has been endemic for many years, why has slim disease suddenly become epidemic, among a rural population that does not seem to have the risk factors that Western patients have for AIDS. It would seem that slim disease is, indeed, recent and that it has spread because of heterosexual promiscuity, which is hard to document in a rural community. Prostitutes and travelling traders are potential sources of infection.

The first recognised cases came from a small village on Lake Victoria, just north of the Tanzanian border. This village was one of many from which goods were traded across the border to Tanzania. The notion that the disease may have been transmitted sexually from Tanzania is interesting since it fits historically with the movements of the Tanzanian army in 1980 and the subsequent regular visits by the Tanzanian traders. Of the 15 traders tested for evidence of HTLV-III antibodies, 10 were positive. These traders admitted to both heterosexual and homosexual casual contacts. Tanzanian soldiers entering Uganda since 1980 have had frequent heterosexual contact with the local population. The only Tanzanian army soldier seen also had serological and clinical evidence of HTLV-III infection. If the virus did indeed come from Tanzania, where did Tanzania get it from? There have been no studies as yet to show whether the virus is endemic in Tanzania and, if so, whether it has been introduced from Uganda via traders and soldiers.

Although the subjects in our study deny overt promiscuous behaviour, their sexual behaviour is, by Western standards, heterosexually promiscuous. The seropositivity in 4 who were sexually immature children raises the possibility of non-sexual spread. However, sexual immaturity is not a reliable indication of sexual inactivity, and we saw few children during our surveys. Paediatricians in Uganda have not seen a change in pattern of disease consistent with HTLV-III infection, but surveys of HTLV-III infection in children are in progress. HTLV-III antibodies were not detected in 34 children attending the main Kampala hospitals.

The virus may also be transmitted by (1) insect vectors, such as mosquitos, bed bugs, or lice, and (2) therapeutic injections. The fact that the disease was first recognised in an exceptionally squalid village suggests that lack of hygiene may be important in its pathogenesis—for example, stagnant water collections may be a breeding ground for mosquitos and overcrowding increases the risk of transmission by insect vectors. If insects are important in transmitting HTLV-III infection, then one would expect a larger pool of infected peoples outside the sexually active group as well as household clustering. Malaria was identified as a major risk factor for Burkitt's lymphoma because the lymphoma did not occur at altitudes where the *Anopheles* mosquito did not occur. Since all our subjects came from the lowlands, we are not able to draw conclusions about an association of HTLV-III infection with altitude or a specific type of mosquito.

Injection needles are commonly re-used in this part of Africa and the frequency of injection abscesses in Uganda suggests that not only are injections common but also that needle sterility is often lacking.[10] However, less than 10% of our study population could remember having had an intramuscular or subcutaneous injection within the preceding 5 years.

Ritual scarification and blood letting practices are not a feature of the peoples of this region of Uganda. Our results suggest that the most likely means of transmission of HTLV-III infection in Uganda is by heterosexual and homosexual contact, and this conclusion is supported by the finding that in Rwanda prostitutes are important in HTLV-III transmission.[11] However, other routes of transmission cannot be ruled out, and further studies are required to clarify the route of transmission and the origin of the virus.

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