Diarrhoea and malabsorption in acquired immune deficiency syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations

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SUMMARY Chronic diarrhoea is frequent in acquired immune deficiency syndrome (AIDS) but has been poorly investigated so far. We report four patients with AIDS in whom diarrhoea and malabsorption were outstanding features, and who underwent extensive digestive investigations. Diarrhoea was a presenting symptom in all subjects and was of secretory type in three of them. D-xylose and vitamin B₁₂ were malabsorbed in all cases; steatorrhea was found in the two patients who could ingest significant amounts of fat. Faecal α 1-antitrypsin clearance was increased in all subjects. Search for digestive pathogens showed unusual protozoans in all patients: in case 1, optical and electron microscopy revealed the presence in the cytoplasm of villous enterocytes of *Microsporidia* protozoans still unreported in AIDS. Stool and jejunal fluid examination showed *Isospora belli* in case 2 and *Cryptosporidium* in cases 3 and 4. On histological and ultrastructural study the former was localised in the cytoplasm of a few enterocytes and the latter was scattered throughout the villus and crypt brush border. Otherwise small intestinal histology only showed minor non-specific changes and the enterocytes were ultrastructurally normal. In patient 3 the slow marker intestinal perfusion technique showed a profuse fluid secretion in the duodenum and proximal jejunum. All patients needed prolonged total parenteral nutrition. *Cryptosporidium* and *Microsporidia* could not be eradicated despite multiple drug trials. *Isospora belli* was transiently cured by pyrimethamine-sulphadiazine. Only patient 2 is presently at home, and patients 1, 3, and 4 died after two, six, and nine months of total parenteral nutrition, respectively.

Diarrhoea has been recently recognised as a frequent symptom in the acquired immune deficiency syndrome (AIDS), especially in Haitian patients;^{1,2} however, its type and mechanism have not been investigated as yet. Within the last two years we had the opportunity to study four AIDS patients in whom diarrhoea and malabsorption were outstanding clinical features. The aim of the present paper is to report the detailed digestive investigations carried out in these patients in order to better characterise the digestive abnormalities in AIDS. It emphasises the frequency of unusual protozoan infestations of the gut and the usefulness of precise morphological studies (optical and electron

microscopy) of small intestinal biopsy specimens for their diagnosis.

Case reports

CASE 1

A 29 year old Haitian was admitted in February 1983 with a five month history of diarrhoea, weight loss (17 kg), fever, and epigastric pain. He had been living in France for four years. He denied any homosexual practice, drug addiction, and blood transfusion. On admission he experienced one to three bowel movements per day and 24 hour faecal output fluctuated between 0.3 and 1.4 l of solid to watery non-bloody stools. Physical examination showed diffuse lymphadenopathy and acquired ichthyosis. Immunological investigations and

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intestinal function tests are summarised in Tables 1 and 2. Examination of stools – including the flotation technique³ – only found *Giardia lamblia*. Examination of the jejunal fluid was negative for parasites. Upper endoscopy (up to the proximal jejunum) and colonoscopy with ileoscopy were unremarkable. Small bowel barium radiography only showed parallel mucosal folds. Light microscopy studies of four duodeno-jejunal and two ileal biopsies (Fig. 1) showed in addition to minor non-specific mucosal changes (partial villous atrophy and mild inflammatory infiltrate of the lamina propria), small (1–5 μm) round or ovoid basophilic bodies which were variably stained by Giemsa, Grocott, and Ziehl stains. They were seen in about one half of villous enterocytes, mainly in the apical area of the villus; they were exclusively located in the supranuclear portion of the cytoplasm of these cells. They were diagnosed as *Microsporidia* under the electron microscope on the following characteristic features:^{4,5} (a) absence of mitochondria; (b) various stages (Fig. 2) of sporogenesis (uni or multinucleated sporonts, uninucleated sporoblast and spores); (c) highly specific coiled polar filament (Fig. 3). No parasitophorous vacuole was seen. Furthermore an abnormal accumulation of neutral fat was found within dilated intercellular spaces of the epithelium and in perivascular areas of the lamina propria. Gastric, rectal, and colonic biopsies were normal. No viral particle was seen in the digestive biopsy specimens. Pyrimethamine (50 mg daily $\times$ 5 days), sulphadiazine (2 g daily $\times$ 15 days) and metronidazole (1.5 g daily $\times$ 7 days) were administered; giardiasis was eradicated but

Fig. 1 Case 1. Duodenal biopsy. Numerous microsporidians in the supranuclear area of villous enterocytic epithelium (arrows) (haematoxylin-eosin $\times$ 1100 original magnification).

diarrhoea and intestinal function tests did not improve. Several extradigestive opportunistic infections (diffuse facial ulcerations caused by *Herpes simplex* virus, *Mycobacterium kansasii* pneumonia, *Pneumocystis carinii* and cytomegalovirus lung infection) and unexplained bouts of fever occurred repeatedly and were treated by multiple oral and parenteral antibiotics. Finally he died 16 months after the onset of the disease. Necropsy revealed in addition Kaposi's sarcoma of inguinal lymph nodes, lung aspergillosis and total destruction of adrenal glands by fibrosis and necrosis.

Table 1 Immunological investigations.

	Absolute lymphocyte count (n/ μl)	Lymphocyte subsets			Skin tests*	Lymphocyte response to non-specific mitogens†	Lymphocyte response to specific mitogens‡	Serum immunoglobulins (g/l)
		% OKT4	% OKT8	ratio OKT4/OKT8				
Patient 1	80–1580	5	68	0.07	0	0	0	IgG = 32.1 IgA = 7.35 IgM = 2.3
Patient 2	320–1470	4	56	0.07	0	+	0	IgG = 20.4 IgA = 9.9 IgM = 2.2
Patient 3	150–2050	2	60	0.03	0	+	0	IgG = 14.7 IgA = 5.9 IgM = 2.9
Patient 4	375–1360	4	80	0.05	0	↘	ND§	IgG = 12.4 IgA = 4.8 IgM = 1.6

* Tuberculin, candidin, streptokinase, trychophyton, tetanus, diphtheria and proteus antigens. † Phytohaemagglutinin, pokeweed mitogen and concavalin A. ‡ Tuberculin, candidin, varidase, CMV and tetanus antigens. § ND = not done. 0, ↘ and + mean respectively negative, diminished and normal responses to all the antigens tested.

Table 2 Intestinal function tests.

	D-xylose* N>1.66 mmol/l	Faecal fat N<21.6 mmol/d	Schilling test with intrinsic factor (N>10%)	Alpha-1-antitrypsin clearance (N<15 ml/d)	Stool electrolytes‡ (mM/litre)		Stool‡ osmolality (mOsm/kg)
					Na	K	
Patient 1	0.53	60.5	3	57	ND§	ND	ND
Patient 2							
initial values	0.26	86.4	3	112	73	31	ND
After eradication of <i>I belli</i>	2.20	20.9	25	32.5	ND	ND	ND
Patient 3	0.40	2.15+	3	45	68	63	299
Patient 4	0.46	9.36+	0.3	28	88	51	297

* Blood D-xylose 120 min after 25 g oral load. † Low and not controlled lipid ingestion. ‡ These data were measured during fasting.
§ ND = not done.

CASE 2

A 30 year old man, living in Green-Cape Island, first presented in January 1982 with diarrhoea and intermittent fever. Within the ensuing 18 months diarrhoea persisted, he lost 23 kg in weight and developed typhoid fever, *Giardiasis intestinalis*, *Salmonella enteritidis*, septicaemia, and *Pneumococcus pneumonia*. The patient was referred to us in June 1983: he appeared chronically ill and dehydrated. He denied homosexuality, drug addiction, and blood transfusion; physical examination was normal. He experienced 8–10 watery non-bloody bowel movements per day; stool volume ranged from 1–6 l per day (average: 2 l) when he was eating and was not markedly reduced by fasting (average: 1.3 l). Immunological and intestinal function tests are shown in Tables 1 and 2.

Additional routine blood tests were normal. Jejunal aspirate and stool examinations for parasites showed *Isospora belli* oocysts; *Candida albicans* was also present in the stools. Bacterial count in the jejunal fluid was normal. Upper digestive endoscopy showed oesophageal candidiasis which was confirmed by biopsy; colonoscopy with ileoscopy and small bowel barium radiographs were normal. Histological examination of five duodenojejunal and one ileal biopsy specimens showed villi of normal or slightly reduced height, with a moderately increased lamina propria infiltrate; in addition a few (less than three per villus) *Isospora belli* maturing schizonts with their merozoites [1–3 elongated (8×3 µm)] bodies, within a vacuole at the basal pole of villi enterocytes were seen (Fig. 4). The presence of *Isospora belli* was confirmed by electron microscopy

Fig. 2 Case 1. Portion of an enterocyte with its nucleus (N) and, in the cytoplasm two developmental stages of microsporidians: one multinucleated (n) plasmod (P) and 6 mononucleated sporoblasts (*) (electron microscopy ×19 000).

Fig. 3 Case 1. Part of a sporogonial microsporidian plasmod showing the juxtannuclear origin of polar filament (PF). N = nucleus. P = polaroplast. (Electron microscopy ×50 000).

Fig. 4 Case 2. Duodenal biopsy. Two merozoites of *Isospora belli* in villous enterocytic epithelium (arrows). (May Grunwald Giemsa $\times 1000$).

(Fig. 5). Gastric, colonic, and rectal biopsies were normal. No viral particle was seen on the gastro-intestinal biopsies. The patient received a three week course of total parenteral nutrition and the following drugs were given orally: metronidazole (0.75 g daily $\times$ 5 days), amphotericin B (3 g daily $\times$ 40 days), pyrimethamine (50 mg daily $\times$ 26 days), sulphadiazine (4.5 g daily $\times$ 26 days). The diarrhoea disappeared progressively and two months later the patient had gained 23 kg; absorption tests had returned to normal (Table 2), *Isospora belli* had disappeared from the stools, duodenal aspirate, and

Fig. 5 Case 2. Intra enterocytic merozoites of *Isospora belli* (arrows) showing nucleus (N), rhoptries (R), micronemas (M) and granules (G). (Electron microscopy $\times 12\,500$).

duodenojejunal mucosa; he was discharged in July 1983. In September 1983 he was readmitted for pulmonary tuberculosis and recurrence of diarrhoea. *Isospora belli* was present in stools and small intestinal biopsies. He responded again to a course of pyrimethamine and sulphadiazine.

CASE 3

This case has been reported in detail elsewhere.⁶ Briefly this 31 year old Frenchman presented in April 1982 with diarrhoea (3–7 daily stools), vomiting, abdominal pain, and weight loss (10 kg) of eight months' duration. The only risk factor for AIDS was a trip to French West Indies and Haiti where he received blood transfusion after a road accident in 1978. On admission physical examination was normal; the daily stool output averaged 0.720 l when he was eating and was not influenced by prolonged total parenteral nutrition (average: 1 l). Routine blood tests were normal. Immunological and intestinal function tests are shown in Tables 1 and 2; Standard stool examination for parasites only found *Candida albicans* but thereafter *Cryptosporidium* sp oocysts were seen in faecal smears stained by modified Ziehl-Neelsen technique.⁷ The protozoan was also found in the jejunal fluid. Upper digestive endoscopy showed whitish granulations of the duodenal mucosa; colonoscopy was normal and small bowel barium radiography showed moderate mucosal fold coarsening. Multiple duodenojejunal biopsy specimens showed normal villous height or partial villous atrophy; crypts were of normal size with occasional indications of cryptitis. There was a dense inflammatory infiltrate of the lamina propria consisting mainly of plasma cells with a few polymorphs and macrophages. *Cryptosporidium* organisms were seen (Fig. 6) as multiple round (2–4 μ m) basophilic bodies, variably stained by periodic acid/Schiff and Grocott's silver impregnation, scattered throughout the villus and crypt brush border in nearly all small intestinal biopsy specimens. *Cryptosporidiosis* was confirmed by electron microscopy (Fig. 7). *Cryptosporidium* sp protozoans were also found in some of the gastric biopsy specimens. Rectal and colonic biopsy samples were normal. No viral particle was seen in the gastrointestinal biopsy samples. A slow marker intestinal perfusion⁸ was performed to localise the site of intestinal secretion; fasting flow rates were measured for 24 hours in the proximal jejunal and distal ileum. Results are shown in Table 3. Numerous agents were given orally: metronidazole (1.5 g daily $\times$ 15 days), amphotericin B (2 g daily $\times$ 75 days), nystatin (300 000 U daily $\times$ 23 days), sulphamethoxazole trimethoprim (1600 mg and 320

Fig. 6 Case 3: Jejunal biopsy. Numerous cryptosporidians attached to the brush border of enterocytes (arrows) (haematoxylin-eosin $\times 430$).

Fig. 7 Case 3. Trophozoite of *Cryptosporidium* in the apex of an enterocyte with characteristic junctional zone (arrows) and feeder organelle (FO). CM = cytoplasmic membrane of enterocyte. ER = endoplasmic reticulum of trophozoite. (Electron microscopy $\times 35\,000$).

Table 3 Fasting 24 hour flow rate in proximal jejunum and at the ileocaecal junction, and stool output.

	24 h flow rates		24 h stool output
	J*	IC*	
Fluid (ml)			
Patient 3	11 245	6 432	1 000†
Normal	2 650 $\pm$ 806‡	2 246 $\pm$ 403‡	<300
Sodium (mmol)			
Patient 3	1 619	751	47†
Normal	361 $\pm$ 92‡	324 $\pm$ 77‡	<10

* J: proximal jejunum; IC = ileocaecal junction; the distal sampling point migrated from the ileum to the caecum 7 hours after onset of the experiment; hourly outputs measured successively at these 2 sites were identical and results were pooled as 24 h ileocaecal flow rates. † Average of daily faecal outputs during 7 days preceding the perfusion. ‡ Mean $\pm$ 2SD; 4 normal subjects.⁸

mg daily $\times$ 5 days), paramomycin (2 g daily $\times$ 26 days), framycetin (900 mg daily $\times$ 13 days), pyrimethamine (50 mg daily $\times$ 27 days), and sulphadiazine (3 g daily $\times$ 27 days); *Candida albicans* was eradicated but diarrhoea did not improve; *Cryptosporidium* persisted on repeated small bowel biopsies. While on total parenteral nutrition he continued to have vomiting, abdominal pain, and diarrhoea, and developed several episodes of *Salmonella typhi* murium septicaemias (for which he received multiple courses of antibiotics) and *Toxoplasma gondii* cerebral abscesses. He died suddenly on October 24, 1982. Permission for necropsy was not granted.

CASE 4

A 36 year-old Egyptian homosexual male was diagnosed as having AIDS complicated by cryptosporidiosis in another hospital. He was referred in August 1983 for total parenteral nutrition because of intractable chronic diarrhoea (10–15 watery stools per day), with hypokalaemia (2.7 mM/l), vomiting, weight loss (14 kg) and fever since December 1982. On admission his temperature was 38°C; physical examination disclosed left axillary and inguinal lymphadenopathy and mild splenomegaly; daily stool volumes ranged from 1 to 2.5 l (average: 1.7 l) when he was eating and averaged 1.3 l on fasting. Routine blood tests were normal. Immunological and intestinal function tests are shown in Tables 1 and 2; glucose (50 g) breath test was normal. Stool examination for parasites confirmed the presence of *Cryptosporidium* oocysts and *Candida albicans*. Upper digestive endoscopy, colonoscopy, and small bowel barium radiography were normal. Seven duodenojejunal biopsy specimens showed the presence of *Cryptosporidium* with a histo-

pathological and ultrastructural picture identical to that of patient 3. The parasite was also found on colonic biopsy specimens. No viral particle was seen in the gastrointestinal biopsy samples. The following drugs were tried orally: amprolium (1 g daily $\times$ 16 days), sulphadimetoxine (1 g daily $\times$ seven days), nystatin (4000 000 U daily $\times$ 16 days), amphotericin B (3 g daily $\times$ five days). He also received several courses of parenteral antibiotics and ornidazole for unexplained fever. Candidosis disappeared from the stools but repeated small bowel biopsies showed persistent cryptosporidiosis and the diarrhoea did not improve. The patient died on 22 April, 1984.

Discussion

Our four patients met the criteria for AIDS:⁹ (1) They had a profound cellular immune deficiency with low counts of helper T cells and decreased OKT4/OKT8 lymphocyte ratios. (2) They had no other cause for this immunological defect. Patients 1 and 4 belonged to classical high risk groups for AIDS⁹ (Haitians and homosexuals); patient 3 probably acquired the disease by receiving Caribbean blood four years previously; living in Green Cape Island was the only detectable risk factor in patient 2: he represents the first example of AIDS from this island, but a few cases have been reported from Continental Africa.¹⁰

Diarrhoea was a major clinical feature in our patients, leading in cases 2, 3, and 4 to hypokalaemia and requiring massive intravenous fluid and ion replacement. This symptom seems to be very frequent in AIDS^{1,2} (90% of Haitian patients with opportunistic infections).² On the contrary malabsorption has not been clearly documented so far in AIDS except in one case where it was associated with a disseminated *Mycobacterium avium* – *intracellulare* infection that mimicked Whipple's disease¹¹ histologically. Our results do suggest that severe malabsorption (involving fat, D-xylose, and vitamin B₁₂) is frequent in AIDS with diarrhoea, even in the absence of gut infestation with atypical mycobacteria; it may require prolonged courses of total parenteral nutrition. Furthermore our four patients had an exudative enteropathy (heretofore undescribed in AIDS) as shown by the high values of α 1-antitrypsin faecal clearance. Of special interest is the presence in our cases of uncommon (and in one case possibly undescribed) intestinal parasites.

Microsporidians represent a protozoan phylum that includes well known pathogens for arthropodes, fishes,¹² and a few mammals¹³ and is characterised by the presence of a polar filament inside the spore.

Microsporidiosis has not been reported as yet in AIDS; furthermore our patient is the first human case where the parasite was found in the cytoplasm of enterocytes. The number of previously published cases of human microsporidiosis is a matter of debate^{14–16} but does not exceed seven.^{17–23} In fact only four case reports^{20–23} are reasonably well documented though only one²² includes an ultrastructural study: in three of them, the organism was found in brain,²⁰ cornea,²³ and the cytoplasm of pancreatic carcinomatous cells.²¹ The last patient²² was a four month old infant with thymic aplasia who presented with diarrhoea; necropsy revealed disseminated microsporidiosis, involving gut smooth muscle, mesenteric ganglia and nerve fibres; enterocytes were apparently spared. The classification of *Microsporidia* remains uncertain; the organisms found in the human cases have been labelled as *Nosema*^{15,21,22} or *Encephalitozoon*.^{20,23} Morphology of spores and sporogenesis observed in our case differs from that described for these genera of microsporidians: *Nosema* spores are binucleated^{4,5,15} and *Encephalitozoon* sporogenesis, studied by electron microscopy in varied animal species²⁴ was found to take place by dropping of sporoblasts inside the cavity of a large parasitophorous vacuole, not seen in patient 1. Thus the *Microsporidia* involved in our case may represent a new genus.

The diagnosis of microsporidiosis has been especially difficult in our patient: it was not found despite careful examination of stools and jejunal fluid; it may also be overlooked by light microscopy of gut biopsy samples because of its very small size (1–5 μ m), intracellular location, and poor tinctorial properties. Thus electron microscopy is essential for identification of this parasite. The role played by the *Microsporidia* in the pathogenesis of the diarrhoea and malabsorption of patient 1 is unclear. It should be stressed that the parasite inhabited many villous enterocytes, and that no other cause of malabsorption was found: there was no significant villous atrophy, nor ultrastructural lesion of the enterocytes (apart from the presence of the organism); eradication of giardiasis did not improve the absorption tests. The accumulation of neutral fat in the intercellular spaces of the epithelium and in perivascular areas of the lamina propria during fasting is intriguing and points to a disorder of lipid transport somewhere beyond the enterocyte.

Isospora belli is a protozoan located in the enterocyte cytoplasm and usually reported from the tropics; it may be responsible for chronic diarrhoea, malabsorption, and various degrees of villous atrophy²⁵ in immunocompetent subjects. It has also been reported, although rarely, in AIDS.^{26,27} In

case 2, diagnosis was easily made by examination of stools and jejunal fluid, and confirmed by careful histological examination of the duodenojejunal mucosa. Its pathogenicity is very likely²⁵ and supported by our case 2: diarrhoea and malabsorption disappeared in concert with the disappearance of *Isospora belli* from the gut and relapsed when the parasitosis recurred. The pathophysiology of the malabsorption in patient 2, however, is unclear in the absence of severe villous changes observed by others²⁵ and with normal enterocytes on electron microscopy.

Cryptosporidium sp is a protozoan that primarily inhabits the microvillus regions of epithelial cells. Until recently cryptosporidiosis was considered to be rare and to occur mainly in immunosuppressed patients.²⁸⁻³⁰ Over the last two years it has been frequently reported in AIDS patients with diarrhoea³¹ and reached an incidence of 38% in a Haitian series.¹ This tiny parasite is easily overlooked,³⁰ unless faecal smears are adequately stained^{7, 32} and histological sections of intestine examined at high magnification.

Cryptosporidiosis resists any form of therapy in AIDS: it is thus impossible to know whether its eradication would cure the diarrhoea. Several facts, however, do suggest that this parasite is causally related to diarrhoea. (1) Recent reports show that *Cryptosporidium* may be commonly found in the stools of immunocompetent subjects suffering from acute, self-limiting diarrhoea.^{3, 33} (2) An outbreak of diarrhoeal cryptosporidiosis has been reported in previously healthy subjects who were exposed to calves infected with *Cryptosporidium*; organisms isolated from these calves and patients could be experimentally transmitted to normal calves and cause diarrhoea.³ (3) In one case²⁸ withdrawal of immunosuppressive therapy led to simultaneous cure of diarrhoea and cryptosporidiosis. Our findings in patients 3 and 4 show that they had a secretory diarrhoea: on fasting, high stool outputs persisted and the osmolality of faecal water was almost entirely accounted for by sodium, potassium, and their accompanying anions. We have shown in patient 3 that the secretion took place in the proximal small bowel, whereas the ileum and colon reabsorbed important amounts of fluid.

The pathophysiology of the secretory diarrhoea and malabsorption syndrome induced by this parasite in AIDS remains speculative: (1) no major abnormality of villous architecture was found, but the presence of numerous *Cryptosporidia* within the brush border (with microvilli loss and displacement) of otherwise well preserved enterocytes might impair transport through the apical pole of the cells. (2) The synthesis by the parasites of an intestinal

secretagogue is an interesting possibility. Protozoans are known to contain hormone-like materials and neurotransmitters³⁴ which are able to stimulate intestinal secretion^{35, 36} and might exert here a paracrine effect. Very recently lysates of *Entamoeba histolytica* were shown to produce intestinal secretion probably mediated by serotonin.³⁷

The role of other pathogens in the mechanism of our patients' diarrhoea is unlikely: *Giardia lamblia* and *Candida albicans* were eradicated without any improvement of the diarrhoea. Small intestinal bacterial overgrowth was probably not involved in the malabsorption syndrome as (1) patient 2 had a normal bacterial count in the jejunal fluid, and patient 4 a normal glucose breath test; (2) they all received almost continuous oral antibiotics and/or sulphonamides when malabsorption was documented. Finally, CMV gastrointestinal infection has been reported in AIDS,^{38, 39} but not found in our patients' gut biopsy samples.

In conclusion diarrhoea and malabsorption represent severe and often intractable manifestations of AIDS in which intestinal parasites probably play a major role. In our experience, only *Isospora belli* can be cured (at least transiently) with some benefit for the patient. Total parenteral nutrition is presently the only therapeutic possibility especially for the secretory diarrhoea of cryptosporidiosis. It has to be maintained usually until the patient's death as in cases 1, 3, and 4.

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