The classical ultrastructural features of Gaucher disease include large numbers of intracytoplasmic, membrane-bound lysosomal inclusions containing characteristic tubular structures on an electron-lucent background, representing the periodic acid schiff (PAS)–positive Gaucher cells identifiable on light microscopy. Following enzyme replacement therapy (ERT), many of the manifestations of the condition are ameliorated, but persistent mesenteric lymphadenopathy has been reported, the ultrastructural features of which previously have not been described. Two children, aged 4 and 8 years old, respectively, both presented with persistent abdominal lymphadenopathy whilst receiving ERT for Gaucher disease. Needle core biopsies were carried out, that demonstrated collections of macrophages and only scattered storage-type cells on light microscopy. PAS staining was negative in one case and only focally positive in the other. Electron microscopic examination, however, confirmed the cells represented macrophages, the cytoplasm of which contained scattered abnormal inclusions containing occasional twisted tubular structures of the type reported in classic Gaucher disease. ERT in Gaucher disease appears to reduce accumulation of the metabolic products at many sites. But for uncertain reasons, abdominal lymphadenopathy may occur containing macrophages that do not form granulomas or classic Gaucher cells on light microscopy. These probably represent incomplete clearance, incomplete/partial enzyme replacement, or possibly an unusual response to a relatively small amount of storage material.

Keywords electron microscopy, enzyme replacement therapy, Gaucher disease, glucocerebrosidase deficiency, glucosylceramidase deficiency, ultrastructure
INTRODUCTION

Gaucher disease (GD; glucocerebrosidase EC3.2.1.45/glucosylceramidase EC3.2.1.62 deficiency) may manifest with a broad range of clinical features ranging from perinatal lethal presentation to asymptomatic osteopenia. Previously, the classification was wholly based on the clinical phenotype until a wide spectrum of clinical findings were recognized with broad variability in presentation. Currently, clinical subtypes are still useful in determining prognosis and management, such as the absence or presence of primary central nervous system disease: nonneurological visceral disease (type 1, GD1 (OMIM#230800) or neurological phenotypes (types 2 & 3, GD2 (OMIM#230900) & GD3 (OMIM#231000) [1]. Enzyme replacement therapy (ERT) in type 1 (GD1) has efficacy but ERT is unlikely to be useful in type 2 (GD2) because the pathogenesis represents cell death/apoptosis rather than direct effects of storage product accumulation. The perinatal lethal subtype (OMIM#608013), presenting as nonimmune hydrops inutero, and cardiovascular subtypes also are recognized, although cardiopulmonary complications are seen in all types.

Currently, ERT is primarily used to treat type 1 Gaucher disease (GD1) to overcome the block in the catabolic pathway and allow clearance of accumulating storage product [2]. The native enzyme (glucocerebrosidase/glucosylceramidase) is widely distributed in a variety of mammalian tissues, including spleen and liver, and involved in the breakdown of complex glycosphingolipids (e.g., gangliosides and globoside). ERT provides exogenous recombinant enzyme preparation based on the human gene sequence but is modified to expose the carbohydrate residues for enhanced uptake by macrophages.

Diagnosis of Gaucher disease is made on clinical grounds, enzyme (acid beta glucosylceramidase) activity assay (using leukocytes/fibroblasts or other nucleated cells), and morphology of characteristic storage “Gaucher” cells (macrophages with fibrillary cytoplasm and an eccentrically placed nucleus) in bone marrow and solid organs [3]. Although the molecular pathology of GD has been thoroughly investigated, genotype-phenotype correlations are imperfect, leading to a suspicion that epigenetic and modifying phenomena are involved in the pathogenesis. Molecular mutation analysis of GBA (glucosidase, beta acid, OMIM#606463, locus 1q21) has been used in at-risk families where the specific mutation is known and in high-risk populations where the carrier frequency of disease-causing alleles is significant. Where the mutation is known, molecular testing also can be used to accurately identify carriers. The four most common GBA mutations (N307S, L444P, 84GG, IVS2 + 1) account for 90% of disease-causing alleles in the Ashkenazi Jewish population and 50–60% in non-Jewish populations [4].
The typical ultrastructural features of GD include large numbers of intracytoplasmic, membrane bound lysosomal inclusions containing characteristic packed tubular structures within an electron-lucent background, representing the periodic acid schiff (PAS) positively stained Gaucher cells seen on light microscopy (Figure 1). Following ERT with imiglucerase, many of the manifestations of the condition are ameliorated, but persistent mesenteric lymphadenopathy may occur [5], the ultrastructural features of which have not been described previously.

**CLINICAL CASES**

Two children, aged 4 years (patient A) and 8 years (patient B) old, respectively, both presented with persistent significant abdominal lymphadenopathy while receiving ERT for GD. Needle core biopsies were carried out to investigate the cause of the persistent adenopathy, which in both cases demonstrated similar features. Collections of macrophages showed pale-eosinophilic cytoplasm but only scattered apparent “storage-type” cells on light microscopy. PAS staining was negative in one case and only focally positive in the other, and no classic Gaucher cells were identified on light microscopy (Figure 2). However, electron microscopic examination confirmed the cells to represent macrophages, the cytoplasm of which...
contained scattered abnormal membrane-bound inclusions containing scanty twisted tubular structures of the type reported in classic GD (Figure 3).

Patient A originally presented aged 2 years with hepatosplenomegaly and had splenic and liver biopsies that were contributory to the diagnosis. The spleen showed infiltration of the red pulp by macrophages of classic Gaucher type and the liver showed patchy moderate fibrosis. She had a partial splenectomy/debulking surgical procedure because of the gross distention. At this time, she commenced enzyme replacement therapy. At the age
of 4 she presented with mesenteric lymphadenopathy and the clinical issue was whether this was related to GD or an independent process such as lymphoma. However, as described above, the abdominal lymph node sampled demonstrated collections of histiocytes with foamy cytoplasm, and there was no recognizable evidence of leukemia or lymphoma.

Subsequently, at age 9 the patient had persistent hepatomegaly but the spleen and kidneys had normal dimensions on imaging. She developed further abdominal and nasopharyngeal lymphadenopathy in addition to severe bone disease despite continued ERT. The total duration of ERT from start of treatment to the time of abdominal lymph node biopsy was 21 months.

Patient B initially presented at the age of 2 years with hepatosplenomegaly and thrombocytopenia and bone marrow examination demonstrated numerous histiocytes with the characteristic morphological appearance of GD. Subsequently she was found to be homozygous for L444P mutation. She began ERT as part of a clinical trial, but at age 8, a routine surveillance ultrasound examination revealed enlarged abdominal lymph nodes. A biopsy was performed, that demonstrated the features described above. There was no evidence of malignancy. She has persistent abdominal

**FIGURE 3** Electron micrographs of storage cells from case 1 (top) and case 2 (bottom) from abdominal lymph nodes demonstrate scattered intracytoplasmic inclusions with a similar appearance to those seen in classic untreated Gaucher disease but much less abundant in number and without a clearly defined lysosomal membrane.
lymphadenopathy to date but no other significant manifestations of tissue infiltration. The total duration of ERT from start of treatment to the time of abdominal lymph node biopsy was 7 years 1 month.

**DISCUSSION**

We report two patients developing marked mesenteric lymphadenopathy while receiving ERT for GD and describe the ultrastructural features responsible for this presentation. Although there are consensus recommendations for ERT and monitoring of children with GD, the optimal dose and frequency remains to be fully determined. The enzyme dose may be increased or decreased after initiation of treatment and during the maintenance phase based on clinical response [6, 7]. One of the current problems in ERT monitoring is the limited information regarding tissue half-life and distribution following intravenous infusion and determining whether adequate concentration of exogenous enzyme are reached in the target organs, such as bone, lung, and brain. There are limitations in assessing disease severity/improvement and rate of disease progression. Currently, the clinical course is measured by parameters such as haemopoietic reconstitution, reduction of liver/spleen volume, and improvement in skeletal findings [8], as well as biochemical measures such as plasma tartrate resistant acid phosphatase (TRAP), liver enzymes (aspartate aminotransaminase or alanine aminotransferase), and plasma chitotriosidase.

There is an enzyme dose-dependent decrease in plasma chitotriosidase activity in patients on ERT. Chitotriosidase is a macrophage-derived chitin-fragmenting hydrolase that is elevated in untreated GD (up to two orders of magnitude). Several plasma markers (D-dimer, CCL18 (chemokine, CC motif ligand 18)/PARC (pulmonary and activation-regulated chemokine OMIM*603757), and CD163 (OMIM*605545)) have been used in Gaucher patients as indicators of disease activity/burden that might be used in long-term monitoring and in treatment response to ERT [9–11]. However the prognostic role of these markers is still unclear. For GD1, regular intravenous infusion of imiglucerase has been shown to improve health-related quality of life, and is safe and effective in primary prevention of manifestations and reversal of hematologic and liver/spleen involvement [12, 13]. It is likely that the presence of end-stage histological changes, such as fibrosis and infarction, influence the response to ERT [4]. There may be some benefit of ERT in type 3 patients, but bone marrow transplant (BMT) or combined BMT/ERT may be of more benefit in the chronic neurological types of GD and long-term outcomes remain to be determined. In terms of gene-based therapy, the GBA gene has been introduced into hemopoietic stem cells but with only small, unsustained amounts of enzyme produced [2, 14].
ERT reduces accumulation of the metabolic products at many sites, as demonstrated histologically in previous reports in which macrophages with cytoplasmic inclusions are reduced in the liver and bone marrow [15, 16]. However, for reasons that remain uncertain, abdominal lymphadenopathy due to abnormal collections of macrophages may occur, as demonstrated by the current cases. The collections of macrophages do not form histologic granulomas or collections of classic Gaucher cells on light microscopy. However, ultrastructural examination demonstrates that the cells are macrophages containing abnormal inclusions with twisted tubular structures of the type typically seen in GD. These findings presumably represent incomplete clearance, incomplete/partial enzyme replacement at the cellular level, or possibly an unusual response to a relatively small amount of residual storage material.

CONCLUSION

We present two cases of pediatric Gaucher disease, both treated with ERT, who presented with persistent abdominal lymphadenopathy of unknown etiology. Although ERT prevents or reverses many of the systemic manifestations, we have demonstrated that persistent mesenteric/abdominal lymphadenopathy, due to localized collections of macrophages, with ultrastructural features of atypical Gaucher cells may occur. The reasons for the presence of these storage cells in mesenteric tissue are unclear but understanding this phenomenon may provide further insight into the efficacy of ERT at the cellular level in storage disorders. Light and electron microscopy remain important methods of assessing response to therapy in GD, which could be used in conjunction with other biological markers or as an independent morphological method for therapeutic investigation.

REFERENCES


Ultrastructural Features of Gaucher Disease

247


**Electronic Databases**


Enzyme Nomenclature: http://www.chem.qmul.ac.uk/iubmb/enzyme