Lethal Invasive Cestodiasis in Immunosuppressed Patients

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Using both traditional methods and broad-range 18S ribosomal DNA (rDNA) polymerase chain reaction, we examined 2 cases of lethal cestodiasis, in which the disease agent had been poorly identified or misidentified. In one case, involving a patient with AIDS, we identified the human dwarf tapeworm, *Hymenolepis nana,* as a cause of aberrant metastatic larval disease. In the second case with similar pathologic abnormalities, involving a patient with Hodgkin disease, we identified a larval cestode with a previously uncharacterized 18S rDNA sequence. A prior report of this case nearly 30 years ago, based on tissue examination, had suggested that the parasite was a sparganum.

Well-known pathogens are easily overlooked or misidentified in immunocompromised patients, because the course of infection and pathological consequences may deviate considerably from the more widely appreciated features of infection in immunocompetent hosts. For example, enteric tapeworm (cestode) infections in humans generally follow a benign and recognizable course and are readily treatable. In immunosuppressed individuals, however, cestode infections can result in abnormal parasite development, disseminated disease, and death. These atypical features confound efforts to identify the disease and etiological agent. Moreover, immunosuppressed individuals are also subject to otherwise rare infections. Two such cases associated with human fatality are described here; molecular methods were critical in clarifying the identity of the etiological agents.

Case descriptions. Santamaría-Fríes et al. [1] described a patient with AIDS who died of a disseminated infection with an "unrecognized metazoan parasite" (case patient 1). This 44-year-old man presented with abdominal pain, fever, weight loss, and numerous masses involving the liver and intra-abdominal and intrathoracic lymph nodes. Death ensued within 10 weeks of presentation. Light and transmission electron microscopy and immunohistochemical studies revealed unique cystlike structures that were eventually interpreted as invasive larval parasitic forms of unknown identity. Broad-range 18S rDNA polymerase chain reaction (PCR) and phylogenetic analysis of sequence amplified directly from infected tissues confirmed the presence of a cestode, most closely related to the cyclophyllidean tapeworm of rats, *Hymenolepis diminuta.* Although the partial 18S rDNA sequence from the pathogen was sufficiently distinct from that of *H. diminuta* to suggest that the 2 were not the same organism, the experimental data and existing databases at that time did not permit a more precise identification.

In 1968, a patient with Hodgkin disease (case patient 2) died after repeated courses of cytotoxic chemotherapy and radiotherapy and was subsequently found to have disseminated parasitic cystlike structures throughout the viscera, blood vessels, lymph nodes, and subcutaneous tissues [2]. Morphological features suggested that these structures were those of a distinct and perhaps "mutated" sparganum (e.g., *Spirometra* plerocercoid larvae) but, after later review, were considered to be "similar to or identical with *H. nana* in the aberrant larval form" [3]. Despite minor differences between the saclike structures observed in this case (figure 1) and those in case 1, most histological features suggested that the associated pathogens might be identical, and thus led to more thorough morphological and molecular analyses of the tissue sections from both cases.

Materials and methods. Fresh-frozen and paraffin-embedded, formalin-fixed, grossly diseased liver tissue was available from case patient 1 for PCR studies and morphological studies, respectively. From case patient 2, only paraffin-em-

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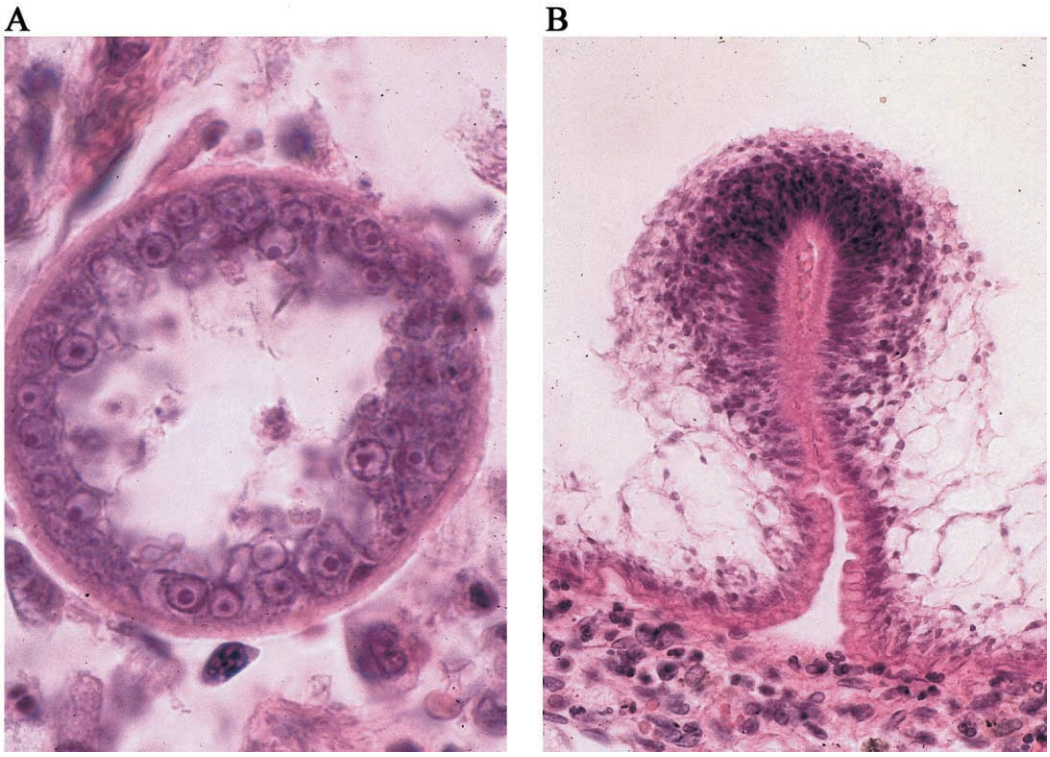

Figure 1. Cestode larval invasion of adipose tissue from case patient 2. *A,* Small spherical sac limited externally by an acidophilic wall. Ultrastructurally, the wall is a syncytium of cytoplasm lined by numerous microvilli. Cells with prominent nucleoli that occupy the periphery of the sac lumen arise from the syncytium. The many small sacs in this tissue closely resemble those of case patient 1 [1] (hematoxylin-eosin stain; original magnification, ×1000). *B,* Detail of a larger sac from case patient 2. The wall, which has the appearance of a thin cuticle, protrudes into the lumen, forming a rudimentary protoscolex. The finding of such protoscolices supports the classification of this parasite as a larval stage of a cestode (hematoxylin-eosin stain; original magnification, ×400).

bedded, formalin-fixed adipose tissue (from 1968) was available. Conventional histological methods were used in the present work; immunohistochemistry [1, 2] and electron microscopy [2] were used in earlier analyses.

Ethanol-preserved adult specimens of *H. diminuta* and *H. microstoma* and eggs of *H. nana* were obtained from laboratory cultures. In addition, ethanol-preserved adult wild-type specimens of *H. nana* were obtained from field collections of *Mus musculus*. Genomic DNA was extracted by grinding the samples in Tris-EDTA with 1% SDS, followed by proteinase K digestion. DNA was extracted from a fresh-frozen liver sample from case patient 1, and paraffin-embedded tissue sections from case patient 2, using methanol extraction and methods described elsewhere [1] and a bead beater (Fast Prep instrument; Bio101/Qbiogene).

From the adult cestode specimens, eggs, and the liver samples from case patient 1, a nearly complete 18S rRNA gene was amplified by PCR in 2 overlapping fragments. A 1100-bp fragment was generated by use of primers 18S-E and 18S-A27′, and a 1500-bp fragment was generated by use of primers 18S-8 and 18S-Cestode-6 [4–6]. PCRs were performed as follows: 3 min at 97°C; 1 min at 96°C, 1 min at 54°C, and 1 min at 72°C (36 times); and 7 min at 72°C. Products were sequenced from both strands. From the tissue from case patient 2, a partial 18S rDNA fragment (V2 region) was amplified using primers designed from conserved regions of all available cyclophyllidean sequences: Hn107F (5′-GGGAATGGGTGCACTTATTAGA-3′) and Hn312R (5′-GTTATCACCATGGTAGGCAGGT-3′). All products from this case were cloned and sequenced directly; at least 3 recombinant plasmid inserts were also sequenced.

An alignment of the nearly complete sequence from case 1 with sequences of all available species of the cyclophyllidean family Hymenolepididae was constructed manually, as was an alignment of the case 2 V2 [5] region with all available cyclophyllidean sequences [6] and 3 closely related, noncyclophyllidean outgroup taxa. The V2 alignment was analyzed by the method of maximum parsimony, and the full alignment by maximum likelihood (ML), using PAUP* software (available at http://paup.csit.fsu.edu). Nodal support was assessed by bootstrap resampling. Reported sequences were submitted to GenBank (accession nos. AY193872–AY193876).

Results. In both cases, a dense fibrous reaction replaced normal tissue architecture in many organs, the lymph nodes in particular, and was accompanied by an extensive inflam-

matory infiltrate characterized by fibrin, macrophages, lymphocytes, and some segmented neutrophils. Throughout the tissues were numerous sacs containing peculiar small cells with prominent, large nucleoli, with average diameters of 85 μm (case 1) and ~100 μm (case 2). Aside from the size difference and the rare rudimentary protoscolices seen in case 2 (figure 1), the results of histologic and parasite morphologic analysis in the 2 cases were identical. Electron microscopy indicated that the sacs were not of human origin and were probably parasitic [1]. Although morphology in both cases was unprecedented, it correctly suggested invasive cestodiasis.

The 18S rDNA sequence (2244 bp) generated from the liver specimen from case patient 1 (GenBank accession no. AY193875) was identical with that of *H. nana,* including highly variable regions where length and base composition are species specific. Similarly, sequences from laboratory isolates (GenBank accession nos. AY193872 and AY193873) and wild-type *H. nana* (GenBank accession no. AY193874) were identical, despite what is presumed to be high levels of inbreeding in laboratory-maintained cultures. Results of ML analysis (figure 2*A*) supported the findings of Okamoto et al. [7], demonstrating that *H. nana* is more closely related to *H. microstoma* than to *H. diminuta.* Our results indicate that *H. nana* was the cause of the fatal tissue-invasive larval parasitic disease process in this patient, despite the rarity of similar reports on human *H. nana*–associated disease [8].

A 183-bp 18S rDNA PCR product (GenBank accession no. AY193876), corresponding to positions ~239–436 in the *Homo sapiens* 18S rRNA gene (GenBank accession no. K03432), was reproducibly generated from the adipose tissue from case patient 2 on several occasions. Sequences from direct product and recombinant insert analyses were identical. Larger gene fragments could not be amplified, presumably because of the age and fixation history of the paraffin-embedded material. The partial sequence was unique among all sequences in the GenBank database but was most similar to that of the cyclophyllidean tapeworm *Skrjabinoporus merops* (figure 2*B*).

The available sequence data from the highly variable V2 region in the tissue from case patient 2 were sufficient to distinguish the family Hymenolepididae from other cyclophyllidean cestodes, including the parasite that infected case patient 2, and to confirm that this parasite is distinct from all others included in this analysis (figure 2*B*). In particular, it is not closely related to the pseudophyllidean genus *Spirometra,* as suggested elsewhere [2]. Of the 11 cyclophyllidean genera previously reported from humans, sequences from species of 6 genera were available for analysis and could also be ruled out, including *Echinococcus granulosus, Dipylidium caninum, Hymenolepis nana, Mesocestoides corti* (not shown in figure 2), and *Taenia solium,* although

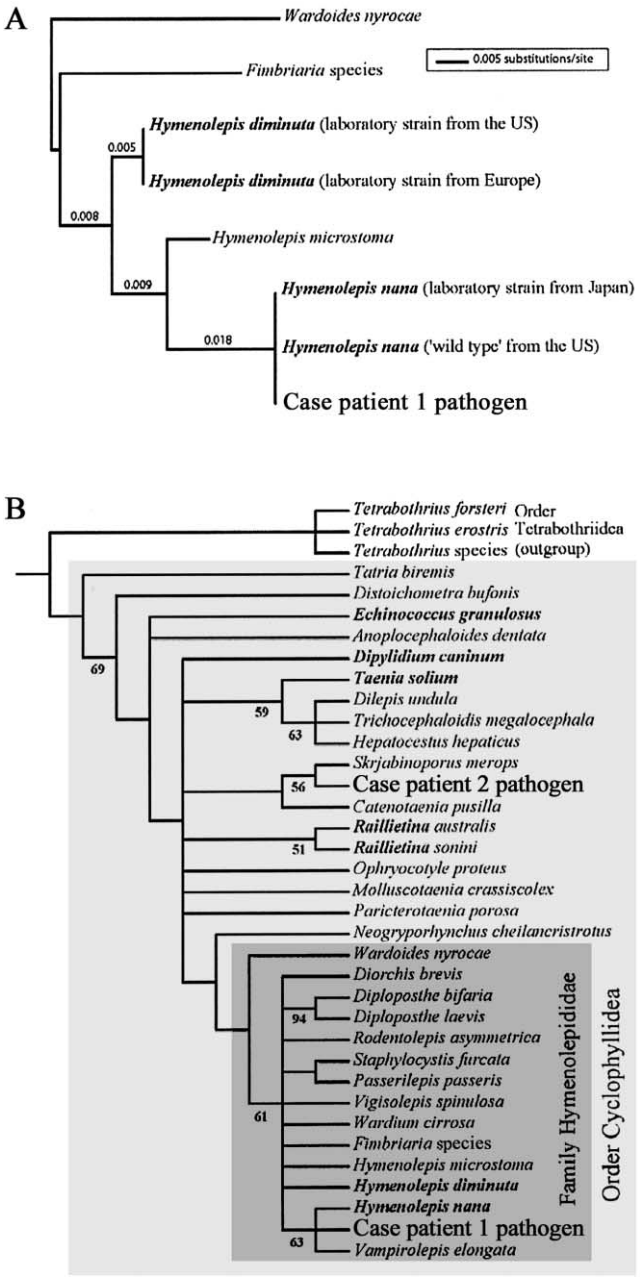

Figure 2. Phylogenetic analyses of pathogen 18S rDNA sequences from cases 1 and 2. *A,* Maximum likelihood analysis of all available complete 18S rDNA sequences of hymenolepid tapeworm species and the pathogen infecting case patient 1. Note that sequences are identical between both laboratory isolates of *Hymenolepis diminuta* and among laboratory and naturally occurring strains of *Hymenolepis nana* and the case 1 pathogen. *B,* Strict consensus of 320 equally parsimonious trees resulting from maximum parsimony analysis of partial (V2 variable region) 18S rDNA sequences of all available cyclophyllidean tapeworm species and both pathogens reported here (numbers show bootstrap support values $\geqslant$50%). Note that the case 2 pathogen is clearly a cyclophyllidean tapeworm but groups outside of the cyclophyllidean family Hymenolepididae and does not match exactly any species currently characterized for the 18S rDNA gene, including some of those commonly reported from humans (shown in bold type).

these data were insufficient to resolve most interrelationships among the cyclophyllidean taxa.

Discussion. Our findings emphasize the capability of metazoan parasites to behave aberrantly in immunocompromised humans and raise the possibility of unrecognized parasitic infections in other patients. The clinical consequences in these 2 cases were severe. These findings also illustrate the utility of molecular methods in characterizing otherwise ill-defined or confusing infectious processes.

Cestode species that infect humans include members of the order Cyclophyllidea and members from the order Pseudophyllidea of the family Diphyllobothriidae. *H. nana* is the most commonly reported cestode of humans, infecting >75 million people worldwide [9], but few cases have been described involving human immunodeficiency virus–infected patients [10]. The sylvatic cycles of both *H. nana* and *H. microstoma* involve infections in murid rodents, including some commonly kept as pets [11].

In general, complex life cycles requiring multiple host species is the rule for cestode parasites. Unique to *H. nana* and *H. microstoma* is the capability of reproducing and completing their life cycles without the need of an intermediate host. This aspect of their ontogeny must bear on the severity of the infection seen in case patient 1 by enabling large worm burdens to accumulate without requiring reinfection. Immunocompetent patients (mainly children) with enteric *H. nana* infection are generally asymptomatic, and larvae are not found in ectopic locations. Moreover, worm burdens are low, and primary infections do not persist after treatment [12]. In contrast, immunosuppression in mice causes larval metastasis and abnormal development, resulting in death of the host [12]. A similar situation appears to have been established in case patient 1, with extraintestinal larval dissemination, presumably via the lymphatic system.

In our initial investigation of case patient 1, 7 years ago, use of the universal 18S rDNA PCR primer 1520RPL [1] resulted in the amplification of an unexpectedly small (~300 bp) product, rather than the intended ~2-kb product, because of misannealing of the terminal 14 nt of this primer. This short sequence was inadequate for high resolution phylogenetic analysis, and, thus, modified sets of primers were used for the current analyses. In addition, public databases at that time contained no 18S rDNA sequence of any cestode. This problem was addressed in the current study by sequencing of 18S rDNA from hymenolepid species.

The parasitic agent in case patient 2 produced pathologic abnormalities similar to those seen in case patient 1 and, yet, is clearly distinct genetically from *H. nana, Spirometra* species, *Taenia* species, and other genetically characterized, known cestode pathogens of humans (figure 2*B*). This pathogen may be an organism familiar to zoologists, despite the absence of previously acquired molecular data that speak to its identity. The genus to which the case 2 pathogen appears most closely related, *Skrjabinoporus,* belongs to the cyclophyllidean family Metadilepididae, which primarily infect nonaquatic, tropical birds [13] and, thus, are unlikely to cause human disease. However, some members of a closely related cyclophyllidean family, Paruterinidae, use rodent intermediate hosts, in which the larval forms proliferate in the liver and body cavities [14]. Such infections result from ingestion of infective eggs dispersed in the feces of owls or other birds of prey common in North America that host the adult worms. Although there is no known report of human infection with larval paruterinid tapeworms, it is conceivable that such an accidental infection could occur via avian fecal contamination, especially in an immunocompromised host.

Despite the expansion of the 18S rDNA sequence database for the known cestode pathogens of humans during the past 7 years, the comparable database for cestode pathogens of animals remains exceedingly small, especially compared with the extraordinary diversity of the latter. Thus, one would expect that the 18S rDNA sequences of most zoonotic cestode disease agents will fail to find a close match in the public sequence databases. We favor the hypothesis that the pathogen that infected case patient 2 is a common cestode of a nonhuman (possibly avian) host and an uncommon human disease agent.

In summary, metazoan parasites may be responsible for previously unrecognized invasive or metastatic disease in humans, especially in immunocompromised hosts. Some of these parasites may be zoonotic agents with unsuspected animal reservoirs. Pathologists should have no problem recognizing invasive larval cestodiasis once they are familiar with the distinct morphologic findings presented in this and other reports [1, 2, 15]; recognition based on surgical pathology may be critical, since early institution of antihelminthic therapy might have saved these 2 lives. The nonspecific histopathological features of invasive cestodiasis emphasize the importance of molecular diagnostic approaches and the routine use of appropriate tissue fixation methods (e.g., pure ethanol). Clinicians and pathologists should maintain a critical and watchful eye on disease and pathologic abnormalities that bear feature of infection but for which an etiologic agent is not readily apparent.

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