

## **Human T-Lymphotropic Retrovirus Type III (HTLV-III) in Danish Homosexuals**

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Sera from 119 patients attending a venereal disease clinic in Copenhagen during May 1983 were screened for antibodies to the newly characterized and isolated retrovirus HTLV-III. Ten out of 45 (22%) homosexual patients and two out of 74 (2.7%) heterosexual patients were found to have antibodies against HTLV-III. The homosexual group (except one case that developed AIDS and died during one year observation period) contained otherwise healthy men some of which had no contact with individuals from high-risk areas and only a few sexual partners. These results strongly suggest that Danish homosexuals with only few contacts are also at risk and that sero-epidemiological studies should also include the healthy homosexual group and probably the heterosexual group attending venereal disease clinics. (Received October 16, 1984.)

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AIDS was initially recognized as a separate disease entity in 1981 (1, 2) and is diagnosed as a severe, unexplained immune deficiency that usually involves a reduction in the number of helper T lymphocytes and is accompanied by multiple opportunistic infections or malignancies.

The acquired immunodeficiency syndrome (AIDS) is known to occur among homosexual or bisexual men, intravenous drug abusers and their infants, female sexual partners of men with the syndrome, Haitians, and patients with hemophilia (1-7). Recently a retrovirus of the HTLV "family" called HTLV-III has been isolated with high frequency from patients with AIDS and pre-AIDS (8), and antibodies to the virus were found in 88-100% of AIDS patients as well as groups with high risk for AIDS (9). A recent sero-epidemiolog-

ical study revealed that 100% of the AIDS sera, 84% of the homosexual men with lymphadenopathy and 21% of healthy homosexuals with an increased risk of AIDS were positive for antibodies to proteins of HTLV-III (10). Similar or identical retroviruses given various names, e.g. LAV, IDAV, IDAV2, ARV, have been identified (11, 12) and subsequently isolated (13-15). Some of these are now being directly compared to several isolates of HTLV-III. In this study we present the results obtained from 119 men attending a venereal clinic in Copenhagen, May 1983. The results demonstrate the presence of HTLV-III antibodies in a high proportion of the healthy homosexuals.

## MATERIALS AND METHODS

*Materials.* 119 consecutive men attending a venereal disease clinic in Copenhagen during May 1983 for suspected venereal disease or for control after treatment of a venereal disease were studied.

*Methods.* Sera were tested for serum antibodies recognizing HTLV-III in two stages. First all sera were tested by indirect ELISA for quantitative levels of IgG binding to disrupted HTLV-III virions coated onto the wells of microtiter plates (16). HTLV-III virions were produced in high quantity by specific clones from a permissive human neoplastic T-cell line (17). Test results were normalized to the value for standardized normal human control sera and all sera with normalized values exceeding a cut-off level of 4.2 (the mean  $\pm$  2SD determined from 356 normal Danish donors) were confirmed by the demonstration of antibody binding to virus-specific proteins separated by SDS-PAGE and electrophoretically transferred to nitrocellulose strips (9, 18, 19) using a modification of the "unlabelled antibody" - "peroxidase enzyme" procedure (Saxinger et al., submitted).

## RESULTS

Of the 119 men attending the venereal clinic 45 were homosexuals and 74 were heterosexuals. Of the total number studied for the presence of antibodies against HTLV-III, 12 were found positive. Retrospectively it was found that 10 were homosexuals and 2 were heterosexuals (Table I). Thus 22% of the homosexuals and 2.7% of heterosexuals had

Table I. *Clinical data on the 12 HTLV-III antibody positive patients*

Pat. no.	Age	Earlier infections with					No. of contacts			Foreign contacts	Episodes with fever, diarrhoea, weightloss lymphnodes
		Gon.	Sy-philis	Herpes I/II	Hep. B	Amoe-biasis	Last month	Last 12 months	Sex contact		
1	41	5	3	I/-	+	+	4	50	M	U.S. Ibiza London	1982/83
2	42	2	1	-/II	+	+	7	25	M	Germany Austria England	-
3	31	5	1	-/-	-	-	1	1	M	-	-
4	36	5	2	-/-	+	-	2	10	M	Spain	-
5	34	5	1	-/-	+	+	1	8	M	Ibiza Sweden Germany (Berlin)	-
6	26	2	-	-/-	+	-	4	50	M/F	-	1983
7	41	5	1	-/-	+	+	?	?	M	?	-
8	36	1	1	-/-	+	-	1	10	M	Spain	1982
9	34	1	1	-/-	-	-	4	10	M	-	-
10	25	-	-	-/-	-	-	5	50	M	-	-
11	24	-	-	-/-	-	-	-	-	F	-	-
12	38	3	-	-/-	-	-	-	-	F	-	-

antibodies against HTLV-III. The positive homosexuals had between 0 to 5 previous episodes of gonorrhoea and between 0 to 3 of syphilis. Herpes virus infection appeared infrequently in the clinical histories. Seven patients previously had had hepatitis B and four had suffered from amoebiasis. The number of contacts varied between 1 and 50 within the last 12 months and between 1 and 7 within the last month before questioning. Five of the patients had contacts outside Denmark but only one had previously visited New York (case 1). Three cases (cases 1, 6, and 8) had earlier suffered from a longer period with fever, diarrhoea, weight loss and enlarged lymph nodes, but at the time of blood sampling in May 1983 and when questioned in June 1984 they were all in good health. Since May 1983 one patient (case 7) had AIDS diagnosed and is now dead.

## DISCUSSION

The serological data presented here show a high prevalence (22%) of HTLV-III antibodies in healthy homosexuals. The group of heterosexuals had 2.7% of cases with HTLV-III antibodies. A group of 356 normal Danish blood donors had 1.7% (Saxinger, unpublished data). Convincing evidence for HTLV-III as the causative agent in pre-AIDS and AIDS has been reported and seroepidemiological data have revealed antibodies to HTLV-III in almost 90% of patients with AIDS and 80% of those with pre-AIDS (9). Further, a more recent report has demonstrated HTLV-III antibodies in 100% of AIDS patients, 84% of patients with lymphadenopathy and in 21% of homosexuals with an increased risk of AIDS (10). The present data concerns an unselected group of healthy patients visiting a venereal clinic in Copenhagen. The high incidence of seropositivity in this group without clinical disease supports a long incubation period of AIDS. However, one must also consider the possible establishment of immunity against the virus within this group. The present data supports and emphasizes that homosexuals as a group and homosexuals with only Danish contacts are at high risk and that future sero-epidemiological studies should include this group, even if they only have a moderate number of contacts.

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## REFERENCES

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men. *N Engl J Med* 1981; 305: 1425-31.
2. Masur H, Michelis MA, Greene JB, Onorato I, Vandie Stouwe RA, Holzman RS, Wormser G, Brettman L, Lange M, Murray HW, Cunningham-Rundles S. An outbreak of community-acquired pneumocystis carinii pneumonia. *N Engl J Med* 1981; 305: 1431-9.
3. Siegal F, Lopez C, Hammer GS, Brown AE, Kornfeld SJ, Gold J, Hassett J, Hirschman SZ, Cunningham-Rundles C, Adelsberg BR, Parham DM, Siegal M, Cunningham-Rundles S, Armstrong D. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med* 1981; 305: 1439-44.
4. Poon M, Landay A, Prasthofer EF, Stagno S. Acquired immunodeficiency syndrome with pneumocystis carinii pneumonia and mycobacterium avium-intracellulare infection in previously healthy patient with classic hemophilia. *Ann Intern Med* 1983; 98: 287-90.
5. Moll B, Emerson E, Small CB, Friedland GH, Klein RS, Spigland I. Inverted ratio of inducer to suppressor T-lymphocyte subsets in drug abusers with opportunistic infections. *Clin Immunol Immunopathol* 1982; 25: 417-23.
6. Curran JW, Lawrence DN, Jaffe H, Kaplan JE, Zyla LD, Chamberland M, Weinstein R, Lui K-J, Schonberger LB, Spira TJ, Alexander WJ, Swinger G, Ammann A, Solomon S, Auerback D,

- Stoneberger R, Mason JM, Haverkos HW, Evatt BL. Acquired immunodeficiency syndrome (AIDS) associated with transfusions. *N Engl J Med* 1984; 310: 69-75.
7. Fauci AS. The acquired immune deficiency syndrome: the ever-broadening clinical spectrum. *JAMA* 1983; 249: 2375-6.
  8. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984; 224: 500-3.
  9. Sarngadharan MG, Popovic M, Bruch L, Schüpbach J, Gallo RC. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* 1984; 224: 497-500.
  10. Safai B, Groopman JE, Popovic M, Schüpbach J, Sarngadharan MG, Arnett K, Sliski A, Gallo RC. Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome. *Lancet* 1984; i: 1438-40.
  11. Barré-Sinoussi F, Chermann J-C, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983; 220: 868.
  12. Montagnier L, Chermann JC, Barré-Sinoussi F, et al. A new human T-lymphotropic retrovirus: characterization and possible role in lymphadenopathy and acquired immune deficiency syndromes. In: Gallo RC, Essex ME, Gross L, eds. *Human T-cell leukaemia lymphoma viruses*. New York: Cold Spring Harbor Laboratory (in press).
  13. Vilmer E, Barré-Sinoussi F, Rouzioux C, Gazengel C, Vezinet-Brun F, Dauguet C, Fischer A, Manigne P, Cherman JC, Griscelli C, Montagnier L. Isolation of new lymphotropic retrovirus from two siblings with haemophilia B, one with AIDS. *Lancet* 1984; i: 753-7.
  14. Montagnier L, Gruest J, Chamaret S, Dauguet C, Axler C, Guetard D, Nugeyre MT, Barré-Sinoussi F, Cherman JC, Brunet JB, Klatzmann D, Gluckman JC. Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. *Science* 1984; 225: 63-6.
  15. Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM, Oshiro LS. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* 1984; 225: 840-2.
  16. Saxinger C, Gallo RC. Methods in laboratory investigation. Application of the indirect enzyme-linked immunosorbent assay microtest to the detection and surveillance of human T-cell leukemia-lymphoma virus. *Lab Invest* 1983; 49: 371-7.
  17. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 1984; 224: 497-500.
  18. Schüpbach J, Popovic M, Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* 1984; 224: 503-5.
  19. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76: 4350-4.