AIDS (3)

21.7.84

LOW PREVALENCE IN THE UK OF HTLV-I AND HTLV-II INFECTION IN SUBJECTS WITH AIDS, WITH EXTENDED LYMPHADENOPATHY, AND AT RISK OF AIDS

R. S. TEDDER

Virology Section, Department of Microbiology, Middlesex Hospital Medical School, London

D. C. SHANSON

Department of Microbiology, St Stephen's Hospital, London

D. J. JEFFRIES

Division of Virology, Department of Medical Microbiology, St Mary's Hospital Medical School, London

R. CHEINGSONG-POPOV, P. CLAPHAM, A. DALGLEISH, K. NAGY, R. A. WEISS

Chester Beatty Laboratories, Institute of Cancer Research, London

Summary Antibodies reacting selectively with human T-cell leukaemia virus type I (HTLV-I) were detected in approximately 5% patients with extended lymphadenopathy syndrome (ELAS) and in less than 1% of unselected homosexual patients and drug abusers. None of 22 patients with acquired immunodeficiency syndrome (AIDS) had HTLV-I antibodies and neither did 85 haemophiliacs and 940 blood donors. 3 out of 113 drug addicts had high titres of antibodies to human T-cell leukaemia virus type II (HTLV-II). A T-cell line was derived from 1 of the seropositive ELAS patients. This line was found to be infected with, and releasing, HTLV-I. Infection by HTLV-I and HTLV-II retroviruses thus occurs more frequently in ELAS patients and drug addicts than in the UK population as a whole, but the low prevalence of these infections in ELAS and AIDS patients indicates that these two strains of lymphotropic retroviruses have no aetiological role in ELAS and AIDS.

Introduction

ALTHOUGH there is no certainty about the aetiology of the acquired immunodeficiency syndrome (AIDS), epidemiological evidence strongly indicates that it is caused by a transmissible agent. The transmission of disease by blood or its products, in particular factors VIII and IX, favours this agent being a virus, and so do the close similarities between groups at risk for AIDS and for hepatitis B. A link between human T-cell leukaemia virus type-I (HTLV-I) and AIDS has been suggested. In one study sera from a quarter of the patients with AIDS examined contained antibodies reacting to membrane antigens of HTLV-transformed cells.[1] In addition virus[2] and proviral DNA[3] have been demonstrated in lymphocytes from patients with AIDS. Recently, however, related but distinct retroviruses have emerged as probable causes of AIDS.[4-8]

HTLV-I is a C-type retrovirus[9,10] associated with adult T-cell leukaemia/lymphoma (ATLL) and numerous isolates have been made from tumour cells of ATLL patients. HTLV-II is a distinct but related virus isolated from a single patient with hairy T-cell leukaemia.[11] HTLV-I can be transmitted to lymphoid and non-lymphoid cells by co-cultivation and cell-free filtrates.[10,12,13] Serological surveys indicate that the prevalence of HTLV-I infection varies between different parts of the world. HTLV-I infection is endemic in places such as southwestern Japan,[14] the Caribbean basin,[15] and parts of Africa,[16] and in some regions its prevalence in the population is as high as 20%. Recently the infection has been shown to be transmissible by blood transfusion.[17] Since HTLV-I infection has been reported in American homosexual patients[1-3] we have undertaken a serological survey in Britain of homosexual men and of subjects at risk of blood-borne infections to determine whether HTLV-I infection is incidental, perhaps associated in some patients with persisting lymphadenopathy, rather than a cause of AIDS.

Subjects and Methods

Subjects

Sera were collected from 1982 onwards from 800 homosexual and from 26 promiscuous heterosexual men attending clinics for genitourinary medicine at the Middlesex, St Mary's, and St Stephen's Hospitals in London. Sera from 500 consecutive unselected donors and from 440 selected donors who had been born outside NW Europe were also examined. 85 serum samples were obtained from patients receiving factor VIII replacement therapy. Sera originally received for hepatitis B serology from 113 drug addicts were also tested.

Patients were diagnosed as having AIDS if they satisfied the CDC criteria for life-threatening opportunistic infections and/or Kaposi's sarcoma. The criterion for the extended lymphadenopathy syndrome (ELAS) was lymphadenopathy in two sites other than the inguinal for more than 3 months. Patients in whom lymphadenopathy was found at a single visit and patients with other non-specific symptoms—eg, malaise, night-sweats, and weight loss—were considered separately (LA). Homosexual men attending the clinics for other reasons, mainly for investigation of their hepatitis B status, formed a non-matched control group. Apparently well regular sexual partners of AIDS patients were also considered separately (AIDS contacts).

HTLV-I Antigens (HTLV-IAg)

An established T-cell line producing HTLV-I (C91/PL)[12] was maintained in suspension culture in RPMI 1640 (Flow Laboratories Ltd) supplemented with 10% heat-inactivated fetal calf serum (FCS). Cells were harvested, washed, and pelleted. The pellets were resuspended in distilled water to give a 10% suspension, frozen and thawed three times, pooled, and stored at −40°C. This preparation was used as the HTLV-I antigen (HTLV-IAg) for the competitive radioimmunoassay.

G. SLAMA AND OTHERS: REFERENCES—*continued*

5. Akgün S, Ertel NH. A comparison of carbohydrate metabolism after sucrose, sorbitol, and fructose meals in normal and diabetic subjects. *Diabetes Care* 1980; **3**: 582–85.
6. Jenkins DJA, Wolever TMS, Taylor RH, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* 1981; **34**: 362–66.
7. Bantle JP, Laine DC, Castle GW, Thomas JW, Hoogwerf BJ, Goetz FC. Postprandial glucose and insulin response to meals containing different carbohydrates in normal and diabetic subjects. *N Engl J Med* 1983; **309**: 7–12.
8. Slama G, Klein JC, Tchobroutsky G. The artificial pancreas: a tool for pharmacological studies in type I diabetes. In: Brunetti P, Alberti KGMM, Albisser AM, Hepp K, Massi-Benedetti M, eds. Artificial systems for insulin delivery. New York: Raven Press, 1983: 567–72.
9. Crapo PA, Insel J, Sperling M, Koltermann OG. Comparison of serum glucose, insulin and glucagon responses to different types of complex carbohydrates in non insulin-dependent diabetic patients. *Am J Clin Nutr* 1981; **34**: 184–90.
10. Crapo PA, Reaven G, Olefsky J. Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes* 1976; **25**: 741–47.
11. Conn JW, Newburgh LH. The glycemic response to isoglucogenic quantities of protein and carbohydrate. *J Clin Invest* 1936; **15**: 665–71.
12. Editorial. High-fibre diets and diabetes. *Lancet* 1981; **i**: 423–24.
13. Slama G, Klein JC, Delage A, et al. Correlation between the nature and amount of carbohydrate in meal intake and insulin delivery by the artificial pancreas in 24 insulin-dependent diabetics. *Diabetes* 1981; **30**: 101–05.
14. Keen H, Thomas BJ. Dietary factors in the aetiology of diabetes. In: Turner M, Thomas B, eds. Nutrition and diabetes. London: John Libbey, 1981: 15–22.
15. Dehlinger C, Laffitte A, Truffe P, Slama G, Costagliola D, Celanie E. Goût sucré et consommation de sucres simples chez 98 diabétiques de type 1 et de type 2. *Diabete Metab* 1983; **9**: 169.

21.7.84.

Competitive Radioimmunoassay (RIA)

The competitive RIA was performed essentially in the same manner as previously described for the detection of antibody to varicella-zoster virus[18] but with HTLV-I-specific reagents. Briefly, 100 μl of an optimum dilution of the globulin fraction prepared from an anti-HTLV-positive serum from a British ATLL patient were used to coat strips of flat-bottomed polystyrene wells ('Removawell', Dynatech Ltd). IgG was purified by ion-exchange chromatography from a similar serum and labelled with ^{125}I (Amersham International). After the free binding sites on the solid phase had been washed and blocked with bovine serum albumin (BSA), 100 μl of a dilution of HTLV-IAg were incubated overnight in the wells to immobilise HTLV-IAg via the intermediate antibody on plastic. The antigen-coated solid phase was then washed.

25 μl of anti-HTLV positive and negative sera (controls) and test sera were added separately to single wells. 75 μl of a dilution of ^{125}I-anti-HTLV IgG (containing approximately 60 nCi ^{125}I) were added to each well and the mixture then incubated overnight at room temperature. The wells were then washed and the binding of ^{125}I-anti-HTLV measured. The test variables were arranged so that sera negative for anti-HTLV bound between 1% and 2% of the label while sera containing high levels of anti-HTLV consistently bound less than 0·1%. Sera or serum dilutions were considered to contain anti-HTLV if they reduced the specific label-binding by >50%.

Lymphocyte Cultivation

Fresh heparinised blood was collected from patients whose serum contained anti-HTLV. Lymphocytes were prepared by 'Ficoll-Hypaque' separation and washed in RPMI 1640. Lymphocyte-cultures were set up at a viable cell concentration of 10^6 cells/ml in RPMI 1640 culture medium supplemented with 20% FCS, 10% T-cell growth factor (TCGF), and 1 μg/ml phytohaemagglutinin (PHA, Wellcome Reagents Ltd). The cultures were fed with fresh medium (containing only FCS and TCGF) twice a week. Once a week the cells were harvested and examined for expression of HTLV antigens by indirect immunofluorescence (IF) and for ability to induce syncytium formation with human osteosarcoma (HOS) indicator cells known to be susceptible to HTLV-induced cell fusion.[19]

Indirect Immunofluorescence (IF)

Approximately 2×10^6 cultured lymphocytes were harvested and washed three times with cold RPMI 1640. The final cell pellet was resuspended in 50 μl of RPMI 1640 medium containing 5 μl of anti-HTLV-I-positive serum from a British ATLL patient and incubated for 30 min at 4°C. After being washed the cell pellet was mixed with FITC-conjugated anti-human IgG (Miles-Yeda Ltd) for another 30 min at 4°C. The cells were washed again with cold RPMI before examination for membrane fluorescence under incident UV light.

Syncytium Induction and the Syncytium Inhibition Assay (SIA)

10^5 HOS cells were cocultivated with an equal number of either HTLV-I-producing C91/PL cells, HTLV-II-producing Ton 1 cells,[20] or patients' lymphocyte-culture in 28 mm^2 flat-bottomed microtitre plates as described previously.[19] The cells were incubated at 37°C for 18 h and then stained with methylene blue and basic fuchsin. Cells with 5 or more nuclei were scored as syncytia. To perform the SIA, dilutions of heat inactivated sera were added at the same time as HOS and C91/PL cells were seeded. Sera inhibiting syncytium formation by more than 80% were considered to contain significant levels of anti-HTLV. All sera reactive by RIA were tested by SIA for anti-HTLV-I and anti-HTLV-II.

Pseudotype Neutralisation Assay (PsNA)

Assay for antibody neutralising pseudotype viruses were done with vesicular stomatitis virus (VSV) as a vector of HTLV envelope antigens.[20] All sera reactive by RIA were tested by PsNA for neutralisation of VSV(HTLV-I) and VSV(HTLV-II) pseudotypes.

Results

The proportion of subjects in different groups seropositive for HTLV-specific antibodies by RIA varied (table I). Altogether 11 sera contained anti-HTLV antibodies—7 from homosexual patients and 4 from drug addicts. Anti-HTLV was not detected in sera from patients with AIDS, nor from the haemophiliacs or the blood donors.

The 11 positive sera were further characterised by quantitative titration of antibodies reacting in RIA, SIA, and PsNA. The latter two assays measure antibodies to functional domains of HTLV envelope antigens and can distinguish between anti-HTLV-I and anti-HTLV-II antibody. ELAS, LA, and control homosexuals had serum antibodies predominantly against HTLV-I (table II). However, 3 of the 4 drug addicts had serum antibodies predominantly against HTLV-II. Sera from the addicts gave low-level reactivity in RIA with HTLV-I antigens but in fact contained high titre antibody to HTLV-II. The fourth drug addict had an extremely high titre antibody to HTLV-I.

Attempts were made to isolate HTLV from 2 seropositive ELAS patients and from 3 seronegative AIDS patients by culture of peripheral blood lymphocytes. We could not attempt HTLV isolation from the drug addicts with HTLV-II-specific antibodies because fresh blood was not available. HTLV was detected in culture from only 1 seropositive patient with generalised lymphadenopathy (ELAS 4) whose antibody titre was low. After 1 week in culture lymphocytes from this patient began to express HTLV antigens (0·01% cells by IF with ATLL sera). Within 6 weeks of culture the proportion of cells expressing HTLV had increased to 100%, and a TCGF-dependent, HTLV-producing cell-line, displaying high syncytium induction activity, was

TABLE I—HTLV ANTIBODIES IN PATIENTS ATTENDING GENITO-URINARY MEDICINE CLINICS, HAEMOPHILIACS, DRUG ADDICTS AND BLOOD DONORS

Serum donors	Proportion with antibody to HTLV
AIDS	0/22
ELAS	4/80
LA	1/60
AIDS contacts	0/27
Control homosexuals	2/621
Drug addicts	4/113
Haemophiliacs	0/85
Promiscuous male heterosexuals	0/26
Unselected blood donors	0/500
Blood donors born outside NW Europe	0/440

TABLE II—TITRE AND SPECIFICITY OF HTLV-ANTIBODIES IN HOMOSEXUAL PATIENTS AND DRUG ADDICTS

Serum donors	RIA (HTLV-I)	SIA		PsNA	
		HTLV-I	HTLV-II	HTLV-I	HTLV-II
ELAS 1	300	300	10	1250	<10
ELAS 2	1000	300	300	1250	250
ELAS 3	1000	300	10	1250	10
ELAS 4	30	30	<10	250	<10
LA 1	30	100	<10	50	10
Control homosexual 1	30	100	<10	50	<10
Control homosexual 2	10	30	<10	50	<10
Drug addict 1	10	30	1000	50	6250
Drug addict 2	3	10	1000	10	6250
Drug addict 3	3	30	1000	50	6250
Drug addict 4	3000	300	30	31 250	250

Antibody titres given as reciprocal of serum dilutions.

TABLE III—CHARACTERISATION OF RETROVIRUS PRODUCED BY A T-CELL LINE FROM AN ELAS PATIENT AS HTLV-I BY TYPE-SPECIFIC INHIBITION OF SYNCYTIUM INDUCTION

Human sera	Presence of syncytia at serum dilutions of:	
	1:10	1:50
Control (pooled AB)	+ + +	+ + +
Autologous (ELAS 4)	—	—
Anti-HTLV-I (ATLL)	—	—
Anti-HTLV-II	+ + +	+ + +

Syncytium inhibition was conducted as described previously,[19] with positive controls for HTLV-I and HTLV-II (not shown).
+ + + = >50% nuclei in syncytia.
— = no detectable syncytia.

established. The virus released by this cell-line specifically reacted with anti-HTLV-I in type-specific syncytium inhibition assays (table III) and thus belonged to the HTLV-I serotype.

Discussion

The isolation of HTLV-I from ELAS and AIDS patients[2,3] raised the possibility of a causal relation between HTLV-I and AIDS. However, our data, that HTLV-I infection is rare in subjects at risk of AIDS, do not support this notion. Moreover, the new retroviruses described by Gallo's and Montagnier's groups[4-7] seem to be much more likely aetiological agents of AIDS and ELAS than are HTLV-I and HTLV-II. The high proportion of individuals seropositive for anti-HTLV-I observed by Essex et al[1,21] may be the result of a weak cross-reaction between the membrane antigens of HTLV-I and the new retrovirus strains.

The narrowness of the specificities of the three immunological assays we employed precludes the detection of more distantly related retroviruses, but it is advantageous for screening and titrating antibodies specific to HTLV-I and HTLV-II. Clearly these two serotypes are endemic at low prevalence in male homosexuals and drug addicts in the UK. Previously only HTLV-I infection had been found in Britain and this was exclusively in the Black West Indian community.[22,23]

The prevalence of HTLV-I and HTLV-II among AIDS risk groups may, like infection by hepatitis A and B viruses, reflect the lifestyle of this population. Among homosexuals, the prevalence is highest in patients presenting with the extended lymphadenopathy syndrome (ELAS), of whom 5% are infected. None of the AIDS patients had HTLV-I antibodies, but AIDS and Kaposi's sarcoma later developed in the single seropositive LA patient. Whether HTLV-I infection contributes to the manifestation of ELAS and AIDS is not known since patients with these syndromes tend to be among the more promiscuous in a community and hence at risk of acquiring any infection that is endemic. This question may be answered by longitudinal studies, which should reveal seroconversion rates. With the cause of AIDS and ELAS likely to be another retrovirus, about 5% of patients with these syndromes will be infected with two distinct yet related T-lymphotropic retroviruses, and the possibility of viral recombination merits investigation.

It seems likely that HTLV-I is transmitted sexually among homosexual men. The prevalence of anti-HTLV in drug addicts suggests that HTLV is also spread parenterally; it has already been shown to be transmissible by whole blood transfusions.[17] Yet it is remarkable that 3 of the 4 HTLV-reactive patients who were drug addicts had serum antibody reactive predominantly to HTLV-II. This virus has been reported from only one patient with a T-cell form of hairy-cell leukaemia[11] living in Seattle, USA. A further isolate has recently been obtained from a New York AIDS patient, whom was also an addict on intravenous drugs (Popovic M and Gallo RC, personal communication). Further screening and serotypic analysis of HTLV infection in drug addicts and other patients subject to blood-borne infections may help to elucidate whether infection with this virus is widespread. The difference in serotypes infecting ELAS patients and drug addicts parallels the subtype differences in hepatitis B infections in the two groups in London, where the HBsAg subtype *ay* is predominant in drug addicts and subtype *ad* in homosexuals, indicating little social contact in the UK between the groups.

Whereas the AIDS retrovirus seems to be cytotoxic to $OKT4^+$ T-cells,[4-6] HTLV-I and HTLV-II are associated with T-cell malignancies and are able to transform the same type of cells in vitro.[10,12,24] It will be interesting to follow up ELAS patients infected with HTLV-I since they may be susceptible to T-cell lymphoma. Our study of HTLV infection in British subjects was largely completed before Montagnier's and Gallo's new retrovirus strains became available to us. Our current evidence shows that almost all British ELAS and AIDS patients have antibodies reacting with the new virus. The prevalence of antibodies specific to this virus in British patients with AIDS, ELAS, or at risk of acquiring these syndromes will be the subject of a separate report.

We thank all our colleagues for helpful discussion and those who provided sera and blood samples. Samples from homosexual patients in London were provided by Prof M. W. Adler and Dr J. Bingham (Middlesex Hospital), Dr A. Lawrence, Dr B. Gazzard, and Prof A. Lant (St Stephen's Hospital), and Prof J. R. W. Harris, Dr D. Goldmeier, Dr A. J. Pinching, and Dr J. Weber (St Mary's Hospital). Sera from haemophiliacs were provided by Dr S. J. Machin (Middlesex Hospital) and from blood donors by Dr J. Barbara (North London Blood Transfusion Centre). The study was supported by the Medical Research Council, the Cancer Research Campaign, and The Wellcome Trust.

Correspondence should be addressed to R. A. W., Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JB.

REFERENCES

1. Essex M, McLane MF, Lee TH, et al. Antibodies to cell-membrane antigen associated with HTLV in patients with AIDS. *Science* 1983; **220**: 859–62.
2. Gallo R, Sarin P, Gelmann P, et al. Isolation of human T cell leukemia virus in acquired immune deficiency syndrome. *Science* 1983; **220**: 865–67.
3. Gelmann EP, Popovic M, Blayney D, et al. Proviral DNA of a retrovirus, HTLV, in two patients with AIDS. *Science* 1983; **220**: 862–65.
4. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983; **220**: 868–70.
5. Vilmer E, Barré-Sinoussi F, Rouzioux C, et al. Isolation of new lymphotropic retrovirus from two siblings with haemophilia B, one with AIDS. *Lancet* 1984; i: 753–57.
6. Gallo R, Salahuddin S, Popovic M, et al. Human T-lymphotropic retrovirus, HTLV-III, isolated from AIDS patients and donors at risk for AIDS. *Science* 1984; **224**: 500–03.
7. Sarngadharan M, Popovic M, Brüch L, et al. Antibodies reactive with a human T-lymphotropic retrovirus (HTLV-III) in the sera of patients with acquired immune deficiency syndrome. *Science* 1984; **224**: 506–08.
8. Weiss R. Retroviruses associated with AIDS. *Nature* 1984; **309**: 12–13.
9. Poiesz BJ, Ruscetti FW, Gazdar AF, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980; **77**: 7415–19.
10. Miyoshi I, Kubonishi I, Yoshimoto S, Shiraishi Y. A T-cell line derived from normal human cord leukocytes by co-culturing with human leukemia T-cells. *Gann* 1981; **72**: 978–81.
11. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, et al. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 1982; **218**: 571–73.
12. Popovic M, Sarin PS, Robert-Guroff M, et al. Isolation and transmission of human retrovirus (human T-cell leukemia virus). *Science* 1983; **219**: 856–59.
13. Clapham P, Nagy K, Cheingsong-Popov R, et al. Productive infection and cell free transmission of human T-cell leukemia virus in non-lymphoid cell line. *Science* 1983; **222**: 1125–27.
14. Hinuma Y, Nagata K, Hanaoka M, et al. Adult T-cell leukemia: Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 1981; **78**: 6476–80.

References continued at foot of next page

10. Smythe PM, Breton-Stiles GG, Grace HJ, et al. Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. *Lancet* 1971; ii: 939–43.
11. Brasseur PH, Agrapart M, Ballet JJ, Briulhe P, Warrel MJ, Savanat Tharavany. Impaired cell-mediated immunity in *Plasmodium falciparum* infected patients with high parasitemia and cerebral malaria. *Clin Immunol Immunopathol* 1983; **27**: 38–50.
12. Ganguly NK, Mahajan RC, Gill J, Koshy A. Kinetics of lymphocyte subpopulations and their functions in cases of amoebic liver abscess. *Trans Roy Soc Trop Med Hyg* 1981; **75**: 807–09.
13. Greenwood BM, Whittle HC, Molyneux DH. Immunosuppression in Gambian trypanosomiasis. *Trans Roy Soc Trop Med Hyg* 1973; **67**: 846–50.
14. Montagnier L, McCormick JB, Quinn T, Piot P. Antibody to lymphadenopathy associated viruses in AIDS patients in Zaire. *Science* (in press).
15. Jaffe HW, Bregman DJ, Selik RM. Acquired immune deficiency syndrome in the United States: The first 1000 cases. *J Infect Dis* 1983; **148**: 339–45.
16. Centers for Disease Control. Immunodeficiency among female sexual partners of males with acquired immune deficiency syndrome (AIDS)—New York. *MMWR* 1983; **31**: 697–98.
17. Harris C, Small CB, Klein RS et al. Immunodeficiency in female sexual partners of men with acquired immune deficiency syndrome. *N Engl J Med* 1983; **308**: 1181–84.
18. Pitchenik AE, Fischl MA, Spira TJ. Acquired immune deficiency syndrome in low risk patients. *JAMA* 1983; **250**: 1310–12.
19. Breman JG, Piot P, Johnson KM, et al. The epidemiology of Ebola haemorrhagic fever in Zaire, 1976. In: Pattyn SR, ed. Ebola virus haemorrhagic fever. Amsterdam: Elsevier, 1978: 103–23.
20. Bygbierg IC. AIDS in a Danish surgeon (Zaire, 1976). *Lancet* 1983; i: 925.
21. Vandepitte J, Verwilghen R, Zachee P. AIDS and cryptococcosis (Zaire, 1977). *Lancet* 1983; i: 925–26.
22. Renoirte R, Michaux JL, Gatti D, et al. Nouveaux cas d'histoplasmose africaine et de cryptococcose observés en Republique Democratique du Congo. *Bull Acad Roy Med Belg* 1967; **7**: 465–527.
23. Lamey B, Melameka N. Aspects cliniques et epidemiologiques de la cryptococcose à Kinshasa: à propos de 15 cas personnels. *Med Trop* 1982; **42**: 507–11.