J. Inher. Metab. Dis. 16 (1993) 263–266

NCL Symposium

Immunolocalization Studies of Subunit c in Late-infantile and Juvenile Batten disease

B. D. LAKE[1]* and N. A. HALL[2]†
[1]*Department of Histopathology, Hospital for Sick Children, Great Ormond Street, London WC1N 3JH;* [2]*Department of Neurochemistry, The National Hospital, Queen Square, London, UK*

The ovine, late-infantile (LIB) and juvenile (JB) forms of Batten disease contain significant amounts of protein in the storage bodies, and subunit c of mitochondrial ATP synthase is a major component of this protein (Palmer et al 1990, 1992). These observations have led to the generation of antibodies to subunit c, and their application to Western blots of tissue extracts and to tissue sections (Hall et al 1991). It was possible to show that the sites of immunoreactivity corresponded to the sites of storage demonstrated by standard histopathological techniques, and that the adult form of Batten disease (Kufs) could also be regarded as a subunit c storage disorder (Hall et al 1991). Infantile Batten disease did not store subunit c, and its protein component has not been identified.

This investigation extends the initial observations to include other tissues and organs.

MATERIALS AND METHODS

Paraffin-wax blocks of formalin-fixed post-mortem tissues from cases of LIB and JB, juvenile Batten disease with GROD (Carpenter et al 1973), a variety of documented lysosomal storage disorders, and Alzheimer disease were available. Immunohistochemistry was performed as described previously (Hall et al 1991).

RESULTS

In LIB and JB the immunoreactivity of the storage material in cells did not appear to be greatly affected by formalin fixation of short or long duration, or by wax embedding. Some loss of sensitivity was suggested by comparison with cryostat sections stained similarly. Immunostaining was not observed in controls or in plaques or tangles in Alzheimer disease. In Sanfilippo disease (MPS IIIA) neurons were sometimes strongly stained, but no immunoreactivity was detected in visceral organs.

The intensity of the neuronal staining was much stronger in LIB in comparison with that of JB, and this was evident not only in brain but in neurons of the myenteric

* Correspondence
† Deceased

and submucosal plexuses of the gut and those found in other sites.

In juvenile Batten disease with GROD, the storage in neurons of the brain was not immunoreactive although a few showed weak diffuse immunoreactivity.

In LIB and JB sites of subunit c accumulation were revealed (a) in epithelial lining cells – apical portions of tracheal epithelium, basal layer of oesophageal epithelium, bile duct epithelium, epithelial cells of the renal pelvis and to a much lesser extent in the renal tubules, ductular epithelium of the parotid glands and the tongue, sweat gland epithelium; (b) in hepatocytes, exocrine and endocrine pancreatic cells, anterior pituitary cells, some lining cells of thyroid follicles; and (c) in smooth, skeletal and cardiac muscle. The degree of involvement was variable from tissue to tissue but in general was less marked in JB.

DISCUSSION

The pattern and distribution of immunoreactivity poses interesting questions. It appears that most epithelial cells are affected by accumulation of subunit c, but that

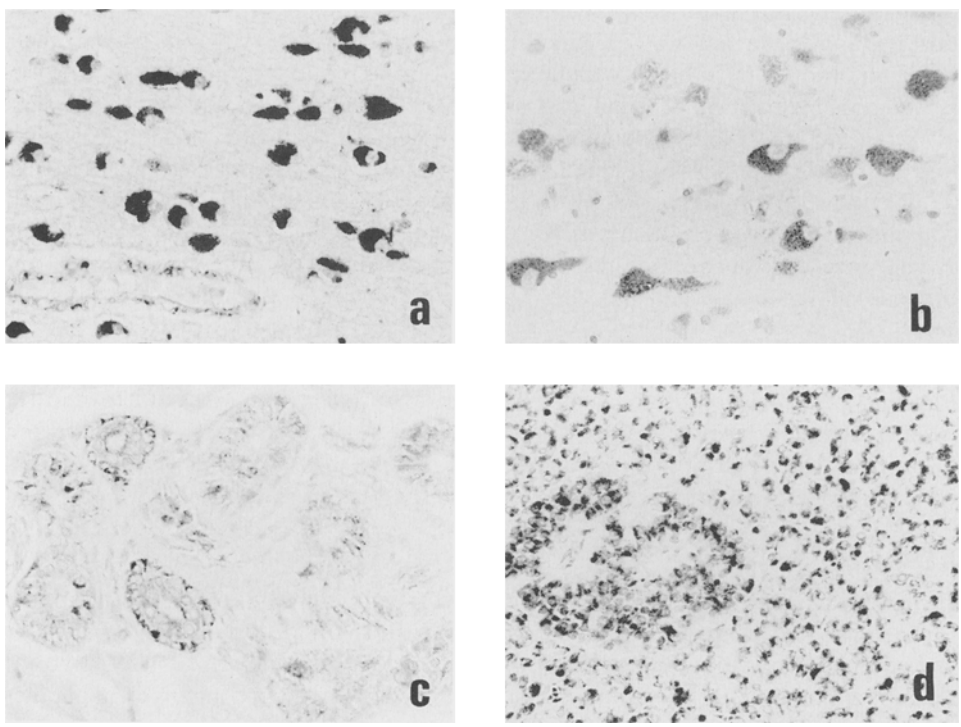

Figure 1 Immunohistochemical localization of subunit c in paraffin sections. (a) LIB brain cortex. The strong staining in the neurons and blood vessels (lower right) contrasts with the weaker staining in JB brain cortex (b). The intensity of staining in (b) is weaker than in LIB. (c) JB skin sweat glands. Subunit c is evident in some but not all of the gland cells. (d) LIB pancreas. Marked accumulation of subunit c is seen in the exocrine and endocrine (islet) cells. A light nuclear counterstain has been added. Incubation and photography were under identical conditions for each. Magnification × 200

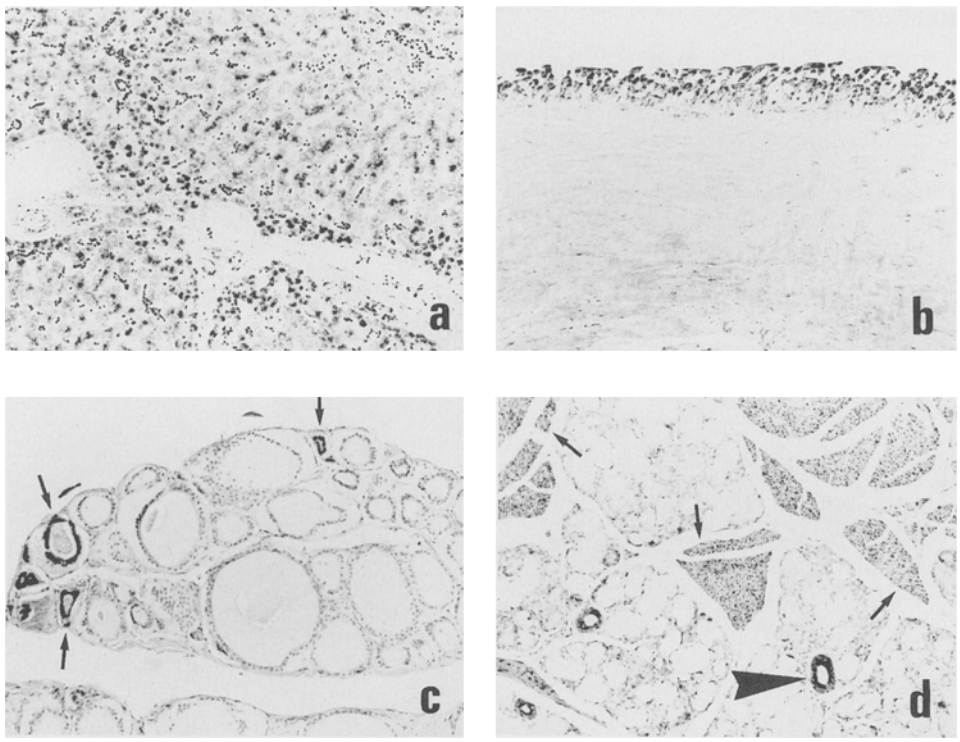

Figure 2 Immunolocalization of subunit c in paraffin sections. (a) LIB liver. Periportal accumulation of subunit c is more prominent than in other areas. Darkly staining red blood cells are present in the sinusoids. (b) LIB, epithelial cells of the renal pelvis. Very marked accumulation of subunit c is found in this site. (c) LIB thyroid. Some follicular lining cells contain abundant subunit c (arrows). (d) LIB tongue. Subunit c is detected in the muscle cells (arrows) and in the ductular epithelium (arrow head). Only scattered cells among the mucous secreting elements are positive. A light nuclear counterstain has been added. Incubation and photography were under identical conditions for each. Magnification × 80

their function is unimpaired. This applies particularly to the exocrine and endocrine cells of pancreas, the pituitary and liver. It is also noteworthy that the basal cells of the oesophageal epithelium are involved while suprabasal cells are not, and this raises the question of whether there is some degradation of subunit c similar to the situation in neutral lipid storage disease (Radom et al 1987).

The failure to demonstrate subunit c in the storing neurons of juvenile Batten disease with GROD places this disorder in the same category as the infantile form. It is possible that this category may also include some Kufs disease in which GROD has been demonstrated.

The significance of the positive immunoreactivity in mucopolysaccharidosis is at present unknown but may be similar to the general inhibition of lysosomal enzyme activity already known in the mucopolysaccharidoses.

REFERENCES

Carpenter S, Karpati G, Wolfe LS, Andermann F (1973) A type of juvenile cerebromacular degeneration characterized by granular osmiophilic deposits. *J Neurol Sci* **18**: 67–87.

Hall NA, Lake BD, Dewji NN, Patrick AD (1991) Lysosomal storage of subunit c of mitochondrial ATP synthase in Batten's disease (ceroid-lipofuscinosis). *Biochem J* **275**: 269–272.

Palmer DN, Fearnley IM, Medd SM et al (1990) Lysosomal storage of the DCCD reactive proteolipid subunit of mitochondrial ATP synthase in human and ovine ceroid lipofuscinoses. *Adv Exp Med Biol* **266**: 211–223.

Palmer DN, Fearnley IM, Walker JE et al (1992) Mitochondrial ATP synthase subunit c storage in the ceroid lipofuscinoses (Batten's disease). *Am J Med Genet* **42**: 561–567.

Radom J, Salvayre R, Maret A, Negre A, Douste-Blazy L (1987) Metabolism of 1-pyrenedecanoic acid and accumulation of neutral fluorescent lipids in cultured fibroblasts of multisystemic lipid storage myopathy. *Biochim Biophys Acta* **920**: 131–139.