The Lancet · Saturday 1 September 1984

PREVALENCE OF ANTIBODY TO HUMAN T-LYMPHOTROPIC VIRUS TYPE III IN AIDS AND AIDS-RISK PATIENTS IN BRITAIN

R. CHEINGSONG-POPOV R. A. WEISS
A. DALGLEISH

Institute of Cancer Research, Chester Beatty Laboratories, London

R. S. TEDDER D. C. SHANSON
D. J. JEFFRIES R. B. FERNS
E. M. BRIGGS

Departments of Microbiology of Middlesex, St Stephen's and St Mary's Hospitals and Medical Schools, London

I. V. D. WELLER S. MITTON
M. W. ADLER

Academic Department of Genito-urinary Medicine, Middlesex Hospital Medical School, London

C. FARTHING A. G. LAWRENCE
B. G. GAZZARD

St Stephen's Hospital, London

J. WEBER J. R. W. HARRIS
A. J. PINCHING

St Mary's Hospital Medical School, London

J. CRASKE

Regional Virus Laboratory, Public Health Laboratory, Withington Hospital, Manchester

J. A. J. BARBARA

North London Blood Transfusion Centre, Edgware, Middlesex

Summary 2000 persons in the UK were examined serologically for antibodies to human T-lymphotropic virus type III (HTLV-III). Sera reacting in a membrane immunofluorescence assay (IFA) to HTLV-III were also positive when tested against cells infected with lymphadenopathy virus (LAV-1), and cross-adsorption tests indicated that these retroviruses are probably identical. A competitive radioimmunoassay (RIA), which was wholly concordant with IFA, was used to screen the sera. 30/31 patients with the acquired immunodeficiency syndrome (AIDS) were seropositive, as were 89% patients with persistent generalised lymphadenopathy (PGL), 17% symptomless homosexual men, 34% haemophiliacs receiving pooled clotting factors, and 1·5% intravenous drug abusers. None of more than 1000 unselected blood donors was seropositive. These data confirm the close association between HTLV-III and AIDS and PGL and show that infection with HTLV-III is also prevalent in the populations in whom these syndromes are most likely to develop. However, it would be unwise to presume that AIDS will necessarily develop in seropositive subjects.

Introduction

THE likelihood of the acquired immunodeficiency syndrome (AIDS) being caused by an infectious agent has been apparent for some years. The exponential rise in the number of cases of this disease has been restricted to certain well-defined risk groups in a pattern that strongly suggests an agent transmissible by sexual or blood contact.[1,2] Case-clustering supports this hypothesis. At about the same time that AIDS emerged, a syndrome of unexplained persistent generalised lymphadenopathy (PGL, formerly known as the extended lymphadenopathy syndrome) appeared in the same risk groups. Although the USA has borne the brunt of the AIDS epidemic so far, there is an increasing prevalence of the disease elsewhere, including Europe.[1] In Britain, the number of cases has risen from 13 in June, 1983, to 51 in June, 1984. Similarly there has been a rise in the prevalence of PGL, especially in homosexual men, since early 1983. Initially thought to be a prodromal phase of AIDS, PGL now seems in many instances to reflect a different response to the same agent.[1,2,4]

Recent evidence has strongly implicated newly identified retroviruses as the cause of AIDS and PGL. These viruses have been termed lymphadenopathy-associated or immunodeficiency-associated virus (LAV/IDAV)[5,6] and human T-lymphotropic virus type III (HTLV-III),[7] and they seem to have more similarities than differences.[8] To date the evidence implicating them aetiologically comprises a high frequency of virus isolation from AIDS and PGL patients[7] and high prevalence of specific antibody in these subjects.[9,10] A notable feature of these retroviruses is their tropism for the T "helper" ($T4^+$) lymphocytes,[11] which typically are depleted in AIDS patients.

Only limited studies of antibody prevalence have been reported[9,10,12,13] for the groups at risk for AIDS. These studies have indicated a moderately high prevalence of seropositivity in apparently symptom-free homosexuals, haemophiliacs, and intravenous drug abusers. Evidence of a rapidly increasing prevalence of HTLV-III antibody in symptom-free homosexuals in San Francisco has been

reported,[13] strongly supporting the concept of a "new" infectious agent spreading within that community. We have determined the prevalence in Britain of HTLV-III antibodies in patients with AIDS or PGL, in subjects considered to be at risk for these disorders, and in blood donors. The methods used were similar to those previously applied to HTLV-I and HTLV-II.[14] We also show that antibodies present in sera from patients with AIDS and PGL recognise viral membrane antigens of both HTLV-III and LAV-1.

Materials and Methods

Subjects

The subjects consisted of the following groups of people:

(1) Patients with AIDS, identified clinically as opportunistic infection and/or Kaposi's sarcoma according to the Centers for Disease Control (CDC) definition. Included in this group was 1 homosexual man with B-cell lymphoma, which is outside the precise surveillance definition.

(2) Patients with PGL, defined as unexplained lymphadenopathy in two or more extra-inguinal sites and lasting for more than 3 months.

(3) Symptomatic homosexual patients, being those with transient lymphadenopathy and/or weight loss, malaise, or pyrexia.

(4) Sexual contacts of AIDS patients, some regular and some casual.

(5) Homosexuals-at-risk, consisting of patients being screened serologically for hepatitis B or syphilis at genito-urinary medicine clinics and two cohorts of symptom-free subjects who volunteered for studies on AIDS.[15,16]

(6) Heterosexual subjects who were recruited from genito-urinary medicine clinics.

(7) Intravenous drug abusers being screened for hepatitis B during 1983 and 1984.

(8) Haemophiliacs undergoing regular clotting factor replacement therapy, sometimes with American commercial factor VIII concentrate.

(9) 1000 unselected blood donors.

The patients were drawn from the Middlesex, St Mary's, and St Stephen's Hospitals. Blood from groups (1)–(6) was collected between June, 1983, and July, 1984, and the sera were stored at −20°C or lower. Sera from haemophiliacs had been collected since 1982.

Virus and Cells

HTLV-I, HTLV-II, and HTLV-III were kindly supplied by M. Popovic and R. C. Gallo (National Cancer Institute, Bethesda, Maryland, USA) and LAV-1 by L. Montagnier (Institut Pasteur, Paris, France). HTLV-III was provided as a persistently infected cell-line HT, clone H9 (HT-H9).[17] HTLV-I and HTLV-II were grown in persistently infected T-cell lines (C91/PL and Ton 1, respectively), as described previously.[14] LAV-1 was provided as cell-free culture supernatant; HT-H9 and CEM cells were also infected with LAV-1 (Clapham P, Weiss RA, and Montagnier L, unpublished). Uninfected HT-H9 and CEM cells were used as negative antigen controls in the serological studies. All cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum.

Immunofluorescence Assay (IFA) for Antibody to HTLV-III Membrane Antigens (HTLV-III-MA)

Approximately 2×10^6 cultured HT-H9 cells infected with HTLV-III or LAV-1 were harvested and washed with complete phosphate buffered saline (PBS), before being incubated with a 1/10 dilution of test serum for 45 min at 37°C. After this the cell pellet was washed, then mixed with FITC-conjugated anti-human IgG (Miles Yega Ltd) for a further 45 min at 37°C. The cells were washed thrice with cold PBS before being examined for membrane fluorescence under incident UV light microscopy. CEM cells infected with LAV-1 (CEM/LAV) were treated in the same way.

Competitive Radioimmunoassay (RIA) for Antibodies to HTLV-III

The assay was conducted in a manner similar to that previously reported[14] for HTLV-I. Briefly, the assay was based on a competitive radioimmunoassay whereby ^{125}I-labelled human anti-HTLV-III competed with test serum for binding to solid-phase immobilised HTLV-III antigen. The following reagents were used:

(i) Anti-HTLV-III globulin. An ammonium sulphate precipitate was prepared from a serum sample selected from among the subjects studied to give high specific binding when radiolabelled (see below). 100 µl at an optimum dilution were used to coat 'Removawell' strips (Dynatech Ltd). Excess binding sites on these wells were quenched by addition of 0·2% bovine serum albumin (BSA) in 0·02 mol/l "tris" buffer containing 0·1% sodium azide (tris-BSA).

(ii) ^{125}I-anti-HTLV-III IgG. An ion-exchange chromatography fraction of IgG (0·02 mol/l phosphate buffer, pH 8·0) was made from the selected high-titre human anti-HTLV-III serum. The IgG preparation was labelled with ^{125}I to a specific activity of 15 µCi per µg protein. For use, this was diluted to 100 nCi per 100 µl in tris containing 2% BSA and 20% normal human serum (NHS) negative for all antibody markers of HTLV-I, -II, and -III.

(iii) HTLV-III antigen (HTLV-III-Ag). Cell pellets from batch cultures of HT-H9 cells infected with HTLV-III were suspended in distilled water at 5% v/v and subjected to three freezing and thawing cycles. Cellular debris was removed by centrifugation and the extract, HTLV-III-Ag, was stored at −20°C. Before use it was diluted in tris-BSA containing 0·1% Tween 20 and incubated for 30 min at 37°C. The same extraction method was used to prepare LAV antigen from CEM/LAV cells.

Solid-phase HTLV-III-Ag was prepared by placing 100 µl of antigen (diluted in tris-BSA containing Tween 20) in globulin-coated wells and incubating it for 2–3 days at room temperature. After aspiration the wells were filled with 1% formaldehyde in tris for 30 min at room temperature, then washed and stored wet before use in the competitive RIA. 25 µl of NHS, positive control sera, or test sera were added separately to individual wells, followed by 75 µl of appropriately diluted ^{125}I-anti-HTLV-III IgG. The mixture was incubated overnight in the wells which were then washed four times with de-ionised water. Bound label was measured in a 16-channel counter (NE 1600, Nuclear Enterprises Ltd). In the presence of NHS and sera from blood donors, approximately 2% of the added label was bound. In contrast, when maximum inhibition occurred only 0·1% of the radiolabel was bound. The binding ratio (BR), representing the ratio of counts per minute (cpm) in wells containing NHS to that in wells containing positive control sera, was influenced by the amount of HTLV-III-Ag present during coating. To conserve supplies of antigen, it was used at dilutions which would routinely give a BR of 10. Sera or serum dilutions reducing the binding of label by more than 67% were considered to contain significant levels of anti-HTLV-III.

Results

Comparison of Tests

Sera from AIDS patients at 1/10 dilution yielded brilliant surface fluorescence on HT-H9 cells infected with HTLV-III or LAV-1 and on CEM/LAV cells, but negligible fluorescence on uninfected HT-H9 and CEM cells. Normal human sera were negative on all these cells. AIDS sera (1/10 dilution) produced only very faint fluorescence on cells infected with HTLV-I and HTLV-II. IFA was thus a specific test for antibody to HTLV-III-MA.

Sera which were reactive by IFA were all positive with RIA. Table I shows the concordance of IFA and RIA for HTLV-III antibodies in 110 sera taken from the different risk groups. 30 sera were tested for antibodies to HTLV-III and LAV-1 by both RIA and IFA; sera positive in any assay gave positive reactions in all four assays. Moreover, one high titre serum, diluted 1/10 000, was adsorbed to excess HT-H9 control cells, or to the same cells infected with HTLV-III or

TABLE I—CORRELATION OF MEMBRANE IMMUNOFLUORESCENCE ASSAY (IFA) AND COMPETITIVE RADIOIMMUNOASSAY (RIA) FOR HTLV-III ANTIBODIES IN HUMAN SERA

—	RIA +ve	RIA −ve
IFA +ve	80	0
IFA −ve	0	30

TABLE II—HTLV-III ANTIBODIES IN DEFINED PATIENT GROUPS SCREENED BY RIA

Serum donor	Proportion with HTLV-III antibodies
AIDS patients	30/31 *(97%)*
PGL patients	110/124 *(89%)*
Symptomatic homosexuals	41/69 *(59%)*
Contacts of AIDS or PGL	15/36 *(42%)*
Homosexuals at risk	53/308 *(17%)*
Heterosexuals from genito-urinary clinics	0/35 *(0%)*
Haemophiliacs who have received pooled clotting factors	63/184 *(34%)*
Intravenous drug abusers	4/269 *(1·5%)*
Unselected blood donors	0/1042 *(0%)*

with LAV-1. The diluted serum was then tested by IFA on infected cells. Each virus-infected line, but not the control cells, adsorbed antibody reacting with both viruses, thus demonstrating the close similarity between the membrane antigens of HTLV-III and LAV-1.

Prevalence of Antibody

Table II shows the prevalence of antibody to HTLV-III in the subjects studied. All but 1 of the AIDS patients were seropositive. The homosexual patient with B-cell lymphoma was also positive. Although falling clearly into the CDC definition of AIDS, the seronegative AIDS patient was unusual clinically;[17] his illness had remained remarkably benign for 5 years, manifesting only as cutaneous Kaposi's sarcoma.

The prevalence of anti-HTLV-III was high in patients with PGL and in symptomatic homosexuals. Anti-HTLV-III was also detected in 17% of the homosexuals at risk and in 34% of the haemophiliacs. Only 1·5% of the intravenous drug abusers were seropositive, and no antibodies to HTLV-III were detected in the donors.

12 positive serum samples taken from the different risk groups were titrated by RIA. Antibody titres (causing >67% inhibition) ranged from 1/30 to 1/10 000. IFA gave equivalent or slightly lower titres.

Discussion

Two simple, reliable, and specific assays for the detection of antibodies to HTLV-III have been described. Findings obtained by competitive RIA shows complete concordance with those obtained by the membrane IFA, indicating that results obtained by the two assays are comparable and that the RIA detects antibody and not antigen. In addition, the assays indicate that HTLV-III and LAV-1 are indistinguishable. Our preliminary data show a wide range of antibody titres in the groups examined but the significance of this needs further evaluation. We do not know what class of antibodies is being detected and against what viral antigens they are directed at in the RIA. Nevertheless, an analogous RIA has already proved to be of value[14] for serological screening of HTLV-I and HTLV-II, and these data show the validity and usefulness of applying competitive RIA to the detection of HTLV-III.

We detected antibodies to HTLV-III in all but one of our AIDS patients, which strengthens the evidence that HTLV-III is aetiologically related to AIDS. The fact that the homosexual man with B-cell lymphoma was seropositive supports the view that this tumour may be related to AIDS, although the surveillance definition has excluded it. The only patient with AIDS who was seronegative has also been atypical clinically, in that his illness has been unusually benign.[18] The findings in this patient indicate that when a patient satisfies the CDC definition of AIDS, the absence of demonstrable anti-HTLV-III should not at present exclude a diagnosis of AIDS.

The great majority of the PGL patients were seropositive for HTLV-III, despite the non-specific definition of this syndrome. This finding confirms the notion that HTLV-III is not only the cause of AIDS but is also the cause of PGL in epidemiologically related risk groups. Evidence of HTLV-III infection in such a high proportion of PGL patients who lack evidence of opportunistic infection argues strongly against the possibility that the retrovirus is itself acting as an opportunist in this setting. The fact that some PGL subjects were seronegative could indicate either that their lymphadenopathy was unrelated to HTLV-III infection or perhaps that they did not produce a detectable antibody response to HTLV-III. Some of these patients have shown spontaneous resolution of their lymphadenopathy,[19] and the role of HTLV-III in PGL will be the subject of further study. The symptomatic homosexual group was clearly heterogeneous and will require detailed analysis. Some had symptoms resembling those seen in "prodromal AIDS"[4] (unexplained fever, weight loss, diarrhoea, oral candidiasis, and lymphopenia lasting more than 6 months), while in others the symptoms may have been due to intercurrent infections that were unrelated to AIDS or PGL. Serological studies on other disorders in homosexual men, such as idiopathic thrombocytopenic purpura, which may be related to AIDS on epidemiological grounds, will help to clarify the spectrum of disease caused by HTLV-III. The 17% prevalence of HTLV-III antibodies in the homosexuals-at-risk corresponds to that found in San Francisco in 1980.[13] Many members of this group are regular attenders at genito-urinary medicine clinics and may not be representative of the homosexual community as a whole.

The prevalence of seropositivity among members of risk groups is striking and requires careful interpretation. Even if HTLV-III is causally related to AIDS and PGL, as is strongly suggested by the evidence, we should not assume that these disorders will develop in all patients infected with this retrovirus. Symptomless seroconversion or seroconversion accompanied by mild symptoms is often seen for many infections, including retroviruses.[20] Although it is too early to draw firm conclusions, it seems possible that overt disease will not develop in at least some, and perhaps the majority, of seropositive subjects. On the other hand, the long latent period and a possible role for co-factors in determining the expression of disease make such a suggestion tentative.

HTLV-III infection might be accompanied by continuing virus replication and the release of virus into blood and possibly other body fluids, which could lead to a carrier state. Some patients with AIDS and PGL seem to behave as carriers, though many may be infectious before they have symptoms of the disease. The accompanying report by Gazzard et al[21] indicates that direct contacts between AIDS patients were rare, implying that patients with PGL were able to transmit infection.

The relatively low prevalence of HTLV-III antibodies in intravenous drug abusers, among whom needle-sharing is presumed to be the means of spread, indicates that the

retrovirus has not spread widely in this community, possibly because of the infrequent overlap between drug abuse and homosexuality in Britain. It will be important to follow antibody prevalence in drug abusers in Britain, especially since HTLV-II antibodies have been found unexpectedly in them.[14]

The high prevalence of HTLV-III antibodies in haemophiliacs found in this and other studies[12] has to be set against the relatively low incidence of disease in this risk group so far—roughly one per thousand haemophiliacs. This high antibody prevalence also shows that the retrovirus, or its antigen, is present in pooled blood products, especially factor VIII concentrates. The likelihood that infection resulted from commercial rather than National Health Service factor VIII concentrates is increased by our failure to detect HTLV-III antibody in over 1000 blood donors from the North London Blood Transfusion Centre. This finding is also reassuring as to the low risk at present of acquiring HTLV-III infection or AIDS by blood transfusion in Britain.

In the setting of blood transfusion it must be assumed that HTLV-III seropositive donors are infectious, as are those seropositive for antibody to HTLV-I.[22] In homosexual patients, it would also be prudent for the time being to assume that those who are seropositive are contagious and to counsel them accordingly. However, it is likely that not all seropositive subjects will be able to transmit the virus sexually, and it may be misleading to presume that overt disease will develop in all such persons. Our limited knowledge about the significance of seropositivity in persons who are not ill means that great sensitivity is required during counselling. It should also be remembered that some seronegative persons might be infectious. Thus until the whole spectrum of host responses to HTLV-III is better defined, the conclusions that can be drawn from a test for antibodies to this virus are strictly limited. What is certain, however, is that a test for anti-HTLV-III is not the same as a test for AIDS.

We thank Dr M. Popovic, Dr R. C. Gallo, and Dr L. Montagnier for providing HTLV-III and LAV; Mr P. Clapham and Mr M. Exley for propagating virus-infected cells and for producing viral antigen; Dr S. Machin, department of haematology, Middlesex Hospital Medical School, London, Prof L. Luzzato, department of haematology at the Royal Postgraduate Medical School, London, and Dr C. R. Rizza, Haemophilia Centre, Churchill Hospital, Oxford, for providing sera from haemophiliacs; the Virus Reference Laboratory, Central Public Health Laboratory, for sera from drug abusers; and numerous colleagues for sera from individual patients. This study was partly supported by the Wellcome Trust, the Medical Research Council, and the Cancer Research Campaign.

Correspondence should be addressed to R. A. W.

REFERENCES

1. Allen JR. Epidemiology of the acquired immunodeficiency syndrome (AIDS) in the United States. *Sem Oncol* 1984; **11**: 4–11.
2. Pinching AJ. The acquired immune deficiency syndrome. *Clin Exp Immunol* 1984; **56**: 1–13.
3. Biggar RJ, Bouvet E, Ebbesen P, Faber V, Kochi MA, Melbye M, Velimirovic B, eds. The epidemiology of AIDS in Europe. *Eur J Cancer Clin Oncol* 1984; **20**: 157–64.
4. Mildvan D, Mathur U. Prodromal syndromes in AIDS. *Ann NY Acad Sci* (in press).
5. 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.
6. 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.
7. Gallo RC, Salahuddin SZ, 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.
8. Weiss RA. Retroviruses linked with AIDS. *Nature* 1984; **309**: 12–13.
9. Brun-Vezinet F, Rouzioux C, Barré-Sinoussi F, et al. Detection of IgG antibodies to lymphadenopathy associated virus in patients with AIDS or lymphadenopathy syndrome. *Lancet* 1984; i: 1253–56.

References continued at foot of next column

R. CHEINGSONG-POPOV AND OTHERS: REFERENCES—*continued*

10. Safai B, Sarngadharan MG, Groopman JE, et al. Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome. *Lancet* 1984; i: 1438–40.
11. Klatzmann D, Barré-Sinoussi F, Nugeyre MT, et al. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* 1984; **225**: 59–63.
12. Melbye M, Biggar RJ, Chermann JC, Montagnier L, Stenbjerg S, Ebbesen P. High prevalence of lymphadenopathy virus (LAV) in European haemophiliacs. *Lancet* 1984; ii: 40–41.
13. Centers for Disease Control. Antibodies to a retrovirus etiologically associated with acquired immunodeficiency syndrome (AIDS) in populations with increased incidences of the syndrome. *MMWR* 1984; **33**: 377–79.
14. Tedder R, Shanson D, Jeffries D, et al. Low prevalence in the UK of HTLV-I and HTLV-II infection in subjects with AIDS, with extended lymphadenopathy, and at risk of AIDS. *Lancet* 1984; ii: 125–28.
15. Pinching AJ, McManus TJ, Jeffries DJ, et al. Studies of cellular immunity in male homosexuals in London. *Lancet* 1983; ii: 126–30.
16. Weller IVD, Crawford DH, Iliescu V, et al. Homosexual men in London; immunological status, Epstein Barr virus infection and lymphadenopathy. *Ann NY Acad Sci* (in press).
17. Popovic M, Sarngadharan MG, Read E, Gallo RC. A method for detection, isolation, and continuous production of cytopathic human T-lymphotropic retroviruses of the HTLV family (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 1984; **224**: 497–500.
18. Maurice PDL, Smith NP, Pinching AJ. Kaposi's sarcoma with benign course in a homosexual. *Lancet* 1982; i: 571.
19. Janossy G, Pinching AJ, Bofill A, et al. An immunohistological approach to persistent lymphadenopathy and its relevance to AIDS. *Clin Exp Immunol* (in press).
20. Weiss R, Teich N, Varmus H, Coffin J. RNA tumour viruses. New York: Cold Spring Harbor Laboratory, 1982: 1–1396.
21. Gazzard BG, Shanson DC, Farthing C, et al. Clinical findings and serological evidence of HTLV-III infection in homosexual contacts of patients with AIDS and persistent generalised lymphadenopathy in London. *Lancet* 1984; ii: 480–83.
22. Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T-cell leukemia virus by blood transfusion: Seroconversion of recipients. *Vox Sang* 1984; **46**: 245–53.