



Lethal infection by a previously unrecognised metazoan parasite

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Summary

Background New microbial pathogens or variant clinical manifestations of known organisms may be first found in immunodeficient patients. An HIV-infected man developed a rapidly-enlarging abdominal mass, suggestive of a neoplasm, that subsequently invaded his liver and caused death. Initial studies showed unusual tissue morphology that could not be matched with any known disease process.

Methods Tissues obtained from biopsy at laparotomy and necropsy were studied by light microscopy, immunohistochemistry, electron microscopy, and broad-range ribosomal DNA-amplification and sequence analysis.

Findings Tissue lesions were characterised by peculiar cytoplasmic sacs containing minute cells with very prominent nucleoli. The pathological process was recognised as a parasitic infection, although its features were different from those of any known eukaryotic pathogen. Phylogenetic analysis of a 357 bp 18S rDNA sequence amplified directly from the involved tissue indicated that the causative agent was a previously-uncharacterised cestode.

Interpretation Fatal disease produced by this newly recognised cestode may not be limited to immunodeficient hosts. Awareness of this metazoan infection may allow early diagnosis—by morphology and DNA sequence analysis—and perhaps successful treatment of subsequent cases.

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Introduction

HIV infection and AIDS have drawn attention to a number of previously unknown opportunistic infections. Some of the infectious agents have resisted classification or propagation in the laboratory. Parasitic infections contribute to the morbidity and mortality associated with HIV infection^{1,2} and atypical parasite behaviour and clinical presentations have been associated with immunosuppression.^{3,4} We encountered a micro-organism that caused a fatal illness in a patient with AIDS. This organism resisted identification until analysis of a small subunit ribosomal DNA (ss rDNA) sequence from infected tissue, indicated that the pathogen was a previously-uncharacterised plathyhelminth, probably a cestode.

Patient and methods

Clinical history

A 44-year-old man with HIV infection for 5 years and a recent diagnosis of AIDS (CD4+ cell count: $<100 \times 10^{-4}/L$) was admitted to hospital in March, 1994, with a 2-month history of abdominal and low-back pain, weight loss, night sweats, and fever. During the previous 5 years, he had several upper-respiratory tract infections and episodes of diarrhoea but no specific opportunistic infection. Stool examination on two occasions was normal. Current medications were zidovudine, dapson, and pentamidine. He had a previous history of hepatitis B infection and treated syphilis. He had been in a monogamous homosexual relationship for 18 years. He worked as an accountant, lived in the San Francisco Bay Area, and had never travelled outside the USA. He went camping in the area in which he lived. He had two dogs with which he had close contact.

On admission he was febrile (39°C). His abdomen was soft and non-tender; a periumbilical 8×10 cm mass was palpable. An abdominal computed tomogram (CT) scan showed para-aortic, paracaval, and mesenteric nonhomogeneous lobulated masses, up to 9 cm long in their greatest dimension. A CT-directed biopsy yielded no diagnostic material. Colonoscopy and biopsies of rectum and terminal ileum were normal. The patient had an exploratory laparotomy and biopsy of the mesenteric mass 1 week after admission.

Because he was presumed to have a protozoan infection, metronidazole was started, but discontinued after 7 days due to intolerance. Severe abdominal pain persisted. He developed anaemia (Hb 9.5 g/dL) and intractable nausea and vomiting with dehydration. He progressively deteriorated, and died 9 weeks after the laparotomy.

Investigations

Morphology Light microscopy and immunohistochemistry of paraffin-embedded samples from biopsy and necropsy material were done, and a biopsy sample was examined by transmission electron microscopy.

Ribosomal DNA sequence analysis Fresh-frozen liver tissue was divided into abnormal and normal portions by gross inspection. Segments of these two types of tissue measuring 3 mm³ were

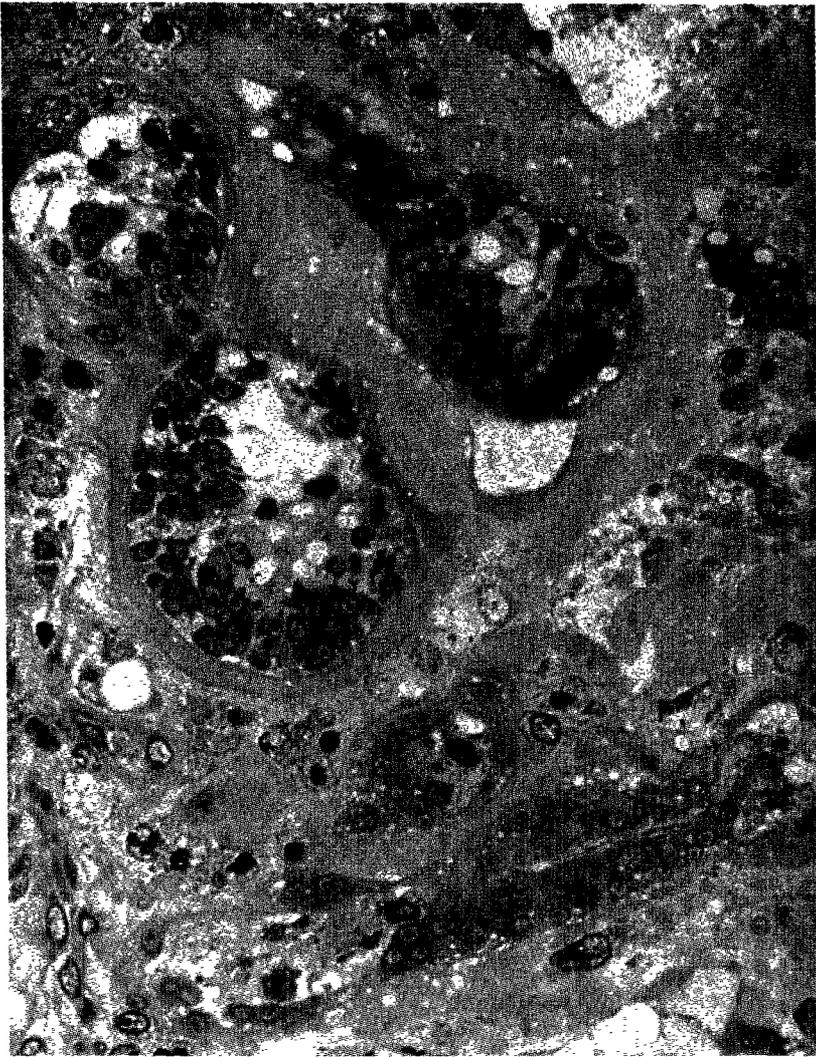


Figure 1: Paraffin section showing several parasitic sacs

Their wall (shell) stains poorly, contains few nuclei and varies considerably in thickness. The profiles of the sacs are oval, flask-shaped, or elongated. All sacs contain numerous cells which do not completely fill the lumina. In this field there are few inflammatory cells. Hematoxylin and eosin. $\times 320$.



Figure 2: Portion of a parasitic sac

The gray-blue shell is acellular in the upper and right fields and contains some nuclei in the lower right. The peculiar cells with round, prominent nucleoli are concentrated on the inner aspect of the shell. Notice marked variation in cytoplasm density. Epon section stained with toluidine blue. $\times 800$

digested and processed in parallel for PCR analysis.⁵ Broad-range eukaryotic ss rDNA amplification reactions included 20 pmol of primers 1 FPL (Eukarya-specific, 5'GCGGATCCGCGGCCGC-TGGTTGATCCTGCCAGT 3') and 1520RPL (universal, 5'GCGGATCCGCGGCCGCYGCAGGTTACCTAC 3'),⁶ 200 nmol MgCl₂, 2.5 U *Taq* polymerase, and 1–10 μ L (1–10%) of the tissue digests in a total volume of 100 μ L. With the hot-start technique, 40 PCR cycles were done with an annealing temperature of 60°C. PCR products were purified and sequenced directly with the Dye Deoxy Terminator Cycle Sequencing Kit (Perkin-Elmer, Foster, City, CA, USA). The ss rDNA sequence obtained in this study was aligned against a database of 80 eukaryotic ss rDNA sequences according to conserved primary and secondary structures. Phylogenetic analyses were based upon comparisons of 303 sites that were judged by these criteria to be in alignment. Phylogenetic trees were constructed with two algorithms: maximum parsimony using the software package PAUP3.1.1;⁷ and maximum-likelihood.⁸ Bootstrap values for the dendrogram generated by PAUP were obtained from 100 resamplings. Distance-matrix analysis followed the least squares method.⁹

Results

Morphology

The open-biopsy material of the mesenteric mass consisted of dense fibroadipose tissue within a rim of lymphatic tissue with an extensive inflammatory reaction characterised by fibrinous exudate, macrophages, and scattered groups of neutrophils forming microabscesses. There were no granulomas or eosinophils.

Randomly dispersed in this stroma were numerous

nests, or sacs, of the microorganism (figures 1 and 2). The sacs measured 85 μ m in average diameter, were oval or flask-shaped, and composed of an outer amphophilic shell (wall) that stained weakly with hematoxylin and eosin, and a central cavity containing characteristic cells. These cells had a mean diameter of 6.7 μ m; their cytoplasm was dense and their most prominent feature was a small spherical nucleus (2 to 4 μ m; clearly smaller than most human nuclei) with a very large round nucleolus. Some parasitic cells were multinucleated. A few sacs had ruptured and their cells were loose in the surrounding stroma. Although invasion of the sacs by histiocytes or neutrophils was seen, phagocytosis was not.

The possible human origin of these cells was explored with immunohistochemical reagents for normal or neoplastic human antigens, including cytokeratins, human chorionic gonadotropin, alpha fetoprotein, epithelial membrane antigen, S-100 protein, vimentin, desmin, and multiple lymphoid markers. All of these markers were negative. The cells did not react with the usual stains for bacteria (such as Gram) or fungi (silver methenamine, Gridley etc). They were acid-fast negative (Ziehl-Neelsen) and did not contain acid mucopolysaccharides (with Alcian blue at pH 1.5). Although the cells were periodic acid-Schiff (PAS)-negative there was abundant PAS-positive and diastase-sensitive amorphous material, presumably containing glycogen, among the cells.

Electron microscopy of the biopsy sample showed that the wall of the sacs was composed of cytoplasmic material containing some glycogen and a few nuclei (figure 3). The

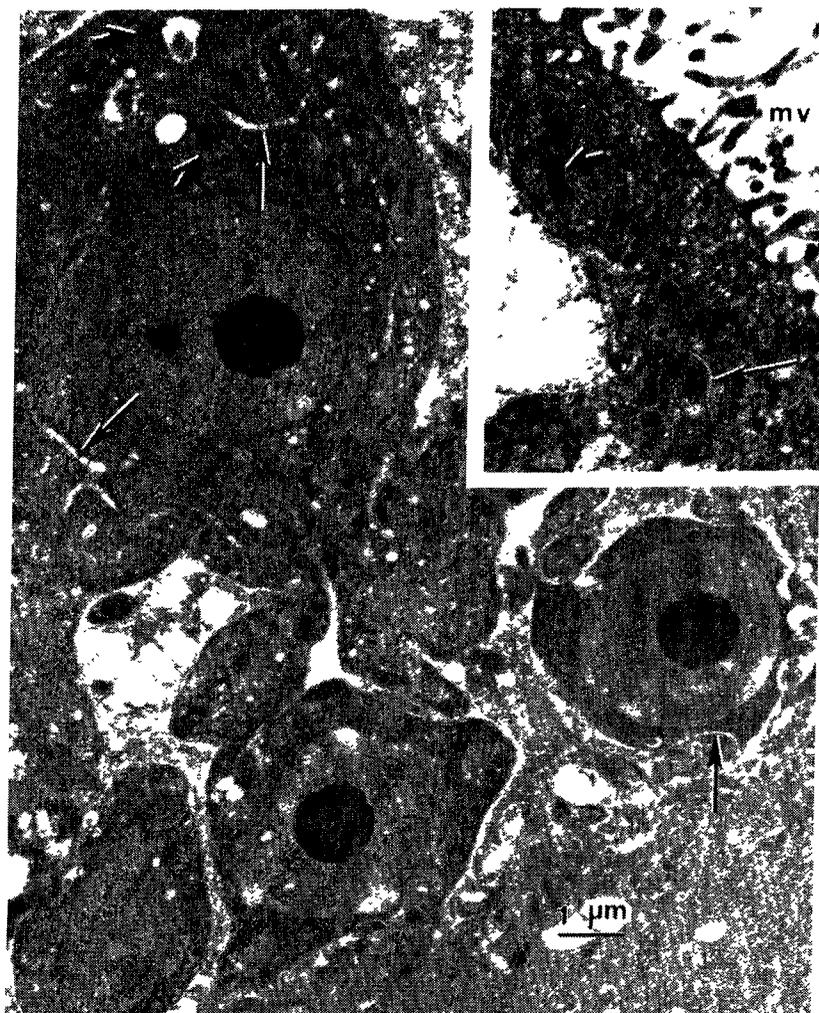


Figure 3: **Ultrastructure of the large (upper left) and small (lower field) parasitic cells described in figs 1 and 2**

The large—presumably immature—cell has small, inconspicuous mitochondria (wide arrows) and multiple flat saccular structures with septations (long arrows). In the smaller cells—presumably older—the saccular structures have become thin channels and the cytoplasm is condensed. Uranyl acetate and lead citrate, $\times 6250$. The inset shows a section of the shell, made of cytoplasm containing a few mitochondria and a septated flat saccular structure. The elongated microvilli (mv) are seen on the outer, convex, aspect. Uranyl acetate and lead citrate. $\times 15\ 000$.

outer surface of the wall was studded with a continuous layer of microvilli, while the inner surface projected lamellipodia toward the lumen. Inside the sacs were the minute cells seen by light microscopy. Those cells in the periphery, between lamellipodia, seemed to originate from

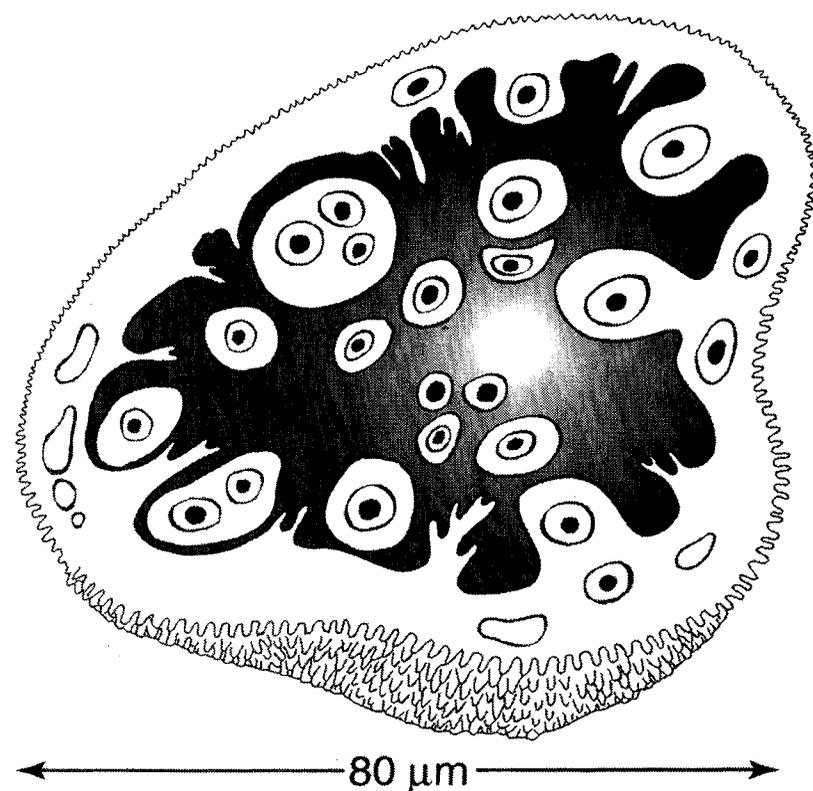


Figure 4: **Three-dimensional reconstruction of a parasitic sac—sectioned in the middle—based on light and electron microscopy**

The cut surface is facing up, while the external surface, studded with microvilli, is partially seen at the bottom. The size of the microvilli has been exaggerated to make them visible at this magnification. The (cytoplasmic) wall contains some nuclei (right and top) and a few vacuoles (left). Within the sac are the characteristic cells, which appear to originate in the wall, detach from it and migrate toward the lumen, becoming smaller and denser in the process. The irregular projections of the wall's cytoplasm toward the lumen (lamellipodia) may serve to support the cells, or may represent the sites of separation of the cells from the common cytoplasm.

the wall and often were continuous with it. They had loosely distributed, small mitochondria and were larger than the cells closer to the centre of the sacs. Those cells in the center had denser cytoplasm, suggesting a process of maturation and/or senescence from the periphery to the centre of the sacs. All cells lacked rough endoplasmic reticulum, phagolysosomes, and junctional complexes (figure 4). Neither cell wall nor chloroplast were seen. Although these features suggested a metazoan parasite, there were no structures such as eggs, tegument, scoleces,

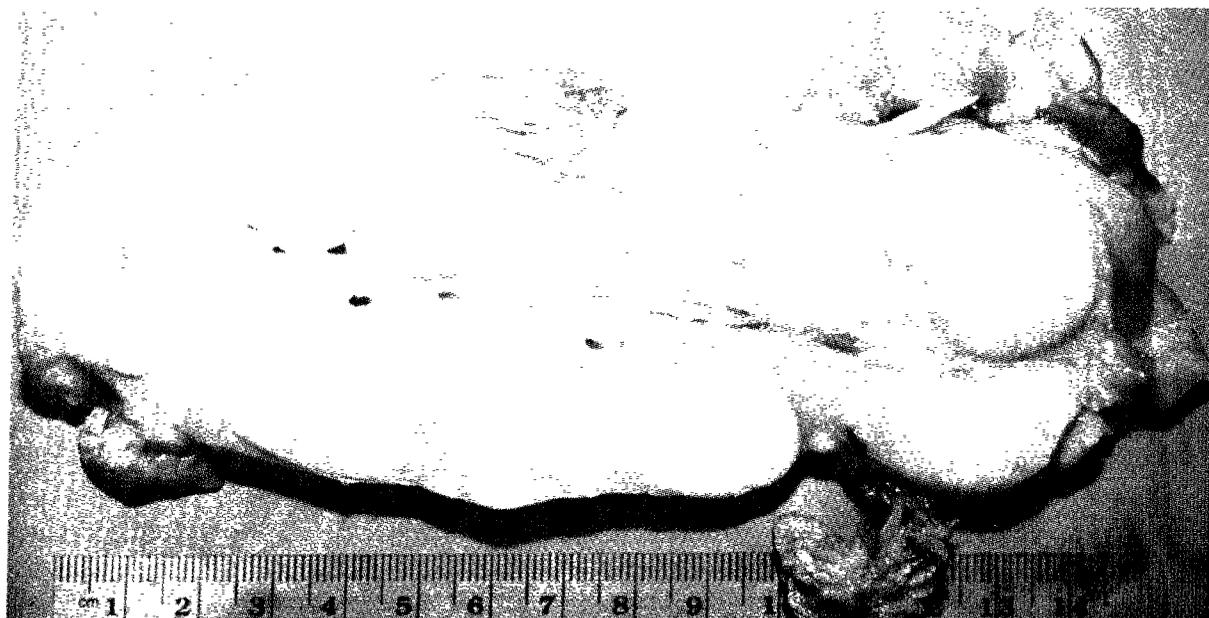


Figure 5: **Section of the retroperitoneal mass**

This large tan-white lobulated tissue is composed of matted lymph nodes. The gross appeared closely resembles that of a neoplastic process such as a lymphoma.

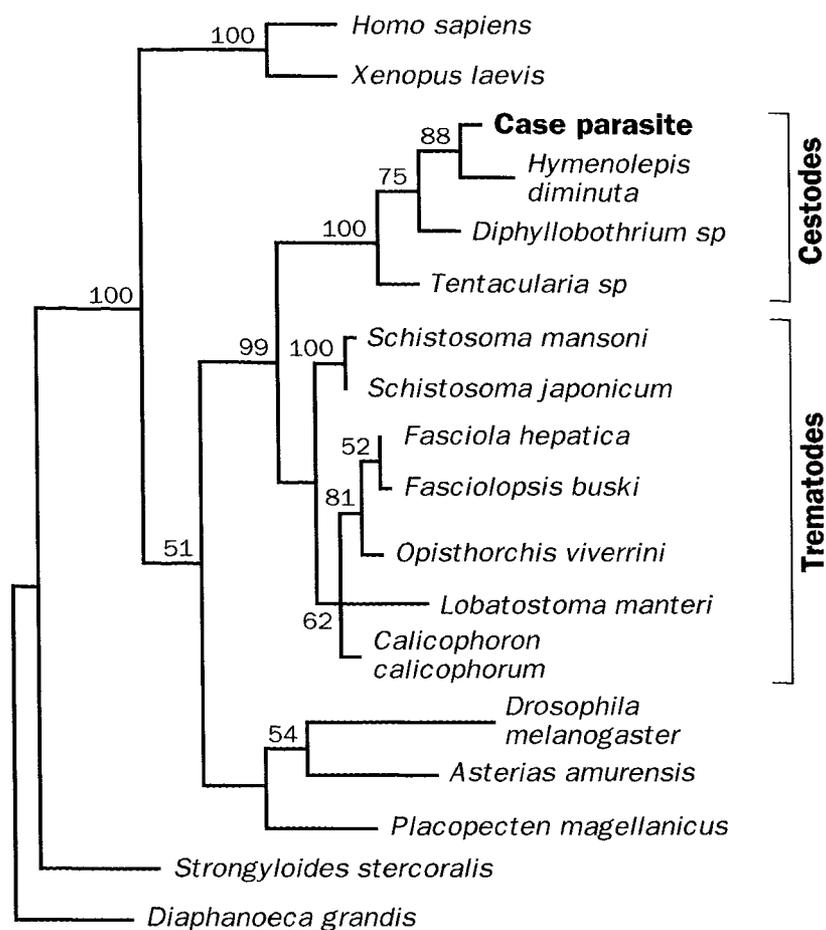


Figure 6: Phylogenetic tree based upon parsimony analysis of partial small subunit rDNA sequences

The analysis suggests that the case parasite is a cestode. This dendrogram represents the best tree among 100 bootstrap replicates. The percentage of the 100 resamplings that corroborate the topology is given at branch nodes (only values greater than 50% are displayed).

or cilia.

Necropsy less than 6 h after death showed large mesenteric and retroperitoneal, lobulated tan masses, up to 20 cm in greatest dimension (figure 5). Smaller nodules were present in the diaphragm and lower retrosternal soft tissue. The liver, which was markedly enlarged, had bulky, irregular tan lesions that involved approximately 75% of the parenchyma. The abdominal masses and the liver had extensive necrosis and contained numerous plastic sacs, most of which were ruptured. Rare cytomegalovirus inclusions were observed in human cells within the mesenteric mass. There was severe bronchopneumonia. No evidence of cancer was found. Cultures from blood, liver, and mesenteric masses were negative for bacteria and fungi, except for light growth of *Candida glabrata* from one of the mesenteric samples.

Small subunit rDNA analysis

A PCR product of approximately 400 bp was consistently detected in digests of diseased liver tissue with reactions using broad range Eukarya ss rDNA primers. No other PCR products were detected. On the other hand, digests of normal portions of liver from the same patient only yielded an approximately 1.8 kb PCR product (data not shown). A 1.8 kb DNA band would have been expected from amplification of human 18S rDNA with these primers. The disease-associated PCR product contained 357 bp, excluding the two PCR primers. This 357 bp sequence corresponded to the ss rDNA of a previously-uncharacterised metazoan (GenBank accession number U 58674). At approximate position 360 within the ss rDNA of the most closely-related organisms, there is a region in which 14 out of 15 nucleotides in a reverse

complementary orientation match the 3' end of primer 1520 RPL, suggesting that 1520 RPL mispriming led to the amplification of this unexpectedly small DNA fragment. Parsimony, distance, and neighbour-joining phylogenetic analyses indicated that the case parasite is most closely-related to the cestodes (figure 6); bootstrap analysis strongly supported this association.

These results and the morphological data, suggest that the nests of cells represent a tissue-invasive larval stage of a cestode-like organism. Given the relatively few cestode ss rDNA sequences that were available for our analysis and the paucity of sequence data from the case parasite, we could not determine precise evolutionary relationships between this parasite and other cestodes.

Discussion

This patient had a progressive, tissue-destructive parasitic infection. Despite rigorous histological examination, the nature of the pathogen could not initially be identified. Efforts to detect signature elements and compounds in the diseased tissues by energy-dispersive and lipid-profile analysis (data not presented) failed to provide specific clues. Small subunit rDNA sequences allow the inference of phylogenetic relationships without reliance on cultivation or growth-based phenotypes. For this reason we amplified these sequences directly from infected tissue. Although there are very few cestode ss rDNA sequences available, our analysis suggests that this parasite is a previously-unknown cestode.

The lack of sequence similarity of this parasite with previously-characterised organisms is consistent with its unique histologic features. We have found no reports of a human-associated parasite morphology or clinical course that resemble those described in this case. In fact neither we, nor any of the experts on human and veterinary parasitic diseases we consulted, have seen this micro-organism before. There is a vague similarity between this case and one reported by Connor et al in 1976 and interpreted as a possible embryonal stage of aberrant spirometra (sparganum).³ In one of their micrographs the minute cells and the enveloping sac slightly resemble our parasitic nests; other aspects, including the ultrastructure, are rather different. The morphology of this parasite suggests that it was in the larval stage.^{3,11} No evidence of maturation (no change in morphology) occurred in the 3-months between biopsy and death.

Assuming that this is a newly recognised human parasite infection, we could only speculate about the possible route of entry and its development within the host. Since the initially detected lesion was in the mesentery, infection through the mouth by eggs or larvae may have occurred, with subsequent penetration through the intestinal wall and dissemination into lymph nodes. The infection may have occurred either from ingestion of contaminated meats, fish, crustaceans, etc, containing proceroid larvae (as in the case of sparganosis) or ingestion of eggs or proglottids as in the case of taeniasis.¹²

As for the source, we have considered the possibility that the dogs with which the patient was particularly affectionate (to the point of biting their paws and noses) may have harboured the organism. Although no parasites were found in the dogs faeces, this source cannot be ruled out. The sexual partner of this patient is apparently healthy.

From studies in mice it appears that helper T

lymphocytes (especially the Th1 subtype) are important in the protective immunity against the cestode *Hymenolepis nana*.¹⁰ Thus, by analogy, this immunodeficient patient might have lacked selective protection against cestode infections.

Awareness of these unusual clinical and pathological features may allow early diagnosis and treatment in subsequent cases. Additional sequence-based studies may be necessary for the complete characterisation of this metazoan.

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Current results of intestinal transplantation

David Grant on behalf of the International Intestinal Transplant Registry*

Summary

Background Intestinal transplantation is an alternative to total parenteral nutrition (TPN) for the treatment of chronic intestinal failure. To determine the current status of small-bowel transplantation, we have reviewed the world experience since 1985.

Methods We built up an international registry by asking twenty-five intestinal transplantation programmes to submit standard data on their cases operated on between 1985 and June, 1995.

Findings One centre (two transplantations) did not use our report form, and these cases were excluded. The remaining twenty-four programmes did 180 transplantations in 170 patients. Two-thirds of the recipients were children. The main indication (64%) was short-gut syndrome; another 13% had a tumour. Of the grafts, 38% were small-bowel with or without colon, 46% were intestine plus liver, and 16% were multivisceral. Graft/patients' survival (%) at 1 and 3 years under cyclosporin immunosuppression was, respectively: 17/57 and 11/50 for small bowel only; 44/44 and 28/28 for intestine plus liver; and 41/41 and

41/41 for multiviscera. The corresponding figures under tacrolimus were: 65/83 and 29/47; 64/66 and 38/40; and 51/59 and 37/43. 78% of the 86 survivors had stopped TPN and resumed oral nutrition.

Interpretation Our approach cannot give data on long-term outcome. The short-term results of intestinal transplantation are similar to those of lung grafting. We conclude that small-bowel transplantation has become a life-saving option for patients who cannot be maintained on TPN and for those who require massive abdominal evisceration for locally aggressive tumours.

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Introduction

Intestinal transplantation is a potential alternative to total parenteral nutrition (TPN) for the treatment of chronic intestinal failure. However, small-bowel transplantation under cyclosporin-based immunosuppression has a high failure rate because of refractory graft-rejection and sepsis.¹ Several centres have reported improved outcomes with tacrolimus.^{2,3} To determine the current status of small-bowel transplantation, we reviewed the world experience since 1985 by using registry data.

Methods

The clinical programmes that had participated in the 3rd International Symposium on Small Bowel Transplantation in Paris, 1993, were contacted by mail and invited to contribute to

*Participants listed at end of report