EVIDENCE FOR TRANSMISSION OF HTLV-III TO EUROPEAN HAEMOPHILIACS VIA US IMPORTED FACTOR VIII CONCENTRATE

MADS MELBYE (1*)
KARIN S. FROEBEL (2)
RAJAN MADHOK (2)
ROBERT J BIGGAR (3)
PREM SARIN (4)
STENER STENBJERG (5)
GORDON D.O. LOWE (2)
CHARLES D. FORBES (2)
JAMES J. GOEDERT (3)
ROBERT C. GALLO (4)
PETER EBBESEN (1)

1. The Institute of Cancer Research, Radiumstationen, 8000 Aarhus C, Denmark.
2. University Department of Medicine, Royal Infirmary, Glasgow G32 2ER, United Kingdom.
3. Environmental Epidemiology Branch, National Cancer Institute, Bethesda, Maryland 20205, USA.
4. Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205, USA.
5. The Blood Bank, Aarhus Municipal Hospital, 8000 Aarhus C, Denmark.

* Correspondence and requests for reprints to: Dr. M. Melbye

ABSTRACT

Seventy-seven Scottish haemophiliacs and 22 Danish haemophiliacs were serologically analysed for antibodies against the human T-cell leukemia virus (HTLV-III). Since 1979 Scottish patients have been treated mostly with factor concentrate enterely produced in Scotland, whereas all,but two Danish patients have regularly received both commercially and locally-manufactured concentrate. All commercially-produced factor VIII concentrate products used in the two populations were manufactured by American compagnies. Overall, 15.6% of Scottish and 59.1% of Danish haemophiliacs were antibody positive ($p<0.001$). None of 11 haemophiliacs not treated in the period 79-84 were seropositive.Two (6.7%) of 30 subjects who had been treated with locally-produced concentrate only were positive, whereas 23 (39.7%) of 58 subjects who had been treated with commercial were HTLV-III antibody positive. Among 52 users of both commercially and locally produced factor VIII concentrate, seropositivity was directly correlated with the consumption of commercial concentrate ($p<0.001$), whereas there was no significant correlation between seropositivity and the used amount of locally produced factor VIII concentrate. Sofar, no Danish and only one Scottish haemophilia patient (a user of commercial factor VIII concentrates) has developed symptoms probably related to AIDS. In conclusion, the data indicate that European haemophiliacs were exposed to HTLV-III via imported factor VIII concentrate from the US.

MATERIALS

Danish haemophiliacs were bled in Aarhus, April, 1984 while attending a regular health evaluation. Plasma was kept at -70 C until testing. Detailed information on medical treatment, was available to determine the amount and origin of each individual's consumption of factor VIII or IX since 1979. Similar data were obtained on Scottish haemophiliacs enrolled in the Regional Haemophilia Reference Centre of Glasgow. These patients were bled between December, 1983 and July, 1984.

Antibody against HTLV-III was measured by an enzyme-linked immunosorbent assay (ELISA) (11,12) in which disrupted whole virus (HTLV-III-H9) (7) was the substrate. Samples were run in duplicate and a known negative control was run eight times on each microtiter plate, with the results for each averaged. Sample results were compared to negative control results through the ratio of the two values. Antibody was considered to be present if the ratio between sample and background was =>5.0, and to be borderline if the ratio was between 3.0 and 5.0. Tests for significance (Chi square and Wilcoxon-Mann-Whitney (W) test (13,14)) compare antibody positive with antibody-negative persons, excluding persons with borderline ratios.

RESULTS

Patient with AIDS-like symptoms

Thirty-five year old Scottish haemophilia A patient with no other risk factors associated with AIDS. Since 1979, he has been treated exclusively on high dosis of

US manufactured factor VII concentrate. Over the past 7 months, he has had a history of malaise, anorexia, weight loss, intermittent fever, lymphadenopathy and night sweats. He has persistent herpetic lesions of the lips and oral cavity and also has candidiasis of the mouth and anus. More recently he complains of dysphagia and central sternal pain. Laboratory values shows persistent lymphopenia, moderate thrombocytopenia, reduced response to several mitogens, reduced number of T helper cells and reduced helper/suppressor ratio (May, 1984: 0.64; July, 1984: 0.29). He is HTLV-III antibody positive.

Healthy haemophiliacs

A total of 22 Danish haemophiliacs (mean=22.8 years, age range 12-46) and 77 Scottish haemophiliacs (mean 34.9 years, age range 13-72) were enrolled in the study.

Twelve (57%) of 21 Danish haemophilia A patients had antibodies against HTLV-III, as did a single haemophilia B patient (total=59% positives) (Table I). Positive subjects with haemophilia A had received significantly ($p<0.05$) larger quantities of factor VIII concentrate manufactured in the USA ($mean_{1979-1984}=498.800$) than seronegative subjects ($mean_{1979-1984}=83.800$), whereas there was no statistical difference between the amount of locally manufactured concentrate used in the two groups (Table II). The only two subjects who had not received US manufactured factor concentrate in the period 1979-84 were both seronegative, whereas the seropositive haemophilia B patient had used only US manufactured factor IX concentrate.

In Scotland, 11 (18%) of 62 haemophilia A patients and one (7%) of 15 haemophilia B patients were HTLV-III

seropositive (Tables I and III). Positive subjects were known to have received commercial factor concentrate in the period 1979-84 except in 2 cases. One travelled yearly thoughout Europe and could have received unrecorded treatment. The other was a citizen of Pakistan who often visited his home country. Seropositive Scottish haemophilia patients had received more commercial clotting factor concentrate than seronegative subjects ($p<0.001$), whereas there was no statistical difference between the two groups as to use of local products.

As shown in Table I, 40% of subjects receiving either commercial factor concentrate alone or in combination with local products had antibodies against HTLV-III whereas only 6.7% of those subjects recorded as receiving only local products were positive. HTLV-III seropositivity was more common in persons more exposed to commercially produced factor VIII (Fig. 1). The percentage of antibodies rose from 11.8% among subjects in the bottom third of commercial product use to 29.4% in the middle third, and to 77.8% in the top third of use (trend analysis, $p<0.001$). In contrast, no significant difference in seropositivity was observed between groups classified according to their use of locally produced factor VIII concentrate.

DISCUSSION

Among haemophiliacs, 59% of Danes but only 16% of Scots had antibodies against HTLV-III. Almost all Danish patients had been treated with factor concentrate manufactured in the United States since 1979, but only 49% of

United States before this date.

In an earlier report we found that 9% of Danish homosexual men back in 1981 had antibodies against HTLV-III and that seropositivity was most strongly correlated with travel to the United States and especially to New York City (15). From this and other studies (3,16) it appears that the prevalence of HTLV-III antibodies and the incidence of AIDS among European homosexuals are approximately 1 to 2 years behind the present epidemic in the United States. However, the prevalence rates of HTLV-III antibodies obtained among Danish haemophiliacs are comparable to what has been found in American haemophiliacs (10) which most likely is due to the worldwide distribution of US plasma products. Futhermore, the estimated incidence of AIDS among American haemophiliacs, 1-2/1.000, is similar to estimates made among European hemophiliacs (1/1.000) (17).

These findings indicate that HTLV-III was most likely distributed through haemophiliac populations by factor VIII concentrate commercially produced in the United States. Although a high proportion of patients exposed to such products were seropositive, the implications with regard to their health remain to be clarified. Both HTLV-III and LAV has been isolated from haemophiliacs with AIDS (18,19). Futhermore, mouse type C retroviruses have proved to withstand the procedures used for factor VIII concentration (20). However, LAV appears relatively labile under certain circumstances (21) and becomes inactivated after 10 min heating (58° C). Futhermore, most factor preparations are stored for months at 4° C before use which might result in inactivated virus that results in immunization rather than infec-

tion. Studies to determine the frequency of viable, complete HTLV-III in factor concentrate products are in progress.

In conclussion, we recommend the use of US commercial factor products to be minimized in the treatment of recently discovered haemophilia children. Most likely, future screening procedures of the donor blood will eliminate the potential risk for the haemophiliacs, but at present our study clearly shows that patients are exposed to active or inactive HTLV-III genomes through the commercial products on which these people are dependent.

REFERENCES

1. Pneumocystis cariniii pneumonia among persons with hemophilia A. Morbid Mortal Wk Rep 31: 365-67, 1982.

2. Marmor M., Risk factors. In: P. Ebbesen, R.J. Biggar, and M. Melbye. AIDS. A basic guide for clinicians. Copenhagen, Philadelphia, London. Munksgaard/Saunders, 1984: 46-47.

3. Melbye M, Biggar R.J., Ebbesen P., Epidemiology. Europe and Africa. In: P. Ebbesen, R.J. Biggar, and M. Melbye. AIDS. A basic guide for clinicians. Copenhagen, Philadelphia, London. Munksgaard/Saunders, 1984:29-41.

4. Lederman MM, Ratnoff OD, Scillian JJ, Jones PK, Schacter B. Impaired cell-mediated immunity in patients with classic hemophilia. N Engl J Med 1983; 308: 79-83.

5. Menitove JE, Aster RH, Casper JT, et al. T-lymphocyte subpopulations in patients with classic hemophilia treated with cryoprecipitate and lyophilized concentrates. N Engl J Med 1983; 308: 83-6.

6. Froebel KS, Madhok R, Forbes CD, Lennie SE, Lowe GDO, Sturrock RD. Immunological abnormalities in haemophilia: are they caused by American factor VIII concentrate? Br Med J 1983; 287: 1091-93.

7. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984; 224: 497-500.

8. Melbye M, Biggar RJ, Chermann JC, Montagnier L, Stenbjerg S, Ebbesen P. High prevalence of lymphadenopathy virus (LAV) in European haemophiliacs. Lancet 1984; ii: 40-1.

9. Ramsey RB, Palmer EL, McDougal JS, et al. Antibody to lymphadenopathy-associated virus in haemophiliacs with and without AIDS. Lancet 1984; ii: 397-8.

10. Goedert JJ, Sarngadharan MG, Eyster ME, Weiss SH, Gallo RC, Blattner WA. Antibodies reactive with human T-cell leukemia viruses (HTLV-III) in the serum of hemophiliacs receiving factor VIII concentrate. (submitted).

11. 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 1984; 49: 371-7.

12. Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 1984; 224: 506-8.

13. Wonnacott TH, Wonnacott RJ. Nonparametric statistics. In: TH Wonnacott and RJ Wonnacott (eds.). Introductory statistics for business and economics, 2nd ed. Santa Barbara, New York, London, Sydney, Toronto. John Wiley and Sons, 1977, pp. 481-500..

14. Goldstein A. Biostatistics. An introductory text. New York, London. The Macmillan Company 1965, pp. 114-7.

15. Melbye M, Biggar RJ, Ebbesen P, et al. Seroepidemiology of HTLV-III in Danish homosexual men: Prevalence, transmission, and disease outcome. Br Med J 1984; 289: 573-5.

16. Cheingsong-Popov R, Weiss RA, Dalgleish A, et al. Prevalence of antibody to human T-lymphotropic virus

type III in AIDS and AIDS-risk patients in Britain. Lancet 1984; ii: 477-80.

17. Bloom AL. Acquired immunodeficiency syndrome and other possible immunological disorders in European haemophiliacs. Lancet 1984; i: 1452-5.

18. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 1984; 224: 500-3.

19. Vilmer E, Barre-Sinoussi F, Roüzioux C, et al. Isolation of new lymphotropic retrovirus from two siblings with haemophilia B, one with AIDS. Lancet 1984; i: 753-7.

20. Levy JA, Mitra G, Mozen MM. Recovery and inactivation of infectious retroviruses added to factor VIII concentrate. Lancet 1984; ii: 722-3.

21. Spire B, Barre-Sinoussi, Montagnier L, Chermann JC. Inactivation of lymphadenopathy associated virus by chemical disinfectants. Lancet 1984; ii: 899-901.

TABLE I - HTLV-III SEROPOSITIVITY IN HEALTHY SCOTTISH AND DANISH HAEMOPHILIACS TREATED WITH LOCALLY PRODUCED OR COMMERCIAL FACTOR PRODUCTS

	Total no. tested	HTLV-III positive	(%)
SCOTLAND			
no treatment	11	0	(0.0)
local	28	2	(7.1)
commercial	4	1	(25.0)
both	34	9	(26.5)
Total	77	12	(15.6)
DENMARK			
local	2	0	(0.0)
commercial	1	1	(100.0)
both	19	12	(63.2)
Total	22	13	(59.1)
BOTH COUNTRIES			
no treatment	11	0	(0.0)
local	30	2	(6.7)
commercial	5	2	(40.0)
both	53*	21	(39.6)
Total	99	25	(25.3)

*Detailed information on use of factor VIII concentrate was missing on one subject (see Fig.1)

TABLE II- MEAN UNITS OF LOCAL AND COMMERCIAL FACTOR VIII CONCENTRATE USED BY HTLV-III SEROPOSITIVE AND NEGATIVE HAEMOPHILIA A PATIENTS, 1979-1984.

	HTLV-III		
	Positive	Negative	p-value*
Scottish			
local	202.700	98.700	N.S.
commercial	144.900	9.100	$p<0.001$
Danish			
local	195.500	144.200	N.S.
commercial	498.800	83.800	$p<0.05$

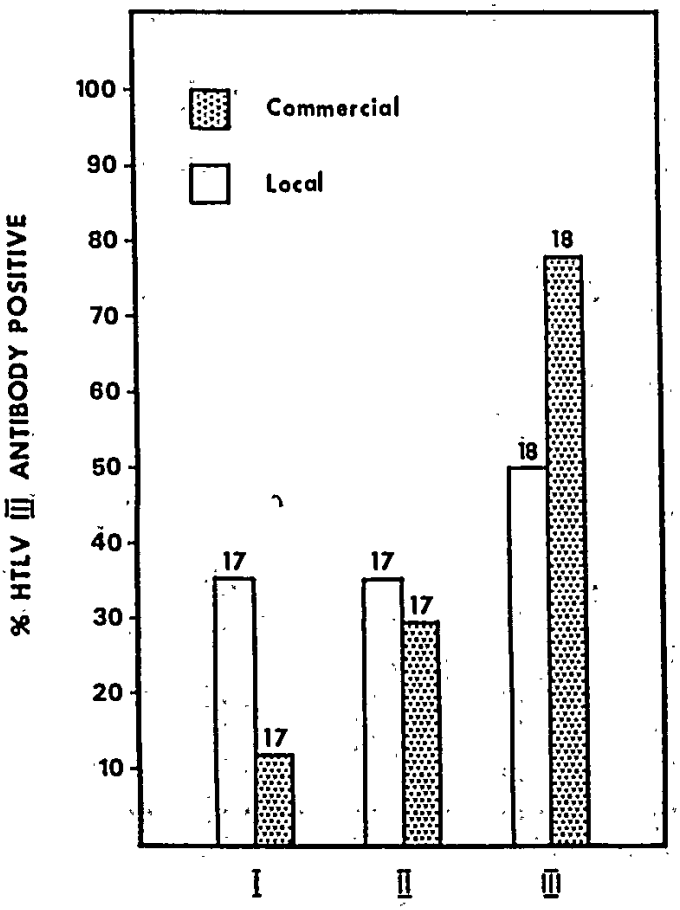

Fig 1 - Percentage of HTLV-III seropositivity among 52 haemophiliacs distributed into thirds according to their use of both commercially and locally produced factor VIII concentrate. Number of subjects tested is given on top of each column. Use: I=lowest, II=middle, III=highest.
Trend analysis: local=not significant; commercial=$p<0.001$