HUMAN MICROSPORIDIOSES AND AIDS: RECENT ADVANCES

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Summary:
Microsporidia, unicellular parasites frequent in animals, were rarely reported in humans before the advent of AIDS. This immunodeficiency syndrome induces the emergence or resurgence of opportunistic infections such as microsporidioses. Since 1985, five species of microsporidia have been found in HIV-infected patients. One of these species was already known in animals whereas all others are new. An increasing number of cases of microsporidioses is detected due to the improvement of methods of diagnosis. According to a study conducted in USA, intestinal microsporidia appear to be the first cause of diarrhea in patients with AIDS. These parasites are intensively investigated as shown by the increasing number of studies published since 1993. Most data concern the diagnosis, pathology, therapy and epidemiology of human microsporidioses as well as the characterization of their agents. Experimental studies aiming to define the immune context of these infections are also reported.

KEY WORDS: microsporidia, microsporidiosis, diagnosis, pathology, therapy, SIDA.

INTRODUCTION

Microsporidia are unicellular parasites which infect a wide range of invertebrates including protozoa such as ciliates (Foissner & Foissner, 1995). Also known in fish, birds and mammals (Canning & Lom, 1986), they were rarely reported in humans before the advent of AIDS. Since 1985, four new species of microsporidia have been found in AIDS patients (Table I) and these parasites are gaining increasing attention as opportunistic pathogens responsible for significant morbidity. Most recent data, concerning human microsporidioses and their agents, are herein reported.

GENERAL CHARACTERS OF MICROSPORIDIA

These protists are considered to be ancient eukaryotes. They have the nuclear organization of eukaryotes but they lack mitochondria, per-

Table I. — Microsporidian species parasite of man and their clinical manifestations according to the literature cited in text.

<table>
<thead>
<tr>
<th>Microsporidia</th>
<th>Clinical symptoms</th>
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<tbody>
<tr>
<td>Enterocytozoon bieneusi</td>
<td>Abdominal cramping, nausea, weight loss, malabsorption, zinc deficiency, chronic diarrhoea, cholecystitis, cholangitis, rhinosinusitis</td>
</tr>
<tr>
<td>Encephalitozoon (= Septata) intestinalis</td>
<td>Diarrhoea, nausea, malabsorption, weight loss, fever, cholecystitis, rhinosinusitis, bronchiolitis, nephritis</td>
</tr>
<tr>
<td>Encephalitozoon bellem</td>
<td>Keratoconjunctivitis, sinusitis, bronchiolitis, nephritis, urethritis, dysuria, prostatitis</td>
</tr>
<tr>
<td>Encephalitozoon cuniculi</td>
<td>Dysuria, nephritis, sinusitis, bronchitis, hepatitis, peritonitis Myositis</td>
</tr>
<tr>
<td>Trachipleistophora blomiatis</td>
<td>Disseminated infection</td>
</tr>
<tr>
<td>Nosema conmori</td>
<td>Corneal stroma infection</td>
</tr>
<tr>
<td>Nosema ocularum</td>
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<tr>
<td>Vittaforma corneae (= Nosema corneum)</td>
<td>Corneal stroma infection</td>
</tr>
</tbody>
</table>

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oxysomes, Golgi membranes, and their ribosomal RNA sequence is similar to that of prokaryotes (Vossbrinck et al., 1987). Their development occurs within cells of their hosts. It consists of a division phase followed by a spore producing phase. The only extracellular stages are mature spores which are released in the extracellular medium after the host cell death. Microsporidian spores possess a typical polar tube of which extrusion ensures the inoculation of the infecting sporoplasm into a host cell (Fig. 1).

Fig. 1. – Transmission electron micrograph showing three spores of Enterocytozoon bieneusi at different stages of maturation in the cytoplasm of an enterocyte. Sections of the polar tube (arrowheads) are seen in the sporoplasm. These spores measure approximately 1.5 x 0.8 μm. Scale bar = 0.5 μm.
AIDS RELATED MICROSPORIDIOSES

Clinical manifestations of microsporidioses depend on the site on the infection. Enterocytozoon bieneusi, (Desportes et al., 1985), develops in the enterocytes of the small intestine. The species causes chronic diarrhea, abdominal cramping and nausea; it is also responsible for the malabsorption and the severe weight loss reported in AIDS patients, and appears to be involved in zinc deficiency as shown by the low levels measured in patients (Asmuth et al., 1994). This microsporidian could thus contribute to the progression to AIDS since deficiency in zinc is known to be related with progression to this syndrome (Graham et al., 1990 cited in Asmuth et al., 1994). The parasite has also been found in HIV infected patients without chronic diarrhea (Rabeneck et al., 1995). Although small intestine is the primary site of infection, Ent. bieneusi can also spread to the biliary ducts and nasopharyngeal epithelium, causing thereby some cases of cholangitis and rhinosinusitis (Weber et al., 1994). Encephalitozoon (= Septata) intestinalis is the second species known to cause diarrhea in AIDS patients (Cali et al., 1993; Hartskeerl et al., 1995). Encephalitozoon bellem has been isolated from ocular biopsies of patients with keratoconjunctivitis (Didier et al., 1991). Whereas Ent. Bieneusi appears to be restricted to epithelia, all species of the genus Encephalitozoon develop in a variety of cells including macrophages thus causing disseminated infections. Encephalitozoon intestinalis, Enc. bellem and Enc. cuniculi, a species already known to infect mammals, can cause rhinosinusitis, bronchiolitis, nephritis, ureteritis, hepatitis as well as peritonitis (Cali et al., 1993; Weber et al., 1994; Gunnarsson et al., 1995; Sobottka et al., 1995; Didier et al., 1996). Three cases of myositis were imputed to Pleistophora sp. (Ledford et al., 1985; Chupp et al., 1992; Hollister et al., 1995). Peculiarities observed in the sporogony of this parasite have lead to the creation of the genus Trachipleistophora with the species T. boninis (Hollister et al., 1995). Of all these species, Ent. bieneusi is the most frequent in AIDS patients. It is reported in 14 to 40 % of HIV-infected patients with weight loss and chronic diarrhea (Asmuth et al., 1994; Weber et al., 1994). A recent evaluation of the prevalence of enteric pathogens in HIV-infected patients has shown that microsporidia are the major cause of diarrhea and other gastrointestinal manifestations (Kotler, 1995).

The interest of biologists and clinicians is mainly focused on the diagnosis and treatment of human microsporidioses. Most information concerning the characterization of these pathogens and their identification is provided by the study of in vitro models of microsporidial infections.

TAXONOMICAL DATA

The comparison of isolates of Encephalitozoon (= Septata) intestinalis obtained from cultures on RK13 cell line (Van Gool et al., 1994) with those of Encephalitozoon cuniculi and Enc. bellem was performed by SDS-PAGE, western blotting and DNA analysis (Hartskeerl et al., 1995). Due to the homology (90 %) found between gene sequences encoding for small subunits of ribosomal RNA, the species previously described under the generic name of Septata has been reclassified in the genus Encephalitozoon.

Genomic analyses have also shown that the microsporidian isolated from the urine and sputum of an AIDS patient corresponded to Encephalitozoon cuniculi, a species already known to infect carnivora, rodents or lagomorphs (De Groote et al., 1995). Similar results were obtained by Hollister. Interestingly the human isolate differed from canine and murine isolates (Hollister et al., 1995). A rabbit isolate has been also recently characterized (Didier et al., 1995).

DETECTION

The identification of intracellular stages of microsporidia requires histological studies of samples obtained by invasive procedures. Therefore, the detection of the spores released in excreta such as faeces, urine, bile, duodenal, bronchial or nasal fluids appears to be the most practical method of diagnosis. However it is hampered by the small-size of microsporidian spores. Actually, the uneasy diagnosis of microsporidia has been a cause of underestimation of the number of infected patients. Electron microscopy remains the best method for the specific identification of developmental stages and also for confirming the diagnosis performed with other techniques such as the staining by the fluorochrome Uvitex 2B (Van Gool et al., 1994) which are extensively used for the identification of microsporidia in stools, urine and any other sample (De Girolami et al., 1995). New specific diagnostic tools have been proposed using immunological characterization or the PCR amplification of genes coding for conserved ribosomal RNA subunits.

IMMUNODIAGNOSIS

The production of specific antibodies directed against microsporidian species as well as the detection of antibodies in patients' sera require large quantities of purified antigens. In vitro cultures of Encephalitozoon...
bellem and Enc. cuniculi provide millions of spores which are released in the supernatants. This material has been used for immunological identification and characterization (Aldras et al., 1994; Didier et al., 1991, 1995; Hollister et al., 1995; Schwartz et al., 1993; Visvesvara et al., 1995). Fluorescent antibody staining enabled the distinction between the two morphologically similar species Enc. bellem and Enc. cuniculi (Schwartz et al., 1995). In the absence of experimental models of Ent. bienesus, other microsporidia have been tested for their serologic cross-reactivity with this species. Both Ent. bienesus and Enc. intestinalis were thus aspecifically detected by indirect immunofluorescent antibody tests using murine polyclonal antisera raised in BALB/c mice immunized with spores of Enc. bellem or Enc. cuniculi (Aldras et al., 1994; Zierdt et al., 1993). A western blot technique using Glugea atherinae, a microsporidian parasitic in fish, has shown the cross-reactivity of sera from patients with microsporidiosis to Glugea antigens (Ombrouck et al., 1995). Since 1994, in vitro cultures of Enc. intestinalis permits the development of diagnostic procedures (Doultree et al., 1995; Hartskeerl et al., 1995). Polyclonal antibodies specifically directed against this species have been raised in rabbits (Aldras et al., 1994; Visvesvara et al., 1995) and mice (Hartskeerl et al., 1995). Ombrouck has recently proposed a method combining the non specific labeling of microsporidian spores by the fluorochrome Uvitex 2B and an indirect immunofluorescent assay using a polyclonal antibody specifically directed against Enc. intestinalis (Ombrouck et al., 1996). Additionally this method enables the distinction between spores of this species and those of Enterocytozoon bienesus which are only stained by fluorochrome.

PCR DETECTION

The genomic amplification by the polymerase chain reaction for the detection and characterization of microsporidial infections in humans has been first investigated in the Netherlands and in the USA (Shuitema et al., 1993; Weiss et al., 1993; Zhu et al., 1994). Primers are selected in the conserved DNA sequence coding for the small subunit ribosomal RNA which contains species-specific sequences and has a characteristic small size. Using these primers, (SSU)rRNA gene fragments of Ent. bienesus were detected in biopsies after amplification by the PCR method. Fedorko et al. (1995) have proposed a PCR assay for the detection of microsporidia in stool specimens. Presently, the species identification of Ent. bienesus and Enc. intestinalis in stool samples can be performed within some hours due to a recent improvement of the PCR method (Ombrouck et al., 1996).

HOST IMMUNITY AND MICROSPORIDIAL INFECTIONS

Clinical manifestations of microsporidioses as those of other opportunistic infections correlate with the intensity of infection and the decrease in the number of CD4+ cells (CD4+ cell counts < 50 per mm3) (Kotler, 1995). Animals models are helpful for understanding human infections and studying the relationships between microsporidia and the host immune system. A model of human encephalitozoonosis (Enc. cuniculi) has been obtained using SCID mice (Hermanek et al., 1993; Koudela et al., 1993). Experimental infections by Enc. cuniculi, Enc. bellem and Vittaforma corneae (= Nosema corneum) were also obtained by Didier et al. (1994) in different murine strains and monkeys. All these microsporidia cause asymptomatic and chronic infection in immunologically competent animals whereas immunocompromised ones die more or less rapidly according to the infecting species. Lethality was observed in generalized infections caused by the dissemination of the parasites by the macrophages. Experimental infections of BALB/c mice have shown that the destruction of Enc. cuniculi by macrophages occurred in the presence of factors released by T-lymphocytes (Schmidt & Shadduck, 1984 cited in Didier et al., 1994, 1995). In vitro studies have demonstrated that murine macrophages could kill Enc. cuniculi when they were treated with IFN-gamma in combination with lipopolysaccharide; a microbiostatic activity was obtained when macrophages were treated with IFN-gamma only (Didier & Shadduck, 1994). According to Didier (1995), microbicidal or microbiostatic activities of activated macrophages occur through the nitric oxyde-dependent mechanism also involved in the killing of Leishmania major and other pathogens (Vouldoukis et al., manuscript in press). These in vitro data suggest that the lack of activating factors such as IFN-gamma due to TH1-cell depletion (Del Prete & Romagni, 1994) is involved in the spreading of Encephalitozoonosis in athymic mice.

TREATMENT

Clinical assays confirmed the selective eradication of all Encephalitozoon species by albendazole. In vitro assays have shown the alteration of the development of Ent. cuniculi. The disassembly of microtubules caused by albendazole inhibits the formation of mitotic spindles and prevents the nuclear divisions thus producing enlarged and disorganised stages (Colbourn et al., 1994). The deve-
development of this species is also inhibited by fumagillin, 5-fluorouracil and sparfloxacin (Beauvais et al., 1994). Patients infected with *Ent. intestinalis* are successfully treated with albendazole as shown by the improvement in symptoms within days of start of therapy and the clearance of spores of the parasite in stools (Sobottka et al., 1995). It is noteworthy that spores of the parasite are still detected in the urine of some treated patients after the improvement of clinical symptoms. The persistence of infected sites could explain the parasitic relapses frequently observed in patients with severe immunodeficiency despite low-dose maintenance therapy with albendazole (Molina et al., 1995). Fumagillin has been used with success in the treatment of keratoconjunctivitis caused by *Ent. bellem* (Dienhouse et al., 1993). Up to this date no therapy has proven to be effective against *E. bieneusi*.

**CONCLUSION**

The increasing number of studies on human microsporidioses have contributed to the better characterization of their agents. They have also improved the diagnosis and the treatment of entero- and intestinal microsporidiosis. However specific treatments of *Ent. bieneusi* are not yet available. In addition the *in vitro* model of *Ent. bieneusi* is still lacking. No doubt that the obtention of long term culture of this species will facilitate the development of specific immunological tests and facilitate pharmacological studies. The method of isolation of microsporidian spores by flow cytometry developed by Challier et al. in 1994 can provide samples of spores of *Ent. bieneusi*. This material is presently selected in our laboratory for the production of antibodies and the infection of cell lines.

The epidemiology of human microsporidioses is under investigation. Spores of *Ent. bieneusi* have been found in stools of an immunocompetent traveller returning from tropical areas and without HIV infection (Sandfort et al., 1993). Similarly a case of infection by this microsporidia has been reported in a Turkish child living in Germany on her return from a vacation trip in Turkey (Sobottka et al., 1995). These patients presented diarrhoea which resolved without treatment. These observations suggest that *Ent. bieneusi* cause intestinal infections which are spontaneously resolutive in immunocompetent people. The presence of *Ent. bieneusi* in an HIV-negative patient who was treated with cyclosporin after transplant therapy confirms the dramatic involvement of the immunodeficiency in the development and pathogenesis of microsporidioses (Monneret et al., 1995). It must be pointed out that some cases of microsporidiosis observed in immunocompetent patients are limited to immunoprivileged sites as shown by corneal stroma infections caused by *Nosema oculatorum* (Cali et al., 1991) and *Vittaforma corneae* (Silveira & Cannings, 1995). These data confirm the opportunistic character of microsporidial infections.

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