Cytochrome c oxidase deficiency

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Cytochrome c oxidase (complex IV of the respiratory chain) is a complex enzyme: the prosthetic group of cytochrome $a_2$ is a unique iron porphyrin, haem $a$, and cytochrome $a_3$ contains copper (Tzagoloff, 1982). The apoprotein consists of multiple subunits: in lower eukaryotes seven polypeptides have been demonstrated (Tzagoloff, 1982), while in preparations from higher animals the number of subunits varies from seven (Ludwig et al., 1979) to 13 (Kadenbach & Merle, 1981) and up to 15 (Griffin & Landon, 1981), depending on the technique of polyacrylamide-gel electrophoresis employed. There is general agreement that the three larger subunits (I–III) are encoded by mitochondrial DNA, and the small subunits by nuclear DNA (Tzagoloff, 1982). There is also evidence that the essential properties of the enzyme are related to the three larger subunits: the two haems seem to be localized in subunits I and II, the two copper atoms in subunit III, and the proton-pumping activity in subunit I (Kadenbach et al., 1982). On the other hand, the functions of nuclear-encoded polypeptides are controversial. In higher animals some subunits appear to differ in different tissues, and they may confer tissue-specificity to cytochrome $c$ oxidase (Jarausch & Kadenbach, 1982; Kadenbach et al., 1982; Merle & Kadenbach, 1982).

Clinical heterogeneity

Given the complexity of the enzyme, it is not surprising that cytochrome $c$ oxidase deficiency should be characterized by remarkable clinical heterogeneity: different clinical expressions were already described 2 years ago (DiMauro et al., 1983a,b) and the spectrum has considerably expanded even in this brief interval of time (Table 1). The most common clinical picture appears to be fatal infantile myopathy and renal dysfunction: seven patients with this syndrome have been described (Van Biervliet et al., 1977; DiMauro et al., 1980; Heiman-Patterson et al., 1982; Minchom et al., 1983; Muller-Hocker et al., 1983; Zeviani et al., 1985). All patients had severe generalized weakness present at birth or in the first weeks of life, hypotonia, hyporeflexia, respiratory failure, lactic acidosis, and De Toni–Fanconi–Debré syndrome. All patients but one died before 4 months of age: one patient died at 8 months after prolonged assisted ventilation (Zeviani et al., 1985). Family history was non-informative in three cases; twin sisters of one patient (Van Biervliet et al., 1977) and a brother of another (Minchom et al., 1983) had died in infancy with similar symptoms, and two patients were siblings (Muller-Hocker et al., 1983).

There were myopathy and respiratory failure without renal dysfunction was seen in two children: one of them died at age 7 months after 5 months of supported ventilation (Bresolin et al., 1985), the other is still alive but respirator-dependent at 4 months of age (P. L. Peterson, personal communication).

The association of myopathy and cardiopathy was described in two patients (Rimondini et al., 1982; Skedros et al., 1984). In collaboration with Dr. David Van Dyke, we studied a baby girl with severe weakness since birth, cardiopathy and lactic acidosis: her strength seemed to increase gradually, but she died of cardiac failure at 34 months. Myopathy and liver disease were manifested in the same family but not in the same patient: two siblings had severe myopathy without liver dysfunction, and their second cousin died of hepatic failure at the age of 9 months (Boustanly et al., 1983).

Myopathy, encephalopathy and renal dysfunction (De Toni–Fanconi–Debré syndrome) characterized the clinical picture of a child whose post-mortem tissues were kindly sent of us by Dr. Aliza Gutman (Hadassah University, Jerusalem, Israel). This boy presented at 2 months of age with failure to thrive, hypotonia, seizures and nystagmus; he also had lactic acidosis and De Toni–Fanconi–Debré syndrome. He died at 16 months. He was the product of a consanguineous marriage and his first cousin died in infancy with a similar syndrome.

Subacute necrotizing encephalomyelopathy (Leigh’s disease) has been attributed to cytochrome $c$ oxidase deficiency in four patients (Wasserman et al., 1977; Miyabayashi et al., 1983; Hoganson et al., 1984), and the same enzyme defect has been documented in a patient with progressive sclerosing poliodystrophy of childhood (Alper’s disease) (Prick et al., 1983). In trichopoliodystrophy (Menkes’ disease) cytochrome $c$ oxidase deficiency was reported in multiple tissues of one patient (French et al., 1983).

All the syndromes described above are fatal in infancy or childhood. In contrast, we have studied a patient with a severe but ultimately benign myopathy due to a reversible defect of cytochrome $c$ oxidase (DiMauro et al., 1983a,b). This baby boy presented at 2 weeks of age with generalized weakness, hypotonia, hyporeflexia, macroglossia and severe lactic acidosis. He improved spontaneously and walked at 16 months; at 33 months, he had only mild proximal weakness. Blood lactate declined to normal values by 14 months of age.

Biochemical heterogeneity

Differential biochemical involvement of different tissues. The clinical heterogeneity described above is accompanied by biochemical heterogeneity. In one case of myopathy alone we found that cytochrome $c$ oxidase activity was almost undetectable in muscle, while it was normal in heart,
liver, kidney and brain. Accordingly, reduced minus oxidized cytochrome spectra showed that the cytochrome $a_2$ peak was lacking in mitochondria isolated from muscle but present in heart mitochondria (Bresolin et al., 1985). In three cases of myopathy and renal dysfunction studied post mortem, data from our own laboratory (DiMauro et al., 1980) and from other laboratories (Van Biervliet et al., 1977; Minchom et al., 1983) have shown that cytochrome c oxidase activity is markedly decreased or absent in muscle and partially defective in kidney, but normal in heart. Of the three cases with myopathy and cardiopathy, the heart was studied in two: cytochrome c oxidase activity was reported normal in one (Rimoldi et al., 1982), while we found markedly decreased activity (12% of normal in isolated mitochondria) in the patient studied in collaboration with Dr. David Van Dyke. We received from Dr. Aliza Gutman specimens of muscle, heart and brain from one patient with myopathy, encephalopathy and renal dysfunction: cytochrome c oxidase activity was decreased in skeletal muscle and brain but normal in cardiac muscle.

In the patient with Leigh’s disease reported by Hoganson et al. (1984), we found markedly decreased cytochrome c oxidase activity in all tissues studied, including liver, brain, kidney, as well as in cultured fibroblasts. This is in agreement with the data of Miyabayashi et al. (1985), while in the patient of Willems et al. (1977) the liver appeared to be spared.

Differential tissue involvement was also demonstrated in muscle biopsies by histochemistry and immunocytochemistry. In a patient with myopathy and renal dysfunction, both enzyme stain and immunoperoxidase reaction were virtually absent in extrafusal fibres, but present in intrafusal fibres of the muscle spindle and in smooth-muscle cells of blood vessels (Zeviani et al., 1985).

Evaluation of immunologically cross-reacting material (CRM). In an attempt to clarify the biochemical basis for the different clinical presentations and to understand the molecular defect in some of these syndromes, we have conducted immunological studies of cytochrome c oxidase in muscle-mitochondria and post-mortem tissues from 12 patients. Cytochrome c oxidase was purified from human heart by the method of Fowler et al. (1962), modified by Tzagoloff & McLennan (1965); by SDS/polyacrylamide gel electrophoresis, the purified enzyme showed seven major subunits, similar to those of beef-heart cytochrome c oxidase (Bresolin et al., 1985). When these proteins were transferred to nitrocellulose paper and subjected to immunoenzymic stain (Western blot), all seven subunits reacted with the antiserum to the holoenzyme, as expected from data in the literature (Jarausch & Kadenbach, 1982).

Antisera or immunoglobulin fractions were used for the following tests: (1) quantitative immunocytochemistry of frozen tissue sections; (2) enzyme-linked immunosorbent assay (ELISA) of muscle-tissue extracts or isolated mitochondrial preparations; (3) immunoblotting of mitochondrial proteins separated by SDS/polyacrylamide gel electrophoresis; (4) SDS/polyacrylamide gel electrophoresis analysis of mitochondrial proteins isolated by immunoprecipitation (Bresolin et al., 1985). These studies are intended to clarify whether or not cytochrome c oxidase deficiency is accompanied by a decrease of immunologically reactive enzyme protein and whether the subunit composition of the enzyme is altered in different forms of cytochrome c oxidase deficiency.

In one patient with myopathy alone (Bresolin et al., 1985) and in another with myopathy and De Toni–Fanconi–Debré syndrome (Zeviani et al., 1985), the amount of immunologically cross-reacting enzyme protein in muscle was markedly decreased by immunocytochemistry, antibody-consumption test and e.l.i.s.a. (Fig. 1). The amount of CRM was also decreased in both muscle and brain mitochondria from the two cousins with myopathy and cardiopathy, and in all tissues from the patient with Leigh’s disease (Fig. 1).

Immunocytochemistry of the three muscle biopsies obtained from the child with benign reversible cytochrome c oxidase deficiency at 1, 7 and 33 months of age showed positive stain in all fibres (E. Bonilla, unpublished work). This is in sharp contrast to the cytochrome c oxidase stain, which was positive in fewer than 5% of fibres in the first biopsy, in approx. 60% of fibres in the second, and in all fibres in the third biopsy (DiMauro et al., 1983a,b).

Thus the genetic heterogeneity of cytochrome c oxidase deficiency extends to the presence of CRM: some patients lack immunologically detectable enzyme protein, others appear to have normal amount of enzymically inactive protein.

Subunit composition of mutant enzymes. In the patient with myopathy and renal dysfunction, the enzyme activity was decreased but not absent immunologically reactive enzyme. SDS/polyacrylamide gel electrophoresis of mitochondrial extracts followed by immunoblotting did not show clearly detectable bands because the content of CRM was too small. However, SDS/polyacrylamide-gel electrophoresis of immunoprecipitated mitochondrial proteins showed the presence of all seven subunits and the relative intensity of the bands appeared normal (Bresolin et al., 1985). Immuno blotting of mitochondrial extracts and electrophoretic analysis of immunoprecipitates in patients with normal amounts of CRM have also shown normal subunit patterns.

**Fig. 1. Immunoreactivity by e.l.i.s.a. of muscle mitochondrial extracts (10 μg of protein/ml) from a patient with myopathy without renal dysfunction (Bresolin et al., 1985) and a patient with Leigh’s disease (Hoganson et al., 1984)**

(a) Immunoreactivity of mitochondrial extracts from a patient with myopathy without renal dysfunction (●) and from control muscle (▲). (b) Immunoreactivity of mitochondrial extracts from a patient with Leigh’s disease (●) and from control muscle (▲).
Genetic considerations. Because three subunits of the enzyme are encoded by mitochondrial DNA, some forms of cytochrome c oxidase deficiency could be due to genetic mutation of the mitochondrial genome. Such disorders ought to be transmitted by non-Mendelian, maternal inheritance (Fine, 1978; Giles et al., 1980).

However, pedigree analysis in the different fatal forms described above has been either non-informative or suggestive of autosomal recessive inheritance. In Menkes’ disease, cytochrome c oxidase deficiency is compatible with a predominance of mutant mitochondria (and low or absent cytochrome c oxidase activity). Such disorders remain hypothetical, and the exclusive involvement of muscle is puzzling.

Conclusions

The remarkable biochemical heterogeneity demonstrated in patients with different clinical expressions of cytochrome c oxidase deficiency remains to be elucidated at a molecular level. The involvement of muscle alone in some patients and of muscle together with one or more other tissues in other patients suggests that there are tissue-specific isoforms of cytochrome c oxidase, and that different molecular errors may affect different tissue-specific subunits or determinants. In one patient with fatal infantile cardiomyopathy and reduced amount of immunologically reactive enzyme protein, we did not find a selective defect of one or more subunits. However, it is possible that defective synthesis of a muscle-specific, presumably nuclearly coded, subunit may cause impaired assembly of the entire complex. Polyclonal antibodies raised against the holoenzyme may not distinguish tissue-specific subunits: we are therefore raising monoclonal antibodies against the SDS-dissociated enzyme. We have already obtained one cell line that produces antibodies reacting against subunit IV of cardiac, but not skeletal, muscle (M. Nakagawa, A. F. Miranda & M. Zeviani, unpublished work).

Recombinant DNA techniques hold great promise for the study of cytochrome c oxidase deficiency. Human mitochondrial DNA has been fully sequenced (Anderson et al., 1981). If maternal inheritance of cytochrome c oxidase deficiency is demonstrated in some families, studies of mitochondrial DNA by restriction endonuclease fragment analysis may reveal mutations. Nuclear genes encoding cytochrome c oxidase subunits are being cloned: using synthetic oligonucleotides as hybridization probes, a complementary DNA clone has been isolated for subunit IV of beef-heart cytochrome c oxidase (Lomax et al., 1984). Homology in amino acid sequences between bovine and human cytochrome c oxidase subunits may allow one to use the bovine clones as probes for the isolation of human genes.

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