REFERENCES

1. Bothwell TH, Charlton RW, Cook JD, Finch CA. *Iron Metabolism in Man.* Oxford: Blackwell Scientific Publications, 1979.
2. Chanarin I. *The Megaloblastic Anaemias.* Oxford: Blackwell Scientific Publications, 1979.
3. Mayet FGH. Anaemia of pregnancy. *S Afr Med J* 1985; **67**: 804-809.
4. Baynes RD, Meriwether WD, Bothwell TH, Fernandes Costa FJ, Bezwoda WR, MacPhail AP. Iron and folate status of pregnant black women in Gazankulu. *S Afr Med J* 1986; **70**: 148-151.
5. Dommisse J, Du Toit ED. The incidence and treatment of severe pregnancy anaemia in the Cape Town area. *S Afr Med J* 1969; **43**: 244-247.
6. Notelovitz M, Nel M, Southwood B. Latent iron-deficiency anaemia in pregnancy. *S Afr Med J* 1972; **46**: 1300-1304.
7. International Committee for Standardization in Haematology (Iron Panel). Recommendations for the measurement of serum iron in human blood. *Br J Haematol* 1978; **38**: 281-290.
8. International Committee for Standardization in Haematology (Iron Panel). The measurement of total and unsaturated iron-binding capacity in serum. *Br J Haematol* 1978; **38**: 291-294.
9. Conradie JD, Mbhele BEL. Quantitation of serum ferritin by enzyme-linked immunosorbent assay (ELISA). *S Afr Med J* 1980; **57**: 282-287.
10. AVIV. Hematofluorometer model ZPP meter (Manual). Lakewood, NJ: AVIV, 1980.
11. Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency in the US population. *Blood* 1986; **68**: 726-731.
12. Svanberg B, Arvidsson B, Norrby A, Rybo G, Sölvell L. Absorption of supplemented iron during pregnancy — a longitudinal study with repeated bone-marrow studies and absorption measurements. *Acta Obstet Gynecol Scand [Suppl]* 1975; **48**: 87-108.
13. Statistical Analysis System. *SAS User's Guide: Statistics.* Version 5. Cary, NC: SAS Institute, 1985: 156-158.
14. Hoffman RG. Statistics in the practice of medicine. *JAMA* 1963; **185**: 150-159.
15. Cook JD, Alvarado J, Gutnisky *et al.* Nutritional deficiency and anemia in Latin America: a collaborative study. *Blood* 1971; **38**: 591-602.
16. WHO Scientific Group. Nutritional anaemia. *WHO Tech Rep Ser* 1968; No. 405, p. 9.
17. Mayet FGH. The anaemia of pregnancy in Durban. *S Afr J Obstet Gynaecol* 1966; **4**: 15-18.
18. Becker D, Charlton RW, Bothwell TH, Edelstein T, Baumslag N, Metz J. Iron nutrition in pregnant Bantu females. *S Afr Med J* 1970; **44**: 414-417.
19. Ross SM, Read MD, Dhupelia I. Iron prophylaxis in pregnancy — is it useful? *S Afr Med J* 1981; **60**: 698-701.
20. Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. *Am J Clin Nutr* 1976; **48**: 449-455.
21. Metz J, Festenstein H, Welch P. Effect of folic acid and vitamin B_{12} supplementation on tests of folate and vitamin B_{12} nutrition in pregnancy. *Am J Clin Nutr* 1965; **16**: 472-479.
22. Edelstein T, Stevens K, Brandt V, Baumslag N, Metz J. Tests of folate and vitamin B_{12} nutrition during pregnancy and the puerperium in a population subsisting on a suboptimal diet. *J Obstet Gynaecol Br Cwlth* 1966; **73**: 197-204.
23. Hall MH, Pirani BBK, Campbell D. The cause of the fall in serum folate in normal pregnancy. *Br J Obstet Gynaecol* 1976; **83**: 132-136.
24. Rothman D. Folic acid in pregnancy. *Am J Obstet Gynecol* 1970; **108**: 149-175.
25. Chanarin I, Rothman D, Ward A, Perry J. Folate status and requirement in pregnancy. *Br Med J* 1968; **2**: 390-394.
26. Colman N, Barker M, Green R, Metz J. Prevention of folate deficiency in pregnancy by food fortification. *Am J Clin Nutr* 1974; **27**: 339-344.
27. Chanarin I, McFadyen JR, Kyle R. The physiological macrocytosis of pregnancy. *Br J Obstet Gynaecol* 1977; **84**: 504-508.
28. Ek J, Magnus EM. Plasma and red blood cell folate during normal pregnancies. *Acta Obstet Gynecol Scand* 1981; **60**: 247-251.
29. Chanarin I. Folate and cobalamin. *Clin Haematol* 1985; **14**: 629-641.

Isolation of human T-lymphotropic virus type I (HTLV-I) from a black South African with Kaposi's sarcoma

W. B. BECKER, M. C. BOTHA, S. ENGELBRECHT, M. L. B. BECKER

Summary

Serological evidence for HTLV-I infection in the South African population has now been confirmed by the isolation of the virus from the peripheral blood lymphocytes of an adult Tsonga male. The subject was an indigenous black man from the south-eastern Transvaal who had suffered from Kaposi's sarcoma for a decade and in whom serum antibodies against HTLV-I were demonstrated. T-lymphocyte cell lines were established from his peripheral blood lymphocytes and shown to be productively infected with HTLV-I as evidenced by: the characteristic cell morphology; the typical viral morphogenesis on ultra-thin section electron microscopy; the viral genome in DNA extracted from the cell lines; characteristic reverse transcriptase activity and viral specific proteins in the cell culture supernatant fluids. Spread of infection occurs through sexual intercourse, from mother to child, and by blood transfusion. Donated blood should be screened to contain the spread of HTLV-I infection.

S Afr Med J 1988; **73**: 481-483.

Department of Medical Virology, University of Stellenbosch and Tygerberg Hospital, Parowvallei, CP
W. B. BECKER, M.MED. (PATH.), M.D., F.R.C. (PATH.), F.C.M. (PATH.)
S. ENGELBRECHT, M.SC.
M. L. B. BECKER, B.SC. (MED.), M.B. CH.B., PH.D.
Department of Haematology, Medical University of Southern Africa, Pretoria
M. C. BOTHA, F.R.C. (PATH.)

Although the first association of retroviruses with animal cancers dates back to the beginning of this century, it was only in 1978 that the first human retrovirus was isolated in the USA from the cultured T-lymphocytes of a patient with mature T-lymphocyte malignant disease. The characterisation of this virus, which is now the prototype HTLV-I strain, and a second isolate were reported in 1981.[1]

The next important step was to link the virus to the cause of a disease. This could not be done in the early serological

surveys in the USA because very few serum samples reacted positively with the virus. The opportunity to link HTLV-I with adult T-cell leukaemia (ATL) resulted from epidemiological and virological studies in Japan. The Japanese workers found that there was an endemic clustering of ATL in certain areas of Japan. They isolated ATL virus (ATLV) from the peripheral blood lymphocytes of ATL patients and implicated this virus in the disease.[2] ATLV and HTLV-I were subsequently shown to be genetically identical.[3]

The approximately 100 known isolates of HTLV-I are genetically homogeneous, with the exception of a strain which was isolated from a Central African immigrant to Europe. This virus strain differed enough genetically from the other isolated strains to be designated HTLV-Ib.[4] The possible extent of genetic variation in HTLV-I is not known.

HTLV-I does not only cause malignant disease but can also cause lesser degrees of immunosuppression which are rarely as severe as that resulting from HIV infection with its consequent opportunistic infections. The possibility should be considered that the endemic form of Kaposi's sarcoma seen in black Africans may be a result of HTLV-I infection.

Several serological surveys for HTLV-I antibodies have been made in South Africa but no virus isolations have previously been reported.[5-7] The isolation of HTLV-I from an indigenous black South African with Kaposi's sarcoma is reported.

Patient and methods

The patient was a 75-year-old black man from the Tsonga tribe, who presented on 6 April 1976 with painful nodules on the lower legs and feet. Histological examination confirmed the diagnosis of Kaposi's sarcoma. He reported again on 28 March 1979 with a recurrence of the lesions on the lower extremities. Kaposi's sarcoma was again confirmed histologically. He was subsequently seen at intervals of 6 - 12 months with recurrent and new lesions affecting both upper and lower extremities. On 8 November 1985 a nodule had also developed on his chest. His lesions had been treated by excision with skin grafting to the defects or by radiotherapy. His clinical condition otherwise appeared normal. Laboratory investigations revealed that the patient's helper/suppressor lymphocyte ratio and the mitogen response of the lymphocytes were normal. The blood biochemistry and full haematological investigations were essentially normal. However, serum antibodies to HTLV-I were detected.

Lymphocyte cultures

Peripheral blood lymphocyte (PBL) cultures were established and maintained as described previously.[8] Briefly, PBLs were separated from heparinised blood by centrifugation on a Ficoll gradient and cultured in an atmosphere of 5% carbon dioxide in air in RPMI 1640 medium supplemented with 0,2% $Na(HCO_3)_2$, 20% heat-inactivated fetal calf serum, 2 mM L-glutamine, antibiotics, 10 - 20% interleukin-2 and sometimes with 100 μg/ml PHA. Polybrene 2 μg/ml and hydrocortisone 2,5 - 5,0 μg/ml were added to some cultures. Medium was replaced at 1 - 3-day intervals.

Reverse transcriptase assay

The supernatants of infected and control PBL cultures were clarified of cells and cellular debris by centrifugation and then pelleted by ultracentrifugation. The reverse transcriptase activity was assayed on the pelleted virus as described elsewhere.[9] After subtraction of the background reading obtained from uninfected control cultures, the reverse transcriptase activity of infected culture supernatant fluid (SNF) was expressed as incorporated radioactivity in counts per minute per millilitre (cpm/ml) of SNF.

Immunofluorescence assay

Immunofluorescence assay, as previously described,[8] was used either to demonstrate viral antigen in cells using known reference sera or to detect specific antibodies in sera using reference virus-infected cell culture lines.[6]

Electron microscopy

Cells from infected and control PBL cultures were prepared for ultrathin-section electron microscopy as described previously.[8]

Southern blot DNA restriction analysis and specific HTLV-I DNA probing

High-molecular weight DNA was extracted from infected PBL cultures by standard methods. An appropriate aliquot of DNA was digested with Sac 1 endonuclease as recommended by the suppliers (Boehringer Mannheim GmbH — Biochemica, Mannheim, FRG). The DNA digest fragments were separated by gel electrophoresis and transferred to Hybond N membranes (Amersham International, Buckinghamshire, England) by Southern blotting.[10] The membrane-bound DNA was tested by standard methods for hybridisation with the Sac 1 endonuclease digest fragment of the HTLV-I genome[11] kindly supplied by R. Gallo. The membranes were washed under highly stringent conditions, air dried, and exposed onto X-ray film to detect radioactivity which would indicate specific hybridisation between the probe and the test DNA thereby confirming the presence of HTLV-I genome in the test DNA.

Results

Specimens of peripheral blood with anticoagulant were received on 21 August 1985, 22 November 1985, 10 March 1986 and 16 August 1986. Continuous cell lines were established from the PBLs of these samples and some lines have been cultured for more than a year. These lymphocyte cell lines are productively infected with HTLV-I as evidenced by the following criteria: (i) the cell lines show the characteristic morphology of lymphocytes transformed and immortalised by infection with HTLV-I including the formation of multinucleated giant cells with lobulated nuclei; (ii) HTLV-I-specific antigens have been demonstrated in the cytoplasm by the immunofluorescence antibody technique using reference positive and negative sera; (iii) transmission electron microscopy of ultrathin sections of the cells showed the characteristic morphogenesis and morphology of HTLV-I viral particles (Fig. 1); (iv) pelleted virus from the supernatant of the cell cultures showed characteristic reverse transcriptase activity; and (v) DNA extracted from the cultured cell lines and analysed by restriction enzyme digestion and the Southern blot technique hybridised specifically with the reference HTLV-I DNA probe kindly provided by R. Gallo (Fig. 2).

Discussion

The routes of transmission of HTLV-I and HIV infections are similar, but HTLV-I characteristically spreads heterosexually within the family context between spouses and from mother to child. Virus-infected lymphocytes circulate in the peripheral blood so that donated blood is also a means of transmission. These features have been well documented in Japan where the infection rate of the population in certain localities may be as high as 37% and blood donations are routinely screened.[12,13]

Presumably this high prevalence in Japan has arisen over a period of time and a similar trend may eventually follow in other countries which have a low prevalence rate at the moment, unless the routine screening of donated blood is instituted.
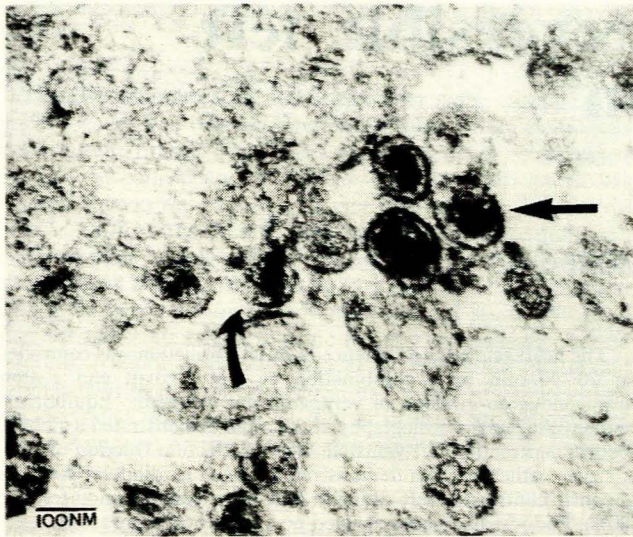
Fig. 1. Transmission electron micrograph: ultrathin section of PBL cell line FS showing characteristic type C retrovirus particles in the intercellular spaces (straight arrow) **and viruses budding from the cytoplasmic membrane** (curved arrow).
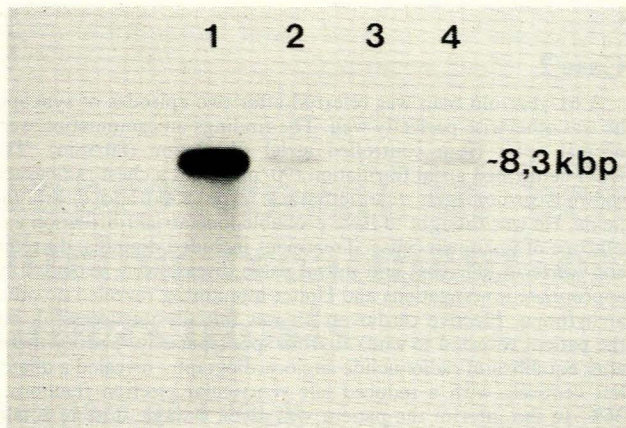

Fig. 2. Specific probing of cellular DNA for HTLV-I genomic DNA. Autoradiograph of Southern blot hybridisation test: 15 μg of each test DNA and 250 pg of pMT-2 positive control DNA were digested with Sac 1 endonuclease, electrophoresed on a 0,6% agarose gel, blotted onto a Hybond N nylon membrane and hybridised with the HTLV-I probe pMT-2. Lane 1 — positive control: HTLV-I pMT-2 probe DNA; size 8,3 kilo-base pairs. Lanes 2, 3 and 4 — high molecuar weight DNA extracted from three different cell lines established with the patient's PBLs hybridise with the specific HTLV-I probe confirming the content of HTLV-I genomic DNA.

A recent phenomenon has been the increasing incidence of HTLV-I infection in intravenous drug abusers often in conjunction with HIV infection.[14]

A low prevalence of HTLV-I antibodies in the RSA was revealed by recent serological surveys including apparently healthy persons.[5-7] This sero-epidemiological evidence of the occurrence of HTLV-I infection has now been confirmed by the isolation of HTLV-I from a seropositive indigenous black man.

The routine screening of blood donors should be instituted in South Africa. This would be based on tests for specific serum antibodies to HTLV-I at least until tests for the viral antigens or genome become a practical possibility.

The relative neglect of HTLV-I infection seems to be because of the long incubation period of 5-30 years as well as the less dramatic course of the associated disease compared with HIV. How high must the prevalence rate rise before preventive action is taken?

REFERENCES

1. Poiesz B, Ruscetti FW, Reitz MS, Kalyanaraman VS, Gallo R. Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukaemia. *Nature* 1981; **294:** 268-271.
2. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterisation of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA* 1982; **79:** 2031-2035.
3. Wanatabe T, Seiki M, Yoshida M. HTLV type I (US isolate) and ATLV (Japanese isolate) are the same species of human retrovirus. *Virology* 1984; **133:** 238-241.
4. Hahn B, Shaw GM, Popovic M, Lo Monico A, Gallo R, Wong-Staal F. Molecular cloning and analysis of a new variant of human T-cell leukaemia virus (HTLV-Ib) from an African patient with adult T-cell leukaemia-lymphoma. *Int J Cancer* 1984; **34:** 613-618.
5. Saxinger W, Blattner WA, Levine PH *et al.* Human T-cell leukemia virus (HTLV-I) antibodies in Africa. *Science* 1984; **225:** 1473-1476.
6. Becker WB, Becker MLB, Homma T, Brede HD, Kurth R. Serum antibodies to human T-cell leukaemia virus type I in different ethnic groups and in non-human primates in South Africa. *S Afr Med J* 1985; **67:** 445-449.
7. Botha MC, Jones M, De Klerk WA, Yamamoto N. Distribution and possible spread of human T-cell leukaemia virus type I in human communities in the northern and eastern Transvaal. *S Afr Med J* 1985; **67:** 668-671.
8. Becker MLB, Spracklen FN, Becker WB. Isolation of a lymphadenopathy-associated virus from a patient with the acquired immune deficiency syndrome. *S Afr Med J* 1985; **68:** 144-147.
9. Rubsamen-Waigmann H, Becker WB, Helm EB *et al.* Isolation of variants of lymphocytopathic retroviruses from the peripheral blood and cerebrospinal fluid of patients with ARC or AIDS. *J Med Virol* 1986; **19:** 335-344.
10. Southern EM. Detection of specific sequences among DNA fragments separated by electrophoresis. *J Med Biol* 1975; **98:** 503-517.
11. Clarke MF, Gelman EP, Reitz S jun. Homology of human T-cell leukemia virus envelope gene with class I HLA gene. *Nature* 1983; **305:** 60-62.
12. Hinuma Y, Komoda H, Chosa T *et al.* Antibodies to adult T-cell leukaemia virus-associated antigen (ATLA) in sera from patients and controls in Japan: a nation-wide sero-epidemiologic study. *Int J Cancer* 1982; **29:** 631-635.
13. Kamihira S, Nakasima S, Oyakawa Y *et al.* Transmission of human T-cell lymphotropic virus type I by blood transfusion before and after mass screening of sera from seropositive donors. *Vox Sang* 1987; **52:** 43-44.
14. Weiss SH, Ginzburg HM, Saxinger WC *et al.* Emerging high rates of human T-cell lymphotropic virus type I (HTLV-I) and HIV infection among USA drug abusers. Proceedings of the III International Conference on AIDS, Washington D.C., 1-5 June 1987. Washington D.C.: US Dept of Health and Human Services and the World Health Organization, 1987: 211.