Discussion

Our results show that patients with Crohn's disease have depressed T-cell function. It is interesting to note that our values for the PHA stimulation test in Crohn's disease were intermediate between normal values and the severely depressed values of Hodgkin's disease found in this laboratory (1117±476 cpm/1000 lymphocytes; n=6).[5] Although patients with Hodgkin's disease have increased susceptibility to fungal and viral infections, this is not the case in Crohn's disease. The less severe depression of T-cell function found in our patients may be related to this relative immunity to those infections.

Malnutrition may cause abnormalities of T-cell function. None of our patients had a serum albumin level under 31 g/l, and the mean level was 40·3 g/l. The mean haemoglobin level was 13·8 g/dl with no level below 11·1 g/dl. Folate deficiency can also cause a lowered PHA response. All our patients had normal red cell folate levels (>160 µg/l of red cells).

We have shown a significant improvement in the response to PHA stimulation after giving transfer factor. Three of our patients also showed conversion of their tuberculin test results. One of the questions that this study raises is the significance of T-cell abnormalities in Crohn's disease. The T-cell abnormalities may be causally linked with the disease or may just be an epiphenomenon. To resolve this question and also to study the therapeutic effect of transfer factor it will be necessary to carry out long-term controlled trials.

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Use of liposomes in treating type II glycogenosis

Type II glycogenosis (Pompe's disease) is associated with the absence of a lysosomal α-glucosidase, which normally hydrolyses the linkages between the glucose units in the glycogen molecule. The clinical symptoms of muscular hypotonia and weakness are often apparent at birth but usually become manifest in the first few months of life and most patients soon die from cardiac failure.

Several attempts have been made to mobilise stored glycogen in the glycogenoses by administering fungal or human α-glucosidase, but these have yielded no clear-cut beneficial results.[1] To overcome some of the problems of direct enzyme administration, liposomes (closed concentric bilayers of lipid alternating with aqueous spaces in which soluble substances can be entrapped) were proposed[2] as carriers of enzymes. The localisation of intravenously administered liposomes in the liver and spleen of experimental animals has been shown, and several other therapeutic uses of liposomes[3] have been proposed. We report here the use of amyloglucosidase-containing liposomes in an attempt to treat type II glycogenosis.

Case report

This girl, the second child of healthy unrelated parents, had congenital dislocation of the hip and was admitted to hospital at the age of 3½ months with a chest infection and feeding difficulties. She was noted to be floppy, and hepatomegaly was detected on clinical examination. Chest *x*-ray examination showed massive cardiomegaly. Muscle biopsy confirmed the diagnosis of Pompe's disease, showing a vacuolar myopathy with excess glycogen on histochemical preparation and a high measured glycogen content with no α-glucosidase activity. Liver biopsy was not undertaken since it would not have benefited the child. At the age of 8 months she was readmitted to hospital in a moribund state with heart failure unresponsive to treatment, and it was decided, with the fully informed consent of the parents, to attempt liposome treatment.

Liposomes (lecithin, cholesterol, and phosphatidic acid in a molar ratio 7:2:1) containing entrapped *Aspergillus niger* amyloglucosidase were prepared as described,[2] except that strict aseptic precautions were taken to achieve a sterile, pyrogen-free preparation. Free enzyme was separated from entrapped by four centrifugation steps at 100 000 *g* for 90 minutes. The liposomes were stored under nitrogen at 4°C after passage through a 0·45-µm millipore filter.

Liposomes were administered by daily intravenous injection over seven days with no obvious side effects. Fever occurred on the third day, although the child had been intermittently febrile in the previous week. Altogether 170 mg of liposomal lipid containing 3 mg of enzyme protein (32 units) was administered. The liver decreased in size considerably during the first four days of treatment, but there was no improvement in her general poor condition. She died from cardiac failure eight days after the start of enzyme treatment. Tissues were frozen at necropsy and stored at −20°C. Enzyme and glycogen measurements are summarised in the table. The low post-mortem enzyme levels were not surprising in view of the small amount administered. Heart and skeletal muscle glycogen levels were extremely high, but the liver glycogen was much lower than expected in a child with type II glycogenosis (usually 10-12%) and supported the clinical observations that the enzyme may have had some effect in breaking down liver glycogen and hence reducing liver size. Our results (see table) show that bile may be a route of export of stored glycogen, as the level in the post-mortem sample was well above that in controls with no storage condition.

α-Glucosidase and glycogen levels in various tissues at biopsy and necropsy

Tissue	α-Glucosidase activity (units*)			Glycogen (% by weight)		
	Biopsy	Necropsy	Normal	Biopsy	Necropsy	Normal
Liver		0·02	3·2		5·5	<4
Spleen		0·04				
Skeletal muscle	U	U	0·17	3·0	7·0	<0·2
Cardiac muscle		U	0·17		6·1	<0·2
Lymph node		U				
Skin fibroblasts	U		11·9†			
Bile					1·04‡	0·02‡

*Units = µmol glucose released from maltose/min/g tissue at 37°C (1 µmol glucose/min/g ≈ 0·18 mg/min/g).
†µmol glucose released from maltose/min/mg protein at 37°C.
‡mg glycogen/ml bile.
U = Undetectable on assay.

Discussion

It is, unfortunately, difficult to estimate the true value of this treatment owing to the lack of biochemical data on liver glycogen before treatment began. Nevertheless, there does seem to have been some success in reducing stored liver glycogen. No reduction in muscle glycogen was observed. Thus the future use of liposomes in the treatment of this condition is limited unless a way can be found of directing liposomes to muscle tissue, perhaps by attaching tissue-specific antigens to the liposomal surface as attempted by Gregoriadis.[4]

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Requests for reprints should be addressed to DAT.

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