Recently recognised microbial enteropathies and HIV infection

Michael J. G. Farthing, M. Paul Kelly and Andrew M. Veitch

Digestive Diseases Research Centre, Medical College of St Bartholomew’s Hospital, Charterhouse Square, London EC1M 6BQ, UK

Persistent diarrhoea and small bowel enteropathy are important features of HIV infection. At least 80% of cases of persistent diarrhoea in patients with HIV/AIDS can be attributed to a specific enteropathogen. The coccidian parasites Cryptosporidium parvum, Isospora belli and Cyclospora and the Microsporidia account for at least 50% of cases of persistent diarrhoea in the industrialised and developing world with major contributions from Mycobacterium avium complex and other bacteria and cytomeglovirus. The intracellular protozoa can be detected on faecal microscopy although confirmation may be required by intestinal mucosal biopsies. Although up to 20% of cases of persistent diarrhoea can not be attributed to a specific infection, the question as to whether HIV infection itself can produce enteropathy remains uncertain. Recent developments in the treatment of persistent diarrhoea in HIV include the use of albendazole for microsporidial diarrhoea and co-trimoxazole for the treatment and eradication of Cyclospora.

Introduction

Persistent diarrhoea occurs in up to 75% of patients with HIV/AIDS in Western populations (Dworkin et al., 1985; Antony et al., 1988; Smith et al., 1988; Rene et al., 1989; Rolston et al., 1989; May et al., 1993) and in up to 100% of those infected in some developing countries (Quinn et al., 1987) and has a high morbidity and mortality. At least 80% of cases of chronic diarrhoea can be attributed to a specific enteropathogen of which the coccidial parasites are among the most important. Infective diarrhoea in HIV/AIDS can involve both the small intestine and the colon and the pan-enteric involvement probably accounts for the high volume of faeces lost that is characteristic of cryptosporidial and microsporidial diarrhoea. Disruption of the villous architecture of the small intestine commonly accompanies infective diarrhoea in HIV infection but the specific determinants of this enteropathy and the mechanisms by which it occurs are poorly understood. Further work is required to define the complex interactions between microbial enteropathogens, intestinal immune responses within the mucosa to these infective agents and their interactions with HIV itself. In advanced HIV infection, nutritional factors may also play a part.

Definition of enteropathy

Enteropathy is characterised by a variable reduction in villous height usually associated with a hyperplastic response in the crypts. There is therefore a decrease in the villous
crypt ratio which inevitably is accompanied by a decrease in the surface area of the small intestine. There may also be evidence of damage to the surface epithelial cells (enterocytes) with a reduction in the height of the cell changing it from a columnar to a cuboidal shape. The changes in villous morphology and enterocyte height are almost invariably associated with inflammatory cell infiltrates in the lamina propria and within the epithelium itself (intraepithelial lymphocytes). These morphological changes in the small intestine are often accompanied by diarrhoea but, if only the proximal small intestine is involved, the compensatory effects of the ileum and colon in retrieving water and sodium may render the individual symptom-free. This is well recognised in the classic enteropathy of coeliac disease which is due to the wheat protein, gliadin, in which, the total thickness of the mucosa is generally maintained or maybe increased when crypt hyperplasia is marked. Thus, the term 'villous atrophy' is not strictly accurate. However, in rotavirus infection in which there is primary loss of enterocytes in the mid-villous region there is a 'true' villous atrophy initially and only subsequently does crypt hyperplasia occur during the recovery phase.

Mechanisms of enteropathy

The enteropathy of coeliac disease is thought to be immune mediated. There is compelling evidence to suggest that T cell activation within the lamina propria may be directly associated with the changes in villous morphology although the precise mechanisms by which this occurs have not been clearly delineated (MacDonald & Spencer, 1988; da Cunha et al., 1990; Livnett et al., 1993). Thus, mucosal inflammation may be one of the key factors in the enteropathy associated with intestinal infection and is commonly associated with cryptosporidiosis, microsporidiosis, giardiasis and infection with Cyclospora. Despite the presence of T lymphocytes within the mucosa in patients with HIV and opportunistic infection in the gut, there is little evidence that these T cells are activated (Lim et al., 1993, 1994), a factor which does seem to be important in the production of villous atrophy in coeliac disease and experimental enteropathy in vitro (MacDonald & Spencer, 1988). In a recent study by Snijders et al. (1995) the cytokine profile in jejunal mucosal cultures was studied in patients with HIV and cryptosporidiosis, microsporidiosis and in HIV infected individuals without diarrhoea. In addition, Snijders et al. studied control tissue from subjects who were not injected with HIV. There was, however, no evidence of increases of IL-1β, IL6, IL8 or IL10 production in the HIV-infected individuals nor was there evidence of increased tumour necrosis factor (TNF-α) or soluble tumour necrosis factor receptor (sTNFR) expression. These findings would support immunohistochemical studies which suggest that mucosal immune activation is not a major feature of cryptosporidiosis and microsporidiosis in HIV. Our own studies in Africans infected with HIV conform with these observations although we did find slight but significantly increased expression of the T cell activation marker, HML-1 (Veitch et al., 1995).

As stated previously, there is evidence that certain enteropathogens such as rotavirus can damage enterocytes directly and produce true villous atrophy. It is possible that, coccidia, particularly the intracellular organisms, Microsporidia and Cyclospora could act in this way although there is no direct evidence to support this finding. It has been
proposed that giardia may disturb villous morphology not only by activating T cells through classic antigen stimulated pathways but also by the direct effect of its mannose-binding lectin, taglin, which shares some features with the plant lectin concavanalin A which is a mitogen.

Enteropathy occurs in HIV infection, sometimes in the absence of intestinal infection (Kotter et al., 1984; Ullrich et al., 1989). HIV has been detected within the intestinal mucosa (Fox et al., 1989) and it has been proposed that the virus itself may be responsible, at least in part, for the enteropathy of HIV/AIDS. In-vitro infection has been shown to reduce villous height and induce crypt hyperplasia in fetal small intestinal explants supporting this proposal (Batman et al., 1994). However, no direct evidence that this is the case in vivo has been forthcoming and sceptics favour the suggestion that the so-called 'pathogen negative diarrhoeas' in HIV are actually due to opportunistic infection which has failed to be detected or is due to an, as yet, unrecognised pathogen. It is well established, however, that there is evidence of generalised immune activation in HIV/AIDS as indicated by increased levels of sTNFR and neopterin (Zangole et al., 1993). We have shown a close correlation in African's with AIDS of these markers with villous height and the degree of mucosal inflammation which does not, of course, establish a causal link (Kelly et al., 1994). There is, however, a remarkably good relationship between the intensity of mucosal inflammation and the intra-epithelial lymphocyte count and villous height but it is unclear, how this inflammatory response relates to the abnormalities of villous architecture when the evidence for T cell activation is relatively minor and the cytokine profile is not significantly different from that of normal small intestinal mucosa.

Finally, there is some evidence that nutritional factors may play a part in producing enteropathy. In experimental models, low calorie and low protein diets can produce abnormalities of villous architecture although the small intestinal epithelium is relatively resistant to the effects of nutritional deficits. In addition, deficiencies in micronutrients, e.g. folic acid and vitamin A, can affect intestinal epithelial integrity. In advanced AIDS, the body mass is often dramatically reduced and, in our own recent studies in Africa, we found a weak, but nevertheless significant, correlation between villous height and triceps skin fold thickness (Kelly et al., 1994), a marker of the size of the body fat compartment. Again, one may merely be observing a correlation between two dependent variables rather than any causal relationship.

**Microbial diarrhoea and enteropathy**

A variety of microbial enteropathogens have been linked aetiologically with persistent diarrhoea in patients with HIV infection (Table I). *Mycobacterium avium* complex (MAC) is one of the major bacterial enteropathogens causing chronic diarrhoea and enteropathy, producing appearances in the small intestine reminiscent of Whipple's disease (Roth et al., 1985; Rolston et al., 1989) due to *Tropheryma whippleii*. It is the protozoa however, particularly the coccidial enteropathogens such as *Cryptosporidium parvum*, the microsporidia and *Isospora belli*, that are responsible for the majority of cases of persistent diarrhoea. The role of *Blastocystis hominis* remains uncertain although it is isolated relatively frequently from HIV-infected individuals with diarrhoea.

The relative importance of these enteropathogens is, to some extent, determined by geographic location. *I. belli* for example, is uncommon in AIDS patients in the USA.
and Europe where it accounts only for approximately 1% of patients with chronic diarrhoea, but in tropical and sub-tropical countries such as Haiti and parts of Africa it is more common, accounting for between 15–28% of cases (De Hovitz et al., 1986). Currently in the UK, *C. parvum* and microsporidia account for almost 50% of cases with chronic diarrhoea followed by cytomegalovirus, *Giardia intestinalis* and MAC (Table II) Gazzard & Blanchard (1993). The apparent distribution of infective agents has also changed with time as is well demonstrated by two studies from Zambia reported in 1990 and 1995 (Conlon et al., 1990; Drobniewski et al., 1995). In the earlier study, 43% of cases of chronic diarrhoea with enteropathy were considered pathogen negative (Conlon et al., 1990). *C. parvum* and *I. belli* accounted for more than half of the cases in which a pathogen was identified. However, in the study reported four years later (Drobniewski et al., 1995), only 19% of individuals were considered pathogen negative due, principally, to the identification of microsporidia including *Enterocytozoon bieneusi* and the related member of the sporozoa, *Septata intestinalis*, which was recently identified for the first time in AIDS patients in Southern Africa (Kelly et al., 1994; Drobniewski et al., 1995). As in the previous study, *C. parvum* and *I. belli* were considered to be the major aetiologic agents in the HIV patients with persistent diarrhoea (Figure).

Table II. Proven causes of persistent diarrhoea in HIV/AIDS in the United Kingdom (cf. Gazzard & Blanchard, 1993)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parvum</em></td>
<td>29 (25)</td>
</tr>
<tr>
<td>Microsporidia</td>
<td>24 (21)</td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
<td>11 (10)</td>
</tr>
<tr>
<td><em>M. avium</em> complex</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>7 (6)</td>
</tr>
<tr>
<td><em>I. belli</em></td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Others</td>
<td>18 (16)</td>
</tr>
</tbody>
</table>

Table I. Microbial aetiology of HIV-related enteropathy

<table>
<thead>
<tr>
<th>Microbe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td><em>M. avium</em> complex</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
</tr>
<tr>
<td>Microsporidia</td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
</tr>
<tr>
<td><em>I. belli</em></td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
</tr>
<tr>
<td><em>Blastocystis hominis?</em></td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
</tr>
<tr>
<td><em>C. parvum</em></td>
</tr>
<tr>
<td><em>Microsporidia</em></td>
</tr>
<tr>
<td><em>Cyclospora</em></td>
</tr>
<tr>
<td><em>I. belli</em></td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
</tr>
<tr>
<td><strong>Helminths</strong></td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
</tr>
<tr>
<td>Adenovirus (types 40 &amp; 41)</td>
</tr>
<tr>
<td>CMV</td>
</tr>
<tr>
<td>Rotavirus</td>
</tr>
<tr>
<td>HIV?</td>
</tr>
</tbody>
</table>
Microbial enteropathy and HIV infection

Figure. Microbial aetiology (■, none; □, Microsporidium; ■, Cryptosporidium; ■, Isopora; □, Strongyloides) persistent HIV-related diarrhoea in Zambia for 44 cases in 1990 (Conlon et al., 1990) and 71 cases in 1994 (Kelly et al., 1994).

Newly recognised enteropathogens

Two new protozoan pathogens, microsporidia and cyclospora have been identified during the past few years which produce clinically important diarrhoeal disease and enteropathy. These organisms are found in patients with HIV in many geographic locations and there is now effective or, at least, partially effective, therapy available.

Microsporidia

Microsporidia belong to the phylum microspora which consists of approximately 80 genera and over 700 species. The microsporidia are intracellular spore-forming protozoa which have been found in most animal groups (Bryan et al., 1991). They were first identified in 1857 and have been known to cause disease in fish, rabbits and a variety of other mammals including laboratory rodents and also primates. Their identification as a human pathogen has occurred relatively recently.

The organism. Microsporidia form spores which are the infective form of the parasite and enable the organism to survive in the environment outside its animal host. Spores vary in size from 1–20 μm in diameter although those of the species infecting mammals are generally small with diameters of 1–2 μm. Within the spore, there is a coiled polar tube which acts as the conduit by which the microsporidium is transferred into the intestinal epithelial cell of the host. Once in the host cell the organism divides and produces sporoblasts which in intestinal microsporidiosis pass into the environment via the faeces. Spores remain viable and infective in the environment for up to 4 months. Although the basic structure of the microsporidia is similar among the various genera and species there are some important differences which allow species identification. E. bieneusi and S. intestinalis can be distinguished on the appearances of the polar tube which in the former is present as a double coil but in the latter as a single coil. In addition, S. intestinalis takes up an intracellular location within a parasitophorous...
vacuole, whereas *E. bieneusi* is free within the enterocyte cytoplasm. Microsporidia are considered to be primitive eukaryotes being devoid of mitochondria and containing ribosomes which resemble those of bacteria.

**Epidemiology.** Infection is transmitted by the faecal-oral route. Although data on the world-wide prevalence of human intestinal microsporidia is far from complete, it is evident from studies in the last few years that it is becoming increasingly recognised as an important enteric infection in AIDS patients, perhaps accounting for up to 35% of cases of persistent diarrhoea (Drobniewski et al., 1995). Symptomatic infection in immunocompetent individuals would appear to be rare.

**Pathogenesis.** The mechanism by which this parasite produces partial villous atrophy is unknown. However, the intracellular location would suggest that the organism may have a direct effect on enterocyte function and thus, it seems likely that villous atrophy is due at least in part to a direct effect of the parasite by increasing enterocyte loss. Crypt hyperplasia occurs in microsporidial infection in the small intestine and is usually associated with increased chronic inflammatory cells in the lamina propria (Modigliani et al., 1985; Drobniewski et al., 1995). The main focus of infection appears to be the small intestine with the majority of organisms found in the jejunum. Organisms are either present in very low numbers or absent in stomach, colon or rectum.

**Clinical features.** Clinically, microsporidiosis in HIV patients is indistinguishable from cryptosporidiosis. The cardinal feature is persistent diarrhoea with increased faecal volumes often up to and excess of 1–2 L/24 h. In addition, there may be fever, abdominal discomfort and objective evidence of intestinal malabsorption such as increased faecal fat. When infection is prolonged, anorexia and profound weight loss become increasingly evident. There are no obvious macroscopic changes in the small or large intestine. Disseminated infection has been reported with microsporidia involving the liver (hepatitis), the peritoneum and also the cornea producing keratopathy. In some of these disseminated cases, infection has been attributed to the organism *Encephalitozoon cuniculi*, a genus closely related to enterocytozoon.

**Diagnosis.** Spores can be detected in faeces by light microscopy using a modified trichrome stain (Orenstein et al., 1990). In view of their small size, spores are difficult to detect if the observer is inexperienced. The various forms of microsporidia (sporonts, plasmodia, schizonts) can also be detected by light microscopy in paraffin-embedded sections of small intestinal biopsies stained with haematoxylin eosin. Resolution of the organisms may be improved by using semi-thin plastic sections stained with methylene blue azure II followed by basic fuchsine or toluidine blue (Bryan et al., 1991). However, identification of microsporidian species relies on resolution of their ultrastructural features by electron microscopy. *E. bieneusi* can only be reliably distinguished from *S. intestinalis* by transmission electron microscopy of small intestine (Kelly et al., 1994). Serologic diagnosis is available for *E. cuniculi* but not for other microsporidial species as yet. Although partial responses have been described with co-trimoxazole there is increasing evidence that albendazole is effective in microsporidiosis at a dose of 400–800 mg twice daily for 14–28 days (Blanshard et al., 1992). Infection with *E. bieneusi* can be suppressed but usually not eradicated whereas in infection with *S. intestinalis*, the symptoms of disease resolve rapidly and the infection is usually eradicated.
Cyclospora

In 1983, some Haitian's with AIDS and chronic diarrhoea were found to have acid-fast cryptosporidium-like organisms in faecal specimens. The organism was intermediate in size between C. parvum and I. belli but thought to be morphologically similar to cryptosporidium. This observation was closely followed by an investigation of travellers and residents in Nepal with persistent diarrhoea who were found to have cyanobacterium-like bodies (CLB) in their stools (Taylor et al., 1988; Shlim et al., 1991). In 1993, this organism was eventually shown to be an organism of the genus Cyclospora and has been tentatively named, Cyclospora cayetanensis (Ortega et al., 1993).

The organism. The genus Cyclospora was formally established in 1881 and infection has been described in a variety of animal species including reptiles, insectivores, myriapods and rodents. Human infection has only been described recently and probably involves a new species, since the human Cyclospora is morphologically distinct from those seen in animals. The infective form of the parasite is the oocyst which on light microscopy appear as non-refractile spheres 8–10 μm in diameter containing a cluster of refractile, membrane-bound globules enclosed within a membrane to produce a morula (Ortega et al., 1993). Oocysts sporulate in vitro when cultured for 5 days at 25 or 32°C. Complete sporulation with the appearance of sporozoites within sporocysts was evident by day 7–13. Potassium dichromate promotes sporulation even at 4°C. Electron microscopy of the immature oocysts reveals an outer fibrillar coat beneath which there is a cell wall. Within a sporulated oocyst, two sporocysts can be identified each of which yields two sporozoites. In the intestine, the parasite takes up an intracellular location within enterocytes and are contained within a parasitophorous vacuole. Parasites have a single nucleus with a prominent nucleoli. Intracellular parasites measure 6–8 μm long and 1–4 μm wide.

Epidemiology. Cyclospora was initially identified in humans in Haiti, Nepal and Peru but is now recognised to occur widely in Europe, Asia, North Africa and throughout the Americas (Hoge et al., 1993). Infection has been reported in immunocompetent travellers, children and in HIV-infected adults. Overall, the world prevalence is not known but transmission is assumed to occur by the faecal-oral route, mainly through water. In Nepal, there is good evidence of seasonal variation with peak incidence during the rainy season (Hoge et al., 1993). Well-documented waterborne outbreaks have been described in Nepal and Chicago. Currently, major risk factors for acquiring cyclospora infection appear to be HIV infection and travel in the developing world.

Pathogenesis. The mechanism by which Cyclospora produces diarrhoea is unknown. However, the organism takes up an intracellular location and one might assume that this results in enterocyte dysfunction and possibly enterocyte loss (Bendall et al., 1993). This may account for the partial villous atrophy and crypt hyperplasia that are features of this infection in the small intestine (Conner et al., 1993; Pape et al., 1994). In addition, there is an increase in chronic inflammatory cells in the lamina propria and also focal increases in intra-epithelial lymphocytes. Evidence to support a direct effect on the enterocyte comes from microscopic examination of the epithelial cells in which there is focal vacuolisation, disruption of the microvillus membrane and a tendency for enterocyte shape to change from columnar to cuboidal. Whether the organism secretes enterotoxins to account for the watery diarrhoea remains to be established.

Clinical features. Infection with Cyclospora cannot reliably be distinguished from cryptosporidiosis, microsporidiosis or isosporiasis on clinical grounds. The major
feature is persistent watery diarrhoea which in immunocompetent individuals lasts between 3–6 weeks but may continue for up to 15 weeks in patients infected with HIV (Shlim et al., 1991; Hoge et al., 1993). Diarrhoea may be cyclic or relapsing. Prolonged illness is associated with profound loss of weight and fatigue. In HIV-infected patients, the diarrhoea is usually persistent and unremitting and not usually self-limiting.

**Diagnosis.** Using an acid-fast stain, such as Ziehl-Neelsen, oocysts 7 μm in diameter can be detected in faecal specimens (Ortega et al., 1993). The organisms also autofluoresce blue when examined with ultraviolet epifluorescence microscopy. Inexperienced microscopists can confuse cyclospora with *C. parvum* although the latter are smaller. If there is doubt, ultrastructural examination of the parasite in small intestinal biopsies by electron microscopy can provide confirmatory evidence.

Preliminary evidence suggested that cyclospora infections were responsive to co-trimoxazole. A double-blind placebo controlled trial has recently been reported from Nepal indicating that 960 mg co-trimoxazole given twice daily for 7 days will eradicate approximately 90% of infections in immunocompetent adults (Hoge et al., 1995). If diarrhoea continues and the stools are still positive for cyclospora, then a more prolonged course of therapy may be required.

**Summary and conclusion**

Persistent diarrhoea and small bowel enteropathy are important features of HIV infection. Although HIV infection itself may in some instances produce enteropathy and diarrhoea, at least 80% of cases of persistent diarrhoea in patients with HIV can be attributed to a specific infective agent. The coccidian parasites *C. parvum*, *I. belli*, microsporidia and cyclospora are emerging as important enteropathogens which can now be reliably diagnosed on faecal examination and for some of these infections at least there is curative therapy available. The mechanisms by which they produce enteropathy and diarrhoeal disease remains unknown and largely unexplored.

**References**


