

Lymphadenopathy and Antibodies to HTLV-III in Homosexual Men

Clinical, Laboratory and Epidemiological Features

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Summary. The study provides information on the epidemiology of HTLV-III infection and the lymphadenopathy syndrome (LAP) in 374 German homosexual men. Sexual contacts in the USA and rectal enemas before receptive anal intercourse are the main risk factors associated with virus transmission. HTLV-III seropositivity is significantly correlated with LAP. Prominent clinical signs are infrequent. Immunological and haematological abnormalities are prevalent, and the retrovirus infection is frequently associated with serological markers of other viruses (hepatitis B, herpes group viruses). Lymphadenopathy as a manifestation of HTLV-III infection is discussed within the context of AIDS-related disorders.

Key words: Homosexual men – Lymphadenopathy – HTLV-III – Epidemiological factors

A lymphotropic retrovirus designated human T-cell leukaemia virus type III (HTLV-III) or lymphadenopathy associated virus (LAV) is regarded as the causative agent of AIDS. Along with AIDS another syndrome has emerged consisting of persistent, generalized LAP, which is unexplained by current illness or drug abuse, both of which are known to cause lymphadenopathy. Both conditions were first reported in homosexual men from

Abbreviations: AIDS = Acquired Immunodeficiency Syndrome; CMV = Cytomegalovirus; EBV = Epstein-Barr virus; HBV = Hepatitis B virus; HTLV-III = Human leukaemia retrovirus type III; LAP = Lymphadenopathy

urban centres in the USA, and appeared among the corresponding risk groups in Europe with a lag of approximately 3 years [3, 6, 8, 9].

Several accounts have been published in Germany on the prevalence of antibodies to HTLV-III in subjects with LAP and AIDS [4, 15, 17, 20, 29]. To complement these preliminary observations, we present an epidemiological study on the relationship between clinical and laboratory parameters, in association with behavioural factors, in 374 male homosexuals; 118 of whom exhibited persistent, generalized lymphadenopathy.

Subjects and Methods

The study was planned in cooperation with male homosexual organizations in Berlin. Confidentiality was assured. Between April 1983 and May 1984 male volunteers of homosexual or bisexual orientation were interviewed and examined at the Landesinstitut für Tropenmedizin. Their lymph node status was assessed by a single investigation as defined by the Centers for Disease Control, USA [9]. Data were analysed of subjects according to the following classification: (1) absence of lymphadenopathy, and (2) palpable lymph nodes of unknown aetiology, being 1 cm or larger, and involving two or more extrainguinal sites. Patients with known causes of lymphadenopathy were excluded. All participants underwent a physical examination and gave a detailed medical history. Particular attention was paid to any symptoms of the so-called B-complex (fever, fatigue, night sweats, wasting, diarrhoea, cough), recurrent infectious diseases, drug abuse and the administration of drugs impairing the immune system. They each completed questionnaire on the duration of homosexual activity, number of life-time partners and partners within the past year, patterns of sexual practices, physical hygiene (rectal enemas) and history of travel to the USA [5, 10].

Laboratory examinations included a complete and differential blood count, assay of liver transaminases and the determination of immunoglobulin classes IgG, IgM and IgA. Serological tests were carried out for toxoplasmosis (IFT, CBR), syphilis (TPHA, VDRL), hepatitis A virus (ELISA), hepatitis B virus (RIA, ELISA), cytomegalovirus (CBR), Epstein-Barr virus (IFT) and herpes I virus (CBR).

Sera were screened for antibodies to HTLV-III by an enzyme-linked immunosorbent assay [4]. ELISA microtiter plates were coated with antigen derived from HTLV-III grown in the HT-H9 lymphocyte cell line, which continuously produces the virus¹. After assessment of the purity of viral preparations by polyacrylamide gel electrophoresis (PAGE), 0.1–0.3 µg of protein was placed in each well. Sera, prediluted with PBS-NaN₃, were tested in duplicate at definite dilutions of 1:50 and 1:250. The results were scored against a negative control serum and a strongly positive serum titrated to 1:250, 1:1,250 and 1:6,250. Peroxidase-conjugated goat anti-human IgG was used for detecting antibodies. Sera generating an optical density of more than thrice the negative control value were designated as positive, whereas sera reading two to three times the value of the negative sample were considered weakly positive. The latter was regarded as a doubtful reaction and the 48 individuals with this finding were omitted from the subgroup analysis of HTLV-III-positive versus HTLV-III-negative. Specificity of the ELISA procedure was monitored by radioimmunoprecipitation of viral antigens by ELISA-positive sera [30].

The Tine-Test was applied for skin reactivity to tuberculine. Stool samples were examined for intestinal protozoa by MIFC. Differences in proportions were analysed using the ch² statistic. Group median values were compared using the Wilcoxon-Mann-Whitney test. A multivariate analysis by log linear models was performed when factors were associated with HTLV-III seropositivity and with lymphadenopathy. In this case values of continuous factors have been categorized as either exceeding, or not exceeding, the overall median value [11].

Results

Study Group

374 homosexual or bisexual males aged 19 to 68 years (median 32 years) participated in this study. Information on life style and sexual habits was obtained from the questionnaires.

Sexual Habits

The median duration of homosexual activity as reported by the 344 individuals was 14 years (1-45 years). The number of life-time partners was up to 100 in 105 (29.7%) men 101-500 in 140 (39.5%) men and over 500 in 109 (30.8%) men. Sexual partners during the previous year numbered fewer than 10 in 95 (27.0%) subjects 11-50 in 170 (48.3%) subjects and over 50 in 87 (24.7%) subjects. Insertive anal intercourse was practiced by 229 (91.6%) subjects; receptive anal intercourse by 205 (87.2%) men. Oro-genital sex was reported by 330 (96.2%) men and oro-anal sex by 227 (72.1%) men. Enemas before anal sex were performed by 166 (47.4%) participants and nitrite inhalants were used by 181 (48.6%) subjects. The use of recreational drugs was indirectly associated with active and passive oro-anal sex and anal intercourse, number of partners and physical hygiene (all P values ≤ 0.001), thus providing an index for sexual activity.

Laboratory and Clinical Features

Several laboratory parameters were found to be of no significance in subgroup analysis: Intestinal protozoa (Entamoeba histolytica 28.1%, apathogen amoeba 55.3% and Giardia lamblia 6.6%); antibodies to Toxoplasma gondii (73.7%), HAV (43.1%) and Herpes I virus (97.1%); liver transaminases, immunoglobuline IgA and white blood cell count (all in the normal range). Immunosuppressive drugs were used by very few individuals. Among the clinical symptoms of the so-called B-complex, only fever was reported in appreciable frequency (9.7%).

Lymphadenopathy

Examination and history revealed persistent generalized LAP in 118 men. The group without LAP consisted of 256 men. The median age was slightly lower in individuals with LAP (32 years) than without LAP (33 years).

Clinical, laboratory and behavioural factors were analysed for association with the lymph node status. Most behavioural parameters such as fella-

¹ Kindly provided by R.C. Gallo (Bethesda)

Table 1. Clinical, laboratory and epidemiological features associated with lymphadenopathy in homosexual men

Parameter	LAP (+/-)	LAP (+)	LAP (-)	Level of significance
	(n)	(median or percent)		(<i>P</i> ≦)
Passive anal sex	79/156	92%	83%	0.001
Rectal enemas	108/240	69%	38%	0.001
Nitrite inhalant	118/253	64%	42%	0.001
Sex in USA	109/244	53%	30%	0.001
Fever	118/254	19%	6%	0.001
Haemoglobin (mMol/l)	118/254	9.4	9.8	0.001
Lymphocytes (%)	118/256	48	42	0.01
IgG (mg/dl)	118/255	1,718	1,171	0.001
Tine test positive	111/242	17%	36%	0.001
EBV antibody	112/244	83%	68%	0.01
Anti-HBc	117/254	84%	66%	0.001
HTLV-III antibody	103/222	72%	17%	0.001

tio and anilingus, insertive anal intercourse, duration of homosexual activity, life-time partners and partners during the previous year were not correlated with either of the two groups. In addition to the laboratory variables described, no associations between LAP and platelet count, immunglobuline IgM and HBsAg were found. Various correlations could be shown between LAP and a number of partially interrelated behavioural variables (Table 1).

More LAP patients complained about fever. Laboratory parameters that were altered in association with LAP were lower haemoglobin concentrations, higher IgG levels and lymphocyte counts (all median values were in the normal range). The absolute increase of lymphocytes was less marked than the increase of relative numbers; a median of $2,809/\mu l$ was counted in patients with LAP, but only $2,575/\mu l$ in men without LAP ($P \le 0.05$). Serological markers indicating past and recent infection with HTLV-III, HBV and EBV were associated with LAP. CMV antibodies were present in 95.8% of patients with LAP and in 89.0% patients without LAP ($P \le 0.05$).

Serumantibodies to HTLV-III

Sera collected at the time of admission could later be tested for antibodies to HTLV-III. Of the 374 participants, 214 (57.2%) were negative; 48 (12.8%) weakly positive and 112 (30.0%) definitely positive. Borderline cases were not included in the statistical analysis. The median age was 32 years in seropositive and 33 years in seronegative individuals.

The same variables tested for association with LAP, were further evaluated for their correlation with HTLV-III antibodies. No association was de-

tected for self-reported clinical symptoms, for the behavioural parameters fellatio and anilingus, active anal intercourse, the number of life-time partners or partners during the previous year, or with antibodies to EBV and Herpes I virus. Platelet count was slightly lower ($P \le 0.05$) in HTLV-III-seropositive (median 204,000/µl) than in seronegative men (median 215,000/µl). TPHA was positive in 54.2% of seropositive and 41.3% of seronegative subjects ($P \le 0.05$). Significant correlations were found for most of the factors also associated with LAP. Results are shown in Table 2.

Three behavioural parameters were examined for their association with HTLV-III antibodies. We demonstrated that visits to the USA with sexual contacts and passive anal intercourse were determinants of seropositivity. Rectal enemas were correlated with both of these parameters. Since enemas were almost exclusively performed by subjects practicing passive anal intercourse, we determined the risk of infection for this subgroup and found that it was more than six times higher (ψ = 6.6) than in participants performing receptive anal sex without physical hygiene. A similar result was obtained for anti-HBc positive men (ψ = 4.3), but not for carriers of CMV and EBV antibodies.

LAP and HTLV-III Antibodies: Log Linear Model Approach

With few exceptions the variables associated with LAP were also related to the presence of antibodies to HTLV-III. To analyse whether these associations are caused by the interrelationship of HTLV-III antibody status and LAP, we subdivided the results into four groups based on combined HTLV-III and LAP characteristics (Table 3). The predominant associations have been assessed by

Table 2. Laboratory and epidemiological features associated with HTLV-III antibody in homosexual men

Parameter	HTLV-III (+/-) (n)	(+)	HTLV-III antibody I +) (-) s median or percent) (
Passive anal sex	72/136	94%	82%	0.001
Rectal enemas	105/199	71%	36%	0.001
Nitrite inhalant	112/211	61%	41%	0.001
Sex in USA	106/202	58%	28%	0.001
Lymphocytes (%)	112/214	48	42	0.001
IgG (mg/dl)	112/214	1,697	1,173	0.001
IgM (mg/dl)	112/214	178	152	0.001
Tine test positive	107/205	19%	37%	0.001
CMV antibody	112/214	96%	87%	0.01
Anti-HBc	112/212	91%	65%	0.001
HBsAg	112/214	11%	3%	0.01

Table 3. Associations of laboratory parameters and clinical features with lymphadenopathy (L), HTLV-III (H), both (L, H) or one in dependance of the other ($L \times H$) in homosexual men by log linear analysis

Parameter	LAP/HTLV-III $(-/-)$ $(n=184)$	LAP/HTLV-III $(-/+)$ $(n=38)$	LAP/HTLV-III $(+/-)$ $(n=29)$	LAP/HTLV-III $(+/+)$ $(n=74)$	Variate associated <i>P</i> value
Lymphocytes	42%	44%	41%	50%	L×H*
IgG (mg/dl)	1,138	1,401	1,411	1,786	L, H**
Anti-HBca	63%	95%	75%	89%	H**
Tine test positive ^a	39%	21%	24%	16%	H*

a corrected for age

log linear models taking into account the interdependence of HTLV-III seropositivity and LAP. Tuberculine reaction and anti-HBc status only showed an association with HTLV-III. Nonspecific polyclonal B-cell activation by HTLV-III leading to increase of IgG is superimposed by the effects of LAP. The association between relative lymphocyte counts and HTLV-III showed a dependence on the LAP status; significantly different counts were only found between HTLV-III positive and negative participants with LAP.

Discussion

Coinciding with the appearance of AIDS, a syndrome designated persistent generalized LAP became endemic in the homosexual community of the USA [8, 9]. There is overwhelming evidence that both these conditions are caused by the human T-cell lymphotrophic virus HTLV-III and that the agent is transmitted by sexual contact [12, 13]. A variety of symptoms including fatigue, fever, night sweats, weight loss, chronic diarrhoea and cough, often in combination with LAP, may pre-

cede the full-blown disease. LAP may also occur without other clinical signs [1, 23].

The present study supports the assumption that LAP and HTLV-III seropositivity are significantly associated and that the lymph node enlargement in many instances is a consequence of the viral infection. HTLV-III has been introduced in the German homosexual population and the number of AIDS cases and fatalities is increasing rapdily. Two life-style factors, i.e. the number of homosexual partners and receptive anal intercourse, seem to determine the spread of HTLV-III infection in the USA [13]. We could not demonstrate an association between the number of partners during the preceding year (or life-time partners) and seropositivity [22]. It is conceivable that at the time our investigation was conducted the transmission of the virus from US citizens to Europeans was still of greater impact than its spread amongst German homosexuals. The other factor, passive anal sex, is significantly correlated to seropositivity in our participants. Rectal douching was performed by a majority before receptive anal intercourse. We were able to prove that this procedure significantly

^{*} $P \le 0.05$

^{**} $P \leq 0.001$

increases the risk of contracting HTLV-III infection. The same was found in subjects with seromarkers of HBV, but not CMV and EBV. The irritation and injury of the vulnerable rectal mucosa and removal of protective substances may facilitate the passage of these viruses. The frequent use of nitrite inhalants was found to be in indirect association with the variables mentioned: evidently it is a marker of permissive life-style rather than a determinant of disease.

Infection with HTLV-III may occur unnoticed [28, 29]. In our study most participants could not recall any recent illness of prolonged duration. In many seropositive men with LAP the symptoms of the so-called B-complex were absent. Fever was associated significantly with LAP, possibly caused by other diseases [1, 10], but not with antibodies to HTLV-III. Several laboratory parameters were associated with LAP and HTLV-III seropositivity. Haematological abnormalities such as leukopenia and, more pronounced, lymphopenia, are constantly found in patients with AIDS and are detected in a considerable proportion of subjects with AIDS-related disease [14, 31]. In contrast, we observed slightly higher absolute and relative numbers of lymphocytes in subjects with LAP. This could be expected in longstanding infections and has been described in homosexuals [7, 18, 27]. The increase in lymphocyte counts in HTLV-III seropositive men may also be an early response to concurrent or reactivated viral infection. In AIDS and the AIDS-related complex, thrombocytopenia is frequently encountered [25]. In our participants, platelet counts were only marginally lower in seropositive men. A decrease in the erythrocyte count and haemoglobin concentration was associated with LAP and may be another consequence of chronic infection.

Elevated levels of immunoglobulins IgG and, less frequently, IgM and IgA are common in the AIDS-complex [2]. Lane et al. (1983) concluded from experiments of T-cell assisted and T-cell independent B-cell stimulation that polyclonal activation of nonspecific antibody production led to hyperimmunoglobulinaemia in AIDS patients [21]. In our study, LAP was only associated with elevation of IgG whereas individuals seropositive for HTLV-III also had high IgM levels. The significantly reduced number of HTLV-III seropositive subjects with positive skin test reactivity fits into the pattern of described abnormalities of cellular immune mechanisms [19]. Details of immunological features in participants of this study will be published elsewhere (Kalden et al., in preparation). Of the agents considered to cause LAP other than

HTLV-III only EBV was associated significantly with LAP in our study.

CMV antibody titers were elevated in correlation to HTLV-III seropositivity, suggesting either reactivation of a latent infection or a new infection. Excretion of CMV has been reproted to be increased in AIDS patients [16]. The association of CMV and HBV with HTLV-III may also be explained by the common route of transmission via rectal mucosa [10, 24]. The finding of HBs-antigenaemia associated with HTLV-III seropositivity could be a result of impaired antigen clearance due to immune deficiency [26].

Our results were obtained in a cross-sectional study. They add information to the knowledge on the types of clinical syndromes caused by HTLV-III infection and the epidemiology of virus transmission. The data will serve as baseline for a longitudinal study on the natural history and outcome of HTLV-III infection in homosexual men.

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