

Peripheral blood lymphocyte appearance in a case of I cell disease

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Abstract

In general, peripheral blood smears are performed to obtain information with regard to various morphological features as an aid in the diagnosis of infection or malignancy. This report presents a patient with I cell disease (inclusion cell disease), a fatal lysosomal storage disorder caused by a defect in an enzyme responsible for the transfer of mannose-6-phosphate ligands to precursor lysosomal enzymes. As a consequence, most lysosomal enzymes are transported outside the cell instead of being correctly targeted into the lysosomes, resulting in the storage of macromolecules in lysosomes. I cell disease, with its heterogeneous clinical presentation, can be diagnosed by the presence of intracellular vacuole-like inclusions in lymphocytes and fibroblasts, high serum lysosomal enzyme activities, and a defect of *N*-acetylglucosamine-1-phosphotransferase. This report describes the morphological aspects of peripheral lymphocytes in a blood smear of a patient, the first clue to the final diagnosis of I cell disease. The observed vacuole-like inclusions in lymphocytes of this patient were negative for periodic acid Schiff (PAS) and Sudan black B staining, in contrast to earlier reports.

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Keywords: I cell disease; inclusion bodies; lymphocytes

I cell disease is a rare autosomal recessive lysosomal storage disorder, first described in 1967,¹ which manifests at birth and slowly progresses to a fatal outcome at approximately 4 years of age.^{1–3} The disease, although heterogeneous in its clinical presentation, has some characteristic features, namely: psychomotor retardation, skeletal abnormalities, shortness of stature, and a Hurler-like appearance. Intracellularly, a generalised storage of macromolecules occurs in lysosomes as a result of multiple lysosomal enzyme deficiencies. Because lysosomes are the cellular organelles in which macromolecules are degraded, these macromolecules accumulate, leading to abnormal inclusions in cells such as lymphocytes. Therefore, the diagnosis of I cell disease can be accomplished by the detection of cytoplasmic inclusion bodies in lymphocytes or cultured skin fibroblasts, together with multiple lysosomal enzyme activity abnormalities. Several

groups^{1 2 4 5} have investigated the ultrastructural features of I cell disease. A common finding in these studies was the various cytoplasmic inclusion bodies found in several types of cells. Furthermore, these inclusion bodies contained macromolecules that could be stained with Sudan black B (lipids) and periodic acid Schiff (PAS; glycogen).⁴

Here, we describe a peripheral blood smear from a patient with vacuole-like inclusions in the cytoplasm of lymphocytes, which led to an early diagnosis of I cell disease. The diagnosis of I cell disease was finally confirmed by lysosomal enzyme activity measurements in plasma.

Case report

This girl was born after an uneventful pregnancy at 38 weeks gestation as the first child of non-consanguineous Turkish parents. The mother had previously had three miscarriages at 3 months of gestational age. The birth weight of the child was 2330 g. She was dysmorphic at birth, but on request of both parents no further investigations were performed. At the age of 56 days she was admitted to our hospital with severe respiratory insufficiency, caused by bronchopneumonia. Artificial ventilation was needed for four days. Physical examination revealed the following abnormalities: coarse facies, thick eyelids, hypertelorism, low implanted ears, narrow forehead, micrognathia and retrognathia, laterally bowed legs, and rocker bottom feet. The liver was 3 cm and the spleen was 1 cm palpable. The activity of alkaline phosphatase in blood was increased to as much as 3240 U/litre (reference value, up to 350 U/litre, age and sex related). Most of the alkaline phosphatase originated from the bones. Serum phosphate, serum calcium, and total protein were decreased (0.78 mmol/litre, 1.10 mmol/litre, and 47 g/litre, respectively).

Full blood count showed: haemoglobin, 4.4 mmol/litre; leucocytes, 7.8×10^9 /litre; and platelets, 172×10^9 /litre.

During her stay on the paediatric ward she drank poorly and her increase in body weight was marginal. After three days, she was readmitted to the intensive care unit because of respiratory insufficiency and cardiogenic shock. She was treated with digoxin and diuretics. When she was clinically stable she was discharged from hospital. From then, the parents withdrew her from follow up.

Methods

Air dried blood smears were stained using standard methods of May Grünwald-Giemsa,

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Sudan black B, and PAS. For electron microscopy, Na-citrate blood (3.8% vol/vol) was centrifuged at 750 $\times g$ for 10 minutes at room temperature. Plasma was decanted and the cells were fixed in 2% glutaraldehyde phosphate buffer (140.4 mmol/litre NaCl, 13.8 mmol/litre Na_2HPO_4 , 1.9 mmol/litre NaH_2PO_4 , pH 7.4) at 4°C. After three hours the buffy coat was removed, sliced, and postfixated in 1% osmium tetroxide for one hour at room temperature. After dehydration in graded concentrations of alcohol, the blocks were embedded in Epon. The ultrathin sections (80 nm) were contrasted with uranyl acetate and lead nitrate and examined with a JEOL 1200 EX/II electron microscope.⁶

Lysosomal enzyme activities in plasma and fibroblasts were measured as described previously.⁷

Results

The blood smear revealed 88% lymphocytes, 8% granulocytes, and 4% monocytes. Approximately 25% of the lymphocytes were atypical and had grey blue inclusions (size, 1–2 μm), with a vague azurophilic granulation in the centre (fig 1). In disrupted normal lymphocytes, only a nuclear ghost was visible; however, in disrupted abnormal lymphocytes (as a consequence of preparing a blood smear), the vacuole-like inclusions were still visible (fig 2). These vacuole-like inclusions might suggest a viral infection. Staining with Sudan black B to identify a lipid storage disease and PAS to detect a glycogen storage disease gave negative results.

Electron microscopy of the blood cells revealed a large number (up to 20) of cytoplasmic vacuoles, some of which had no visible content, although most had an aggregation of small globular or possible tubular structures. In addition, a round osmiophilic structure was found in most cells (fig 3).

The diagnosis was finally confirmed by the low activities of β -D-galactosidase (8 nmol/h/mg protein; reference value, 600–1500), *N*-acetyl- β -D-glucosaminidase (1715 nmol/h/mg protein; reference value, 8000–38 000), and α -D-mannosidase (16 nmol/h/mg protein; reference value, 200–720) in fibroblasts and the high activities of β -D-galactosidase

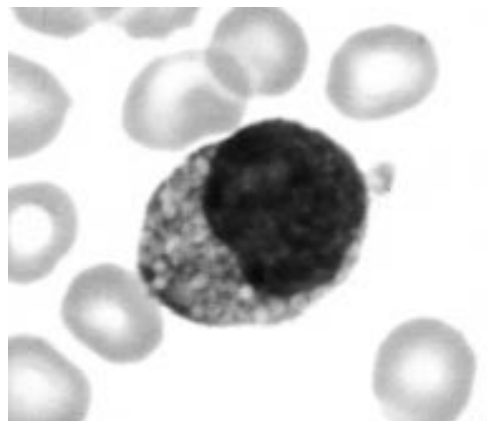


Figure 1 A lymphocyte with many vacuole-like inclusions (original magnification, $\times 900$).

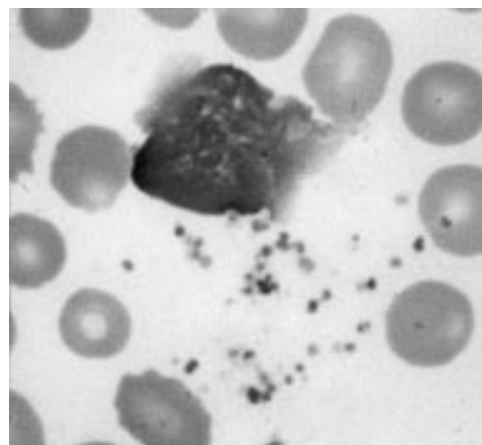


Figure 2 A disrupted cell with the released inclusions around the nuclear remnant (original magnification, $\times 900$).

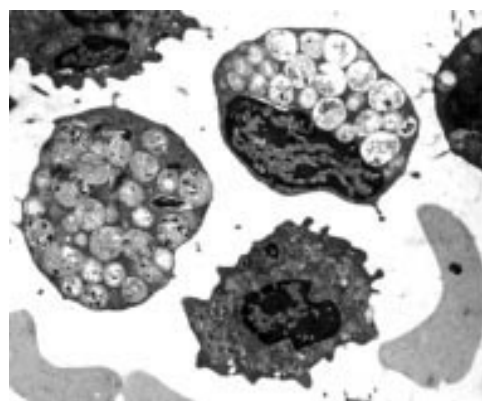


Figure 3 Electron microscopic image of lymphocytic vacuoles containing round osmiophilic structures (original magnification, $\times 15\ 000$).

(117 nmol/h/mg protein; reference value, <20), *N*-acetyl- β -D-glucosaminidase (26 582 nmol/h/mg protein; reference value, 900–7000), and α -D-mannosidase (7000 nmol/h/mg protein; reference value, 50–350) in plasma. These findings are characteristic of an I cell disease.

Discussion

I cell disease is evident from birth and shows a gradual deterioration. The disease has an autosomal recessive mode of inheritance.

Although several authors have described vacuoles or inclusions in the lymphocytes of patients with I cell disease,^{4 8 9} electron microscopic and light microscopic images have only occasionally been presented.¹⁰ In our case, the vacuoles appeared to be inclusions. Neutrophils and monocytes had a normal appearance in our patient's blood smears. Electron microscopy of the lymphocytes revealed that the inclusion bodies had a single membrane and most of them contained an osmiophilic structure. However, in contrast to Leroy *et al*,⁸ PAS and Sudan black B staining were negative. After disruption of the cells these inclusions were clearly visible, whereas in the case of normal lymphocytes only a nuclear ghost was visible.

Although many authors have described I cell disease, only Koga and co-workers⁴ and Leroy and co-workers⁸ have mentioned vacuoles or

inclusion bodies in blood cells. This report confirms these findings. However, in contrast to Koga *et al*, we did not find vacuoles in neutrophils.^{4 8}

Careful microscopic observation of peripheral blood smears may lead to the discovery of rare abnormalities in blood such as intracellular metabolite accumulation. The presence of abnormal granulation or vacuolisation of lymphocytes can facilitate a rapid diagnosis of some inherited metabolic diseases.

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