

Short Communication

Opportunistic Infections with Coronavirus-Like Particles in Patients Infected with the Human Immunodeficiency Virus?

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Summary

From 35 patients infected with Human Immunodeficiency Virus (HIV) and belonging to various risk groups, 60 stool specimens were examined for the presence of Coronavirus-like particles (CVLP) using electron microscopy. CVLP were detected in 5 (8%) stool samples from 5 different patients (14%). Only one of the patients had diarrhoea. The five patients with CVLP-positive stools were all at advanced stages of HIV infection. The remarkable discrepancy between our data and another study, reporting a rate of 50% CVLP-positive HIV patients, most of them persistently shedding the virus, is discussed.

Zusammenfassung

Von 35 mit dem humanen Immundefizienz-Virus (HIV) infizierten Patienten aus verschiedenen Risikogruppen wurden 60 Stuhlproben elektronenmikroskopisch auf die Anwesenheit von Coronavirus-like particles (CVLP) untersucht. In 5 (8%) Stuhlproben von 5 (14%) verschiedenen Patienten wurden CVLP nachgewiesen. Lediglich bei einem Patienten bestand eine Diarrhoe. Alle Patienten mit CVLP-Nachweis befanden sich in einem fortgeschrittenen Stadium der HIV-Infektion. Die auffällige Diskrepanz zwischen unseren Daten und einer anderen Studie, bei der 50% der untersuchten HIV-Patienten überwiegend persistent mit CVLP infiziert waren, wird diskutiert.

Introduction

The clinical relevance of Coronavirus-like particles (CVLP) in acute gastroenteritis of neonates and infants seems to be undoubted (8). In contrast, the significance of

CVLP as an aetiological agent of diarrhoeal disease in adults is far less certain. The virus can be detected at the same rate, i.e. in Europe about 5 to 6%, in faecal extracts from grown-up patients with acute diarrhoea as from healthy adults. Recently, a prospective study of Kern et al. (6) stated the detection of CVLP in 50% (8/16) of adult patients with Acquired Immunodeficiency Syndrome (AIDS) or Lymphadenopathy Syndrome (LAS) without any correlation to enteritic symptoms. Furthermore, in 6 patients the viral shedding continued over a period of 12 months. The high incidence of a persistent CVLP infection in patients with serious immunodeficiency seems to make sense. It was assumed therefore, that CVLP could be another agent of opportunistic infections in immunocompromised hosts, and that CVLP could be one of the causative agents of diarrhoeal disease in AIDS (7).

With the object of confirming the results of Kern et al. (6) we investigated 35 patients infected with Human Immunodeficiency Virus (HIV). However, in contrast to Kern et al., we failed to detect CVLP in the stools of the HIV patients significantly more frequently than it has to be expected in the faeces of normal healthy adults.

Materials and Methods

Stools samples. 60 faecal samples were obtained from 35 HIV-infected adults, whose age ranged from 18 to 47 years. 34 patients were infected with HIV-1, one with HIV-2, as proven by HIV-antibody detection assays (i.e. enzyme linked immunosorbent assay, immunofluorescence test and immunoblot). The patients were at different clinical stages of disease: 5 (8%) stool specimens were taken from patients at stage 1b according to the classification scheme of Brodt et al. (2), 9 (15%) at stage 2a, 18 (30%) at stage 2b and 28 (47%) at stage 3. The patients belonged to various risk groups: 19 male hemophiliacs, 8 male homosexuals, 4 male and 2 female intravenous drug users, and one man whose exposition is unknown. The man infected with HIV-2 had spent many years in North-West-Africa. 14 of these patients (40%) were repeatedly examined over a period of 15 months at the longest. During this period of observation, the clinical situation of 4 patients deteriorated (one patient changed from stage 2a to 3, three patients from stage 2b to 3). As a control group, 860 children with acute gastroenteritis, examined once within 18 months, were used.

Electron microscopy. Stool specimens were suspended in phosphate-buffered saline (approximately 10% v/v) and the suspension centrifuged at $1,400 \times g$ for 10 min to clear it of bacteria and debris. 28 of the faecal supernatants were further processed by the agar-diffusion-filtration technique (5), preparing 2 formvar coated copper grids from each for electron microscopy. In 29 of the faecal specimens one aliquot of the supernatant was processed by the method mentioned above and a second by ultracentrifugation at 50,000 rpm for 20 min in the airfuge (Beckman Inc.) directly onto the copper grids. In three of the faecal samples two preparations were processed by ultracentrifugation only. Grids were treated with 2% phosphotungstic acid (pH 7) and examined with a Siemens Elmiskop 101, magnification $80,000 \times$. The virus was identified by its characteristic morphology: the particles were pleomorphic with diameters ranging from 80 to 230 nm, and displayed the typical radiating surface projections measuring 20 to 30 nm (Fig. 1). The virus was seen regularly in separate particles, virus aggregations were exceptional.

Results

CVLP were only detected in 5 stool samples (8%) of 5 patients (14,3%) (Table 1). The positive specimens came from three hemophiliacs and two homosexuals. Only one of the patients suffered from diarrhoea when the stool sample was taken. Virus shed-

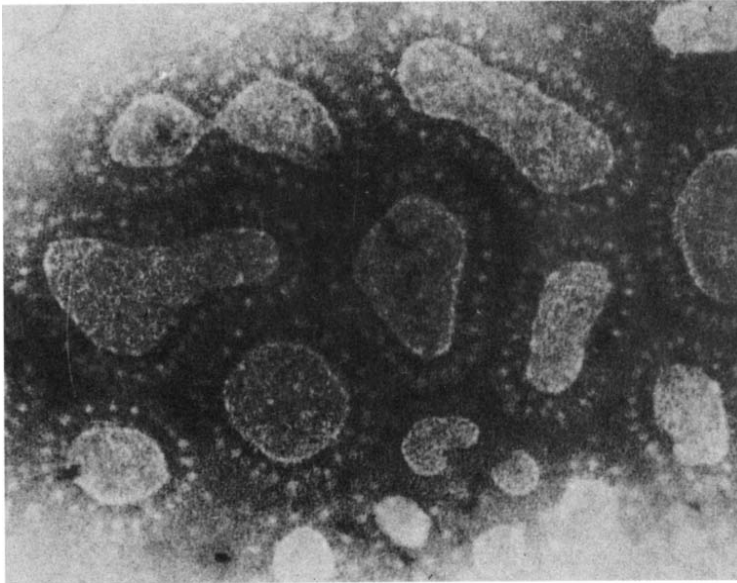


Fig. 1. Pleomorphic CVLP with typical radiating surface projections, detected in the faeces. Magnification 160,000 \times .

ding obviously did not persist, as reexamination of faecal excretions from the enteritic and one other patient two days and four weeks later respectively did not yield CVLP. Two of the CVLP-shedding HIV patients died very soon (i.e. 5 and 18 days after we had received the first stool specimens). CVLP were detected only in patients at an advanced stage of HIV infection: Two patients were at stage 2b, three patients at stage 3. In the control group CVLP were detected in 79 of 860 cases (9.2%).

Table 1. Detection of CVLP in faecal samples of HIV-infected patients

Risk group of the HIV-1 infected patients	Clinical stage* at the time of stool examination			
	1b	2a	2b	3
male hemophiliacs	1	4	16(2) [§]	10(1)
male homosexuals	3	1	0	13(2)
male i.v. drug users	1	3	2	0
male pat. with unknown risk	0	0	0	1
female i.v. drug users	0	1	0	1
HIV-2 infected man	0	0	0	3

* According to the classification scheme of *Brodt et al. (2)*.

[§] Number of examined stools, in brackets number of positive stools (i.e. number of positive patients).

Discussion

The data presented here show that, in contrast to the findings of *Kern et al.*, the proportion of CVLP-positive stools (8%) of HIV-infected patients did not essentially surpass the data reported for healthy adults (3, 8). Even when looking only at the results of patients with advanced immunodeficiency syndrome (stage 2b and 3), no more than 11% of the faecal samples proved to be CVLP-positive. *Kern et al.* concentrated their study on 16 homosexual men as a social group "preferentially exposed to a number of infectious and transmissible agents". Also when focussing on this particular social group in our investigation, the occurrence of CVLP in stool specimens (2/17) did not significantly exceed the rate of the rest of the HIV-infected individuals.

Two factors could be responsible for the discrepancy between our results and those of *Kern et al.*: First, the sensitivity of the methods applied for CVLP detection could be different. In this respect, it is important to note that our detection rate of 9.2% in children suffering from acute gastroenteritis is in the range of other reports (i.e. only 2.2% CVLP-positive faeces in England (3), 15.3% and 8.1% resp. in Germany (1, 8)), and is significantly higher than that in healthy children (1.4%) (8). Furthermore, a comparative investigation (4) revealed that both low-speed centrifugation and ultracentrifugation are the most sensitive techniques for detection of CVLP by electron microscopy.

Certain characteristics of the respective group of patients studied could be another reason for the incongruous results. As documented by several investigators (9, 10, 11) the occurrence of CVLP can vary considerably from one population to the other in various regions and societies of the world. Furthermore, the incidence of CVLP infections seems to be greatly dependent on faecal-oral transmission. In our investigation predominantly hemophiliacs were examined whereas *Kern et al.* concentrated their study on homosexuals.

In summary, one can conclude that HIV-infected patients obviously do not have an increased incidence of CVLP infections and that the high rate of CVLP infections detected by *Kern et al.* is probably related to the social behaviour of the patients involved in the study.

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