Myxobolus sp., Another Opportunistic Parasite in Immunosuppressed Patients?

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During a study of intestinal parasitic infections in human immunodeficiency virus-positive patients, a parasite belonging to the phylum Myxozoa, recently described from human samples, was identified in one sample. When this parasite was stained by the modified Ziehl-Neelsen staining method, the features of the spores were identified: they were pyriform in shape, had thick walls, and had one suture and two polar capsules, with each one having four or five coils. The suture and two polar capsules were observed with the chromotrope-modified stain. The number of stools passed was more than 30 per day, but oocysts of Isospora belli were also found. Upon reexamination of some formalin- or merthiolate-iodine-formaldehyde-preserved samples an identical parasite was found in another sample from a patient presenting with diarrhea. Strongyloides stercoralis larvae and eggs of Hymenolepis nana and Ascaris lumbricoides were also found in this sample. Given that both patients were also infected with other pathogens that cause diarrhea, the possible pathogenic role of this parasite could not be established. The probable route of infection also could not be established.

Parasites of the phylum Myxozoa have been described in lower vertebrate hosts, mainly in teleostean fish and in some amphibians (14), in which they occasionally cause pathological alterations, such as whirling disease in salmon. Two recent reports have described the finding of parasites of the phylum Myxozoa in human fecal samples. The first report, by McClelland et al. in 1997 (7), described the presence of Henneguya salminicola in two patients presenting with diarrhea; in one of these patients the spores of the parasite were initially misidentified as spermatozoa. The second report, by Boreham et al. in 1998 (1), described the finding of spores identical to those of Myxobolus plectroplites, found in the freshwater fish Plectroplis ambiguus. However, there have been no previous reports of the finding of parasites of this genus in immunosuppressed patients. We describe here the finding of spores of Myxobolus in two patients, one of them with human immunodeficiency virus (HIV) infection.

CASE REPORT

In March 1995, a 49-year-old heterosexual, single, HIV-positive male patient consulted the Sexually Transmitted Diseases Program at the Hospital San Juan de Dios in Bogotá, Colombia. He presented with chronic, watery diarrhea, without blood or mucus, of 18 months’ duration that had become more severe in the previous 9 months, with the number of stools passed being more than 30 per day. Other symptoms included postprandial vomiting, anorexia, asthenia, paresthesias in the lower limbs, and dyspnea upon moderate exertion, which progressed to dyspnea upon mild exertion. The clinical diagnosis was malabsorption syndrome. The patient had no previous history of living with animals.

Laboratory findings included the following: white cell count; 7,000/mm3; total neutrophil count, 3,500/mm3; total lymphocyte count, 3,220/mm3; total eosinophil count, 140/mm3; total monocyte count, 140/mm3; total mononuclear cell count, 3,360/mm3; CD4+ lymphocyte count, 504/mm3; CD8+ lymphocyte count, 504/mm3; CD8+ count, 1,515/mm3 (15% CD4+ cells and 45% CD8+ cells); and CD4+/CD8+ ratio, 0.33. Mycobacterium avium-M. intracellulare-M. scrofulaceum complex organisms were isolated from blood samples of this patient.

MATERIALS AND METHODS

Microscopic examination was performed from the sediment of fecal samples subjected to the formalin-ether concentration method with centrifugation at 250 × g for 10 min (3). Pyriform spores with bilateral symmetry and two polar capsules were observed in wet mounts. Smears from the sediment were stained with modified Ziehl-Neelsen (Z-N) stain (3, 4), modified chromotrope 2R stain, and stained spores were 10.5 to 12 μm wide (average size, 11.87 by 4.83 μm). The valves of the spores were smooth and devoid of sutural ridge folds; the polar capsules, located at the apex of the spore, were pyriform. The anterior ends of the polar capsule were well separated, without an intercapsular appendix. When stained with the modified Z-N stain, the polar filament exhibited four to five coils, which are perpendicular to the main axis of the capsule (Fig. 1). An isodinophilous vacule was absent. The sporoplasm occupies one-third of the posterior end of the

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spore (Fig. 2). The parasite was classified as belonging to the genus *Myxobolus*, family *Myxozomatidae*, phylum *Myxozoa*.

Subsequently, upon reexamination of some formalin- and merthiolate-iodine-formaldehyde-preserved specimens from the same hospital, identical spores were found in a sample from another patient, together with *Strongyloides stercoralis* larvae and eggs of *Ascaris lumbricoides* and *Hymenolepis nana*. It was impossible to contact this patient in order to know more about his history.

**DISCUSSION**

We have reported that the spores that we found belong to the genus *Myxobolus* because their morphology complies with the morphologic definition of this genus: species having two polar capsules in the apex of the spore, both of which are located in the sutural plane (2). The spores do not have a posterior extension of the valve. Four hundred sixty-six nominal species of *Myxobolus* have been described, most of them from fish. There is only one previous report of spores of this genus in human fecal samples in Australia. The size of the fresh spores is similar to that of spores of *Myxobolus macrocapsularis* or *Myxobolus cultus* (16), but in order to establish the species, it is necessary to do transmission electron microscopy studies or sequencing of the 18S rRNA gene (9, 11, 13).

Sitja-Bobadilla and Alvarez-Pellitero (12) could not observe the coils of the polar filament upon light microscopy examination of *Myxozoa* spores on a wet mount or when stained with toluidine blue and periodic acid-Schiff stains. For the spores reported here, the use of the modified Z-N stain (3, 4) yielded excellent results because the coils of the polar filament could be seen clearly without the need for other techniques such as transmission electron microscopy, which is expensive and time-consuming, as mentioned by Sitja-Bobadilla and Alvarez-Pellitero (12). In addition, the stain enables the observation of other important features such as the relative position of the polar capsules in relation to the axis of the spore, the distance between the apexes, the intercapsular appendix, and the cover of the polar capsules. With the other stains which were used, such as modified chromotrope, Giemsa, and modified Shaffer-Fulton stains, the only staining observed was on the cover of the polar capsules and in some cases on the suture.

The relationship between the profuse watery diarrhea that this patient presented with and the presence of the parasite in his stool sample cannot be readily established because the life cycle of parasites belonging to the genus *Myxozoa* has two different stages, one which generally develops in an annelid of the genus *Tubifex* (8) and the other one of which develops in a vertebrate host, usually a teleostean fish (6, 15). According to Boreham et al. (1), the spores are highly resistant to environmental conditions and are not affected by gastrointestinal fluids. For these reasons it is difficult to know whether the parasite developed in the human host or if it was acquired from a contaminated environment. However, the latter hypothesis is unlikely because the patient had been in prison for 6 months. On the other hand, because infection of fish in Colombia has been described only for a species of the genus *Heneguya* (5) in a fish commonly known as "cachama blanca" (*Piaractus brachypomus*), even this hypothesis may be invalid. Besides, this patient had an infection with *I. belli*, a coccidian parasite which causes profuse watery diarrhea in immunosuppressed patients. In this case we consider that the most likely cause of the patient’s diarrhea was the infection with *I. belli*. The patient was treated for isosporiasis with trimethoprim-sulfamethoxazole, and at follow-up after 2 months the diarrhea persisted and spores of *Myxozoa* were still present in his fecal samples.
The persistence of *Myxozoa* spores suggests that this was not an incidental finding, as indicated in two previous reports (1, 7).

The possible pathogenic role of this parasite, particularly in immunosuppressed patients, must be elucidated in the future, since in the patient reported on here it was found together with *I. belli*, and in the other sample described, *S. stercoralis* and *H. nana* eggs were found.

This is the third report of spores of a parasite belonging to the phylum *Myxozoa* in human fecal samples and the first one to report this finding in an HIV-positive patient. This is also the first report of *Myxozoa* spores in Latin America, thus broadening the geographic distribution.

The importance of using modified Z-N stain as a suitable stain for the study of these parasites must be emphasized, because it allows the visualization of the convolutions of the polar filament, an important structure from the taxonomic point of view.

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