

## Detection of Coronavirus-Like Particles in Homosexual Men with Acquired Immunodeficiency and Related Lymphadenopathy Syndrome

P. Kern<sup>1</sup>, G. Müller<sup>2</sup>, H. Schmitz<sup>2</sup>, P. Rácz<sup>3</sup>, W. Meigel<sup>4</sup>, G. Riethmüller<sup>5</sup>, and M. Dietrich<sup>1</sup>

<sup>1</sup> Klinische Abteilung, <sup>2</sup> Abteilung für Virologie,

<sup>3</sup> Abteilung für Pathologie, Bernhard-Nocht-Institut für Schiffs- und Tropenkrankheiten, Hamburg

<sup>4</sup> Abteilung für Dermatologie, Allgemeines Krankenhaus St. Georg, Hamburg

<sup>5</sup> Institut für Immunologie der Universität München

**Summary.** Coronavirus-like particles were identified by electronmicroscopy in the feces of homosexual men. The particles banded at a density of 1.21 g/ml after cesium chloride density gradient centrifugation. To determine whether the presence of this virus might be related to clinical symptoms, several patient groups were studied prospectively. In 8 of 16 (50%) homosexual males with acquired immunodeficiency syndrome (AIDS) or unexplained lymphadenopathy syndrome (LAS), coronavirus particles were found. In contrast, such particles were found in none of 18 heterosexual controls and in only 3 of 20 homosexual males without AIDS or LAS. Thus, coronavirus excretion correlated significantly ( $2\alpha < 0.01$ ) with the clinical diagnosis of AIDS or with syndromes belonging to the AIDS-related complex. In addition, such particles identified in the serum of one patient with LAS and diarrhea suggest invasion and systemic spread of the agent and underline that this virus behaves differently from "common cold" human coronaviruses.

**Key words:** AIDS – LAS – CVLP – T-lymphocytes – Neopterin concentration

### Introduction

The outbreak of Kaposi sarcoma, its prodrome – LAS [11, 17], and opportunistic infections in homosexual men from the United States and Europe [4] and in patients from Central Africa [7] has

*Abbreviations:* AIDS = acquired immune Deficiency syndrome; LAS = unexplained, generalized lymphadenopathy syndrome; sp. = species; EM = electronmicroscopy; rIFN  $\alpha$ A = recombinant leukocyte  $\alpha$ A Interferon; CVLP = coronavirus-like particles; CsCl = cesium chloride

initiated intensive search for causative and opportunistic infectious agents. Considerable evidence has recently been accumulated with regard to the etiologic role of human T-lymphotropic retroviruses LAV/HTLV-III [1, 8, 9]. However, high prevalences of antibody to LAV/HTLV-III among homosexuals [5] suggest that the exposure to the virus is much more common than AIDS itself. Thus, host response to infection can be expected to range from subclinical to severe and may be influenced by concomitant infectious agents. Multiple intestinal parasitic infestations and other microbial infections are often seen in homosexual men [19]. In the search for viruses, we observed CVLP in the feces of homosexual men and report here on a prospective study for the presence of these particles in stool and serum specimens of patients belonging to a high risk group of AIDS.

### Materials and Methods

#### *Patients and Controls*

Detailed clinical, parasitological, serological and immunologic data were available from 35 homosexually or bisexually active males originating from Germany and from one African male whose feces were screened for the presence of virus particles. Two of them who showed systemic manifestation of Kaposi sarcoma were studied while under experimental rIFN  $\alpha$ A therapy. One European and one African patient had opportunistic infections and met the criteria for AIDS [4]. Twelve patients had clinical signs compatible with the prodrome of AIDS (LAS) [11, 17], whereas six had unexplained chronic enterocolitis. Of the remaining cases, eight were hospitalized for acute hepatitis B and one for amebic liver abscess which was acquired during the patient's stay in the tropics. In five homosexuals, studied in detail, signs or symptoms of AIDS or LAS could be excluded. Sixteen healthy heterosexual males (including medical staff taking care of patients with AIDS or related diseases) and two females with diarrhea (yersiniosis in one case, unexplained in the other case) were studied as controls and in parallel with patients' specimens.

### Laboratory Investigations

The routine laboratory profile included hematology, hemostaseology, blood chemistry, and urinary analysis. Between one and three stool samples were examined on consecutive days for the presence of protozoa or helminths' eggs; stool cultures were performed to test for pathogenic bacteria, i.e., *Salmonella* sp., *Shigella* sp., *Yersinia* sp., and *Campylobacter* sp. In all symptomatic cases, a proctoscopy and a lymph node biopsy were performed. Serological tests for hepatitis A, hepatitis B, cytomegalovirus, Epstein-Barr virus, syphilis, and others were performed.

### Immunology

Patients evaluated prospectively were tested for cutaneous anergy by a multitest procedure (Multitest Mérieux, Lyon, France) for six different recall antigens. For the enumeration of T-lymphocyte subpopulations, mononuclear cells were separated by density medium centrifugation on Ficoll-Paque (Pharmacia, Freiburg, FRG) and nonadherent lymphocytes were obtained after plastic adherence. The differentiation antigens were identified by indirect immunofluorescence using monoclonal antibodies [20] (T cells: T 411, OKT 3, T-4: T-151, OKT 4, T-8: T 811, Ortho, Heidelberg, FRG). Serum neopterin was measured by a sensitive radioimmunoassay (Henning Berlin, Berlin) [21].

### Electron Microscopy

A suspension of feces in distilled water (up to 1:10 v/v) was homogenized and centrifuged for 10 min at 1,500 g in order to remove debris and bacteria. The supernatant was further diluted 1:3 with water and the suspended material was pelleted at 25,000 g for 30 min in a specially designed centrifuge tube onto pioloform coated grids [18]. The specimens were negative stained with sodium-silico-tungstate and screened for the presence of virus particles using a Philips EM 301 electron microscope. In the same way as feces, serum of nine homosexual and 18 heterosexual males was cleared of subcellular debris and pelleted at 25,000 g for 30 min directly onto grids. Equal areas of all specimens were screened electronmicroscopically for the presence of virus particles until they were considered negative in this respect.

### Purification of CVLP

Two stool samples containing large numbers of CVLP were subjected to further characterization [12]. One volume of the stool suspensions was mixed with nine volumes of a CsCl solution in phosphate-buffered saline (density 1.20 g/ml). After centrifugation at 180,000 g for 48 h the clearly visible band was collected, dialyzed against 0.01 Tris buffer (pH 7.40) and used for negative contrast electronmicroscopic examination.

### Statistics

For statistical evaluation all patients and controls were divided into three groups: *group A*, patients with AIDS or LAS; *group B*, homosexual men with or without clinical symptoms other than those of group A; *group C*, heterosexual patients and controls. Data were analyzed using the chi-square test with Yates' correction. Group A was compared with groups B and C, and group B with group C. These two tests are orthogonal contrasts. Additionally, the 2 × 2 table correlation coefficient was computed and tested.

### Results

The clinical and immunologic findings presented at the time of investigation are shown in Table 1. Patients with AIDS or LAS are clearly distinguished by the inversion of the T<sub>4</sub>/T<sub>8</sub> ratio, the decrease in absolute T<sub>4</sub><sup>+</sup> lymphocytes, and the elevation of the serum neopterin. Cutaneous anergy was observed in 10 of 16 cases. In contrast, the remaining study population of homosexuals or heterosexuals failed to show such abnormalities.

Cultures of three consecutive stool samples examined for the presence of bacterial pathogens in symptomatic homosexuals excluded infections of *Salmonella* sp., *Shigella* sp., *Yersinia* sp., and *Campylobacter* sp. Proctoscopy and mucosal biopsies were performed in symptomatic cases and only

**Table 1.** Clinical and immunological findings of study population

Patient groups	Age: mean and (range)	Clinical diagnosis	n	Neo-pterin bnol/l	T-lymphocyte subsets <sup>a</sup>			
					T <sub>3</sub> μl (%)	T <sub>4</sub> μl (%)	T <sub>8</sub> μl (%)	T <sub>4</sub> /T <sub>8</sub> ratio
Group A AIDS and LAS n=16	33.8 (22–50)	Kaposi sarcoma	2	27.6	1,334 (72)	473 (25)	899 (48)	0.50
		Opportunistic infection	2	22.4	645 (66)	191 (18)	330 (33)	0.47
		Lymphadenopathy and diarrhea	4	17.3	1,944 (79)	690 (28)	1,036 (42)	0.67
		Lymphadenopathy	8	14.6	1,666 (78)	727 (33)	885 (42)	0.86
Group B Homosexual males n=20	33.2 (17–57)	Diarrhea	6	7.7	1,582 (77)	893 (43)	686 (34)	1.35
		Amebic liver abscess	1	20.4	2,843 (84)	1,946 (58)	964 (29)	2.00
		Acute hepatitis B	8	12.4	1,977 (78)	1,069 (43)	835 (31)	1.40
		Asymptomatic	5	5.6	1,977 (77)	1,311 (50)	727 (27)	1.88
Group C Heterosexual controls n=18	34.4 (24–58)	Diarrhea	2	ND	ND	ND	ND	ND
		Asymptomatic	16	4.7	1,588 (75)	927 (44)	534 (25)	1.77

<sup>a</sup> Mean values of absolute or relative (*in brackets*) lymphocyte subset count are indicated; ND: not done

**Table 2.** Laboratory findings in patients with AIDS or LAS (Group A) as compared to controls (Group B and Group C). Percentages in brackets

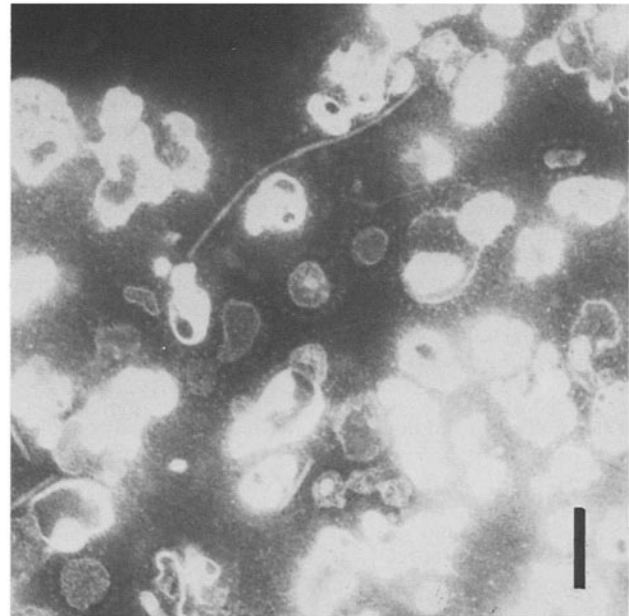
Patient groups	$T_4/T_8$ <0.9	Antibodies against				Intestinal protozoa <sup>a</sup>	Coronavirus- like particles
		HAV IgG	HBc	CMV-VCA IgG	T. pallidum IHA		
<b>Group A</b>							
AIDS or LAS <i>n</i> =16	14 (87%)	6 (37.5%)	10 (62.5%)	15 (93.7%)	8 (50%)	11 (69%)	8 <sup>b</sup> (50%)
<b>Group B</b>							
Homosexual males <i>n</i> =20	0 (0%)	5 (25%)	14 (70%)	15 (75%)	6 (30%)	8 (40%)	3 (15%)
<b>Group C</b>							
Heterosexual controls <i>n</i> =18	0 (0%)	2 (11%)	2 (11%)	10 (55%)	ND	0 (0%)	0 (0%)

<sup>a</sup> Cysts of the following protozoa were identified as joint agents: *Entamoeba histolytica*, *E. coli*, *E. hartmanni*, *Endolimax nana*, *Jodamoeba bütschlii*, and *Giardia lamblia*

<sup>b</sup> There is a significant difference ( $2\alpha < 0.01$ ) of Group A versus Groups B and C, no statistical difference was observed between Group B and Group C. These tests are orthogonal contrasts

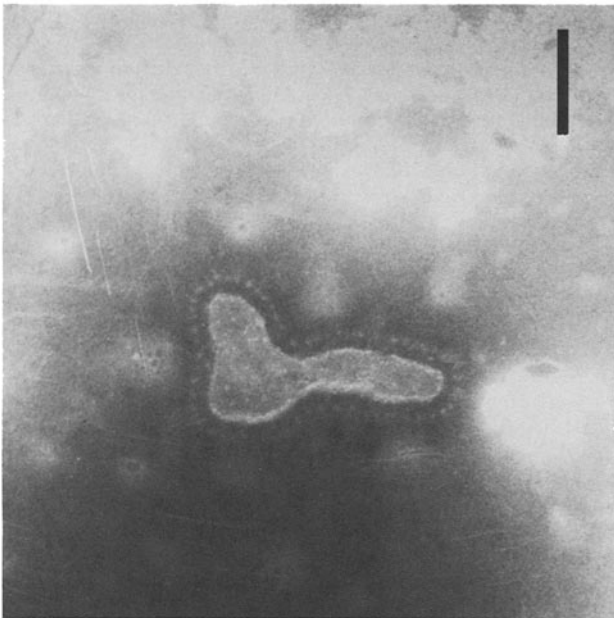
a nonspecific irritation was observed. Lymph node histology in all cases with lymphadenopathy revealed follicular hyperplasia. The serological, parasitological and virological investigations are concluded in Table 2. A high proportion of patients with AIDS or LAS revealed antibodies against hepatitis A, hepatitis B, cytomegalovirus, and syphilis, but so also did homosexuals not belonging to these groups. The parasitological stool investigations revealed that 40% to 70% of homosexual men harbor cysts either of apathogenic intestinal protozoa, i.e., *Entamoeba coli*, *Entamoeba hartmanni*, *Endolimax nana*, *Jodamoeba bütschlii*, or of pathogenic intestinal protozoa, i.e. *Entamoeba histolytica* or *Giardia lamblia* (Table 2).

By the negative staining technique, distinct particles were visualized electronmicroscopically in 11 homosexual patients. These particles are to be classified as CVLP because of their typical pleomorphic appearance and their regular radiating projections (Figs. 1 and 2). No other distinct structural entities resembling known viruses were found in any specimen. In order to distinguish the particles from cellular debris, the material was further characterized by CsCl density gradient centrifugation. A buoyant density of 1.21 g/ml was observed for those particles which clearly identifies CVLP. Six patients were studied repeatedly over a period of 12 months and CVLP could be identified in all samples re-examined. Eight of 16 patients (50%) with AIDS or LAS were found to excrete particles within the feces, a finding which is significantly



**Fig. 1.** Multiple pleomorphical CVLP with typical ring-shaped surface projections in the feces of homosexually active males. The width of the fringe in different particles was approximately 20 nm. Bar represents 100 nm

different from the group of homosexual and heterosexual patients and controls ( $2\alpha < 0.01$ ). Two patients of group B who excreted CVLP had unexplained diarrhea over a long period of time whereas the remaining positive case offered no clinical symptoms at all. The presence of CVLP in the feces correlated with the clinical symptom of



**Fig. 2.** Typical CVLP in the feces of a patient with LAS and diarrhea. Bar represents 100 nm

lymphadenopathy ( $r=0.48$ ,  $P<0.01$ ) or with the immunologic findings of an inverted T4/T8 ratio ( $r=0.46$ ,  $P<0.01$ ), whereas no correlation could be found with enteritic symptoms. Among seven sera from homosexual males with AIDS or LAS, tested for the presence of such particles, one specimen contained a small number of CVLP, whereas no particles were found in 18 heterosexual controls.

### Discussion

Our findings clearly show that homosexual men are preferentially exposed to a number of infectious or transmittable agents including apathogenic intestinal protozoa. With the exception of the clear immunologic aberrations seen in patients with AIDS or LAS, most of the homosexual males were found to have markers of presumably sexually transmitted diseases to a similar extent. In 50% of the patients with AIDS or LAS, however, we found structurally distinct viral particles in the feces by the negative staining technique. They showed the typical morphology of coronaviruses characterized by a corona of widely spaced radiating projections [15, 16] and resembled a buoyant density (CsCl) of 1.21 g/ml characteristic of CVLP [12]. The sensitivity in detecting CVLP by our protocol of stool preparation and the EM exploration is not known. However, the observed frequency must be considered as a minimum because samples with low numbers of particles might have been overlooked by the EM method.

Human coronaviruses (strain 229E and OC43) are associated with lower respiratory tract disease and are responsible for 15% of common colds [16]. These viruses are distributed world-wide and antibodies were present with high prevalences [14, 22]. In addition, some human coronaviruses may be associated with enteric infections [2, 3, 13]. However, their causative role and their serological relationship to other human coronaviruses has not yet been demonstrated [25]. Two distinct groups of enteric coronaviruses can be differentiated: one is a minor group of enteric coronaviruses that has been reported from neonates associated with epidemic gastroenteritis [24], and the other occurs in babies in nursery homes and causes a necrotizing ulcerocolitis [6]. The major group has been reported from many parts of the world in children and adults with gastrointestinal diseases, although a significant proportion of the unaffected population also shed these particles [3, 12]. It is deduced that coronavirus infection might become endemic under low standard of personal hygiene as there is a high incidence found in the rural population in Southern India [15] and among Australian aborigines [23].

While no information is available with regard to the mode of transmission of enteric coronaviruses, similarities to other viral and microbial agents present in the homosexual population become obvious. The particular intimate contacts of homosexuals favor a fecal-oral route of transmission [19]. In epidemiologic studies it has been shown that the enteric coronaviruses are occasionally excreted in the feces over a long period of time (up to 85 months) [3]. This was also found in our patients with LAS or AIDS, even during potent antiviral therapy with rIFN  $\alpha$ A. However, the most striking finding was the presence of the virus in the blood of one patient with LAS and intermittent diarrhea. This illustrates that the organ tropism of most of the known animal or human coronaviruses [25] might not be present in the examined group of homosexual males. In contrast to acute infections caused by most of the known animal and human coronaviruses, some murine and feline types involve multiple organs and have a strong tendency to establish persistent and chronic diseases [10, 25]. It remains open to question whether the detection of CVLP in human feces and blood demonstrates the systemic spread and multiple organ tropism of an up until now unrecognized strain of the CVLP.

It appears that CVLP might act as another infectious agent in homosexual men and that chronic infections might establish themselves because of

the particular immunodeficient state characteristic of AIDS or diseases belonging to the AIDS-related complex.

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Dr. Peter Kern

Klinische Abteilung, Bernhard-Nocht-Institut für

Schiffs- und Tropenkrankheiten

Bernhard-Nocht-Str. 74

D-2000 Hamburg 4

Bundesrepublik Deutschland

## Note Added in Proof

Sera of patients were investigated for the presence of HTLV III antibodies by indirect immunofluorescence [9]. 14/15 (93%) patients of group A and 1/20 (5%) patients of group B were positive, whereas no antibodies could be detected in the control group.