

# Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues

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## Summary

**Background** Zidovudine is commonly administered during pregnancy to prevent mother-to-child HIV-1 transmission. We investigated mitochondrial toxic effects in children exposed to zidovudine in utero and after birth.

**Methods** We analysed observations of a trial of tolerance of combined zidovudine and lamivudine and preliminary results of a continuing retrospective analysis of clinical and biological symptoms of mitochondrial dysfunction in children born to HIV-1-infected women in France. Mitochondrial dysfunction was studied by spectrophotometry and polarography of respiratory-chain complexes in various tissues.

**Findings** Eight children had mitochondrial dysfunction. Five, of whom two died, presented with delayed neurological symptoms and three were symptom-free but had severe biological or neurological abnormalities. Four of these children had been exposed to combined zidovudine and lamivudine, and four to zidovudine alone. No child was infected with HIV-1. All children had abnormally low absolute or relative activities of respiratory-chain complexes I, IV, or both months or years after the end of antiretroviral treatment. No mutation currently associated with constitutional disease was detected in any patient.

**Interpretation** Our findings support the hypothesis of a link between mitochondrial dysfunction and the perinatal administration of prophylactic nucleoside analogues. Current recommendations for zidovudine monotherapy should however be maintained. Further assessment of the toxic effects of these drugs is required.

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## Introduction

The antiretroviral nucleoside analogue zidovudine can lower the rate of mother-to-child transmission of HIV-1 infection.<sup>1</sup> Official recommendations to use this preventive treatment during the second half of pregnancy, labour, and for the first 6 weeks of life in neonates<sup>2</sup> have led to a substantial decrease in the number of infected children in more-developed countries.<sup>3,4</sup> Simplified regimens adapted to the medical and economic conditions in Africa and South Asia have also been effective.<sup>5</sup>

Tolerance of this treatment has been a concern.<sup>6–8</sup> The data from the first protocol<sup>9</sup> and later observational studies were reassuring. The most common secondary effect was, as expected, transient and rarely severe macrocytic anaemia. Whether late side-effects appear after the period during which children are not infected by HIV-1 is not clear. This question prompted long-term follow-up studies<sup>10</sup> and the establishment of national registers for antiretroviral prescriptions during pregnancy. The development of multidrug antiretroviral therapies reinforces the need for such studies because many HIV-1-infected women have received zidovudine in combination with other nucleoside analogues, especially lamivudine, since 1995. We report mitochondrial dysfunction in unrelated children, free of HIV-1 infection, exposed in utero and postnatally to one or two nucleoside analogues.

## Patients and methods

### Patients

The French National Epidemiological Network for studying mother-to-child transmission of HIV-1 (Enquête Périnatale Française) is an epidemiological survey that was started in 1986 and involves more than 90 obstetrics and paediatrics centres throughout France. All participating centres adhere to a common monitoring schedule and include all seropositive women who agree to participate. By Jan 15, 1999, 3779 mother-child pairs were included, of which 1754 were exposed to zidovudine or other drugs as part of the mother's treatment during pregnancy. Children who are not infected are followed up for 18 months. HIV-1-infected children are followed up for as long as possible.<sup>11</sup> Protocol ANRS075 was a large non-randomised phase II trial to assess tolerance to zidovudine and lamivudine administered to prevent mother-to-child transmission of HIV-1. The study was integrated into the French epidemiological network.

### Biochemical and molecular analyses of mitochondria

Spectrophotometric and polarographic studies of liver homogenates, skeletal-muscle and cardiac-muscle mitochondria, and digitonin-permeabilised lymphocytes were done to measure absolute enzyme activities and relative ratios of activities.<sup>12</sup> We compared results with control values taken from normal children or children with metabolic disturbances but proven non-mitochondrial disease, investigated inhouse. Some residual

activities in abnormal tissues in patients fall into control ranges because of the wide variations in the amounts, activities, or both of mitochondria in control human tissues and the frequent accumulation of abnormal mitochondria in respiratory-chain-deficient cells. The measurement of activity ratios enables detection of the presence of defective mitochondria in such cases. A difference of more than three SD from the mean control value was taken to show an abnormal balance between respiratory-chain complexes resulting from a functional enzyme deficiency.<sup>13,14</sup>

Total DNA extracted from lymphocyte or muscle was amplified by PCR to detect mutations 3243 and 3271 (MELAS), 8344 and 8356 (MERF) and 8993 (NARP-LEIGH).<sup>15</sup> Mitochondrial DNA was tested for deletions by long-range PCR amplification.

Quantitative determination of mtDNA was done by Southern blot analysis. Total DNA was isolated from enzyme-deficient tissues and linearised with *PvuII*. Blots were hybridised with a [<sup>32</sup>P]dCTP-labelled single-strand mtDNA probe cloned in our laboratory.<sup>16</sup> Hybridisation signals were measured by densitometric analysis of the autoradiogram after normalisation for the nuclear-encoded 18S rRNA content of each sample.

## Results

### Patients

Eight children with mitochondrial dysfunction were found. The first two patients were identified by the analysis for serious adverse events of ANRS075. The other six patients were identified during continuing retrospective screening for mitochondrial symptoms among patients enrolled in the national epidemiological survey since 1986 and in the therapeutic trial (table 1).

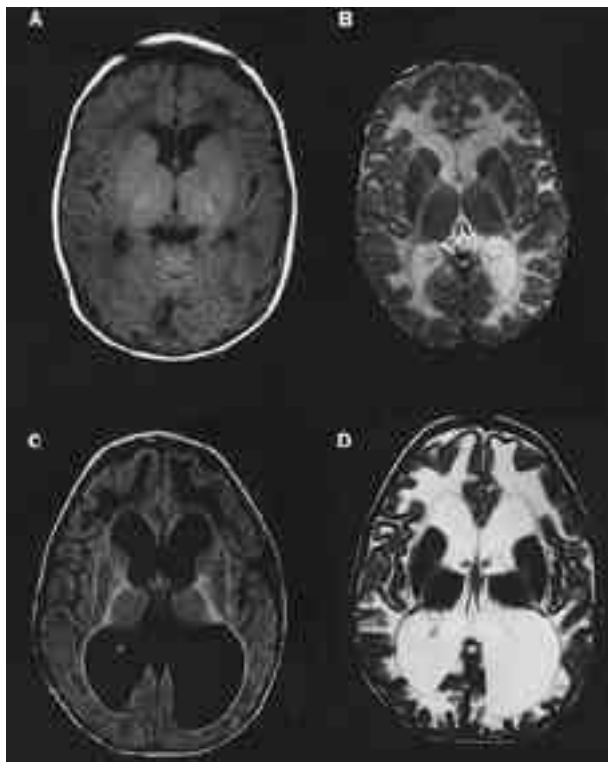
At age 4.5 months, the first patient presented with visual impairment. Cerebral nuclear magnetic resonance imaging showed initially (at age 5.0 months) demyelinating lesions of the brainstem that became more severe and secondarily associated (at 11.0 months) with sustentorial lesions. From age 4.5 months to 11.0 months, the growth was abnormal and associated with vomiting. There were no important hepatic, pancreatic muscle enzyme, or haematological abnormalities, but blood and cerebrospinal fluid lactate concentrations were high (2.5 mmol/L [normal <1.5 mmol/L] and 4.5 mmol/L [<2.0 mmol/L], respectively). The child died aged 13 months because of respiratory and cardiac-rhythm disorders. The symptoms were compatible with Leigh's syndrome and mitochondrial investigations were done at age 12 months.

The second patient, from age 4 months until death at 11 months, had refractory epilepsy and deterioration of cognitive and psychomotor abilities. Cerebral imaging showed diffuse demyelinating lesions associated with massive cortical necrosis (figure). There were no substantial biological abnormalities for liver, pancreas, muscle, or haematological markers. The blood lactate concentration was high (2.5 mmol/L) but cerebrospinal fluid lactate was normal. Several disorders were excluded because of normal results from the following diagnostic procedures: organic-acid chromatography (urine), aminoacid chromatography (serum, urine, cerebrospinal fluid), serum cholesterol, triglycerides, vitamins A and E, pyruvate dehydrogenase activity in lymphocytes, fatty-acid

Patient	Nucleoside exposure			Clinical summary	Follow-up	Lactic acidosis during treatment†	Persistent lactic acidosis‡	Haematological cytopenia§	High plasma liver enzyme activity	High plasma pancreatic enzyme activity	High plasma muscle enzyme activity	Pathological NMR imaging	Abnormal electroretinography	Cardiomyopathy
	Prepartum*	Perpartum	Postpartum†											
1	Zidovudine (32 weeks), lamivudine (32 weeks)	Intravenous zidovudine	Zidovudine (5 weeks), lamivudine (5 weeks)	Brainstem symptoms	To death at 13 months	Not done	+	-	-	-	-	+	-	-
2	Zidovudine (outset), lamivudine (32 weeks), didanosine (outset to 6 weeks)	Intravenous zidovudine	Zidovudine (5 weeks), lamivudine (5 weeks)	Seizures, tetraparesia, cognitive impairment	To death at 11 months	Not done	+	-	-	-	-	+	-	-
3	..	Intravenous zidovudine	Zidovudine (6 weeks)	Seizures, myopathy, transient cardiomyopathy	4 years 4 months	Not done	+	+	-	-	+	-	+	+
4	Zidovudine (14 weeks)	Intravenous zidovudine	Zidovudine (6 weeks)	Seizures	2 years 5 months	Not done	-	-	-	-	-	+	Not done	-
5	Zidovudine (22 weeks)	Intravenous zidovudine	Zidovudine (6 weeks)	Seizures, tetraparesia, cognitive impairment	3 years 4 months	Not done	-	-	+	-	-	+	Not done	-
6	Zidovudine (32 weeks), lamivudine (32 weeks)	Intravenous zidovudine	Zidovudine (2 weeks), lamivudine (2 days)	Symptom-free	1 year 2 months	+	+	+	+	+	-	+/-¶	+/-¶	-
7	Zidovudine (31 weeks)	Intravenous zidovudine	Zidovudine (4 weeks)	Symptom-free except near-miss syndrome	7 months	+	+	-	-	-	-	-	Not done	-
8	Zidovudine (outset), lamivudine (7 weeks), didanosine (outset to 5 weeks)	Intravenous zidovudine	Zidovudine (6 weeks), lamivudine (6 weeks)	Symptom-free	2 years 2 months	Not done	-	-	+	+	-	+	+	-

NMR=nuclear magnetic resonance. \*Start time of treatment during pregnancy in weeks of gestation. †Duration of treatment in days or weeks for neonate; patient seven received half dose zidovudine after 2 weeks of treatment. ‡Blood lactate  $\geq 2.5$  mmol/L. §Haemoglobin <10 mg/L, platelets <100 $\times 10^9$ /L, neutrophils <1.0 $\times 10^9$ /L, or any combination of these. ||1.5 times normal values; all biological abnormalities observed on at least two occasions and after end of treatment. ¶Difficult to interpret at this age.

Table 1: Effects of nucleoside exposure before and during pregnancy, and after birth



**Axial T1-weighted (A and C) and T2-weighted (B and D) magnetic resonance scans at age 4 months (A and B) and 10 months (C and D) in patient two**

Scans show intensity of acquired cortical and subcortical brain atrophy and associated hypersignal of white matter.

oxidation and biotinidase activities (lymphocytes), very-long-chain fatty acids (serum), lysosomal enzymes (galactosidase, galactosylceramidase, arylsulfatase A, mannosidase, GM1 and GM ganglioside), copper and ceruloplasmin (serum), and oligosaccharide excretion (urine). These symptoms were consistent with ALPERS syndrome, and led to mitochondrial investigations between ages 5 months and 7 months.

At age 8 months, during a febrile episode, patient three had a seizure and was thought to be hypotonic. At age

15 months, the child showed symptoms of hypokinetic hypertrophic cardiomyopathy. Blood hepatic and pancreatic enzyme concentrations were normal but the child had neutropenia (neutrophils  $0.9 \times 10^9/L$  [normal  $>1.5 \times 10^9/L$ ]), high concentrations of muscle creatine phosphokinase in blood (350 IU/L [ $<250$  IU/L]), and persistently high blood lactate concentrations (4 mmol/L), although cerebrospinal lactate was normal. Endomyocardial biopsy showed intracytoplasmic vacuolisation in myocytes, but without inflammation. The cardiomyopathy progressively improved and symptoms of peripheral myopathy were seen at age 2.5 years. At age 4.0 years, the child's cardiac function was normal, but moderate muscular deficit persisted; lactate and muscle creatine phosphokinase concentrations in blood remained high. Electroretinography showed macular and peripheral abnormalities. Cerebral nuclear magnetic resonance imaging was normal.

In the fourth patient, early development was normal. Between ages 14 months and 27 months, the child had four episodes of febrile seizures. Neurological assessment at age 27 months showed mild spastic diplegia. Haematological and biochemical findings, including lactate concentrations in blood and cerebrospinal fluid, were normal. Cerebral nuclear magnetic resonance imaging showed moderate hypersignal of the white matter in T2-weighted images, with no evidence of necrosis (figure).

From age 7 months until 15 months, patient five had repeated seizures. Cognitive development and neurological assessments between episodes were normal until age 15 months. The child developed status epilepticus for 4 h, which led to severe neurological dysfunction with cortical blindness and spastic tetraparesis. Biological tests at 15 months showed only high blood hepatic enzyme concentrations (aspartate and alanine aminotransferases 200 IU/L [ $<40$  IU/L]), which progressively returned to normal. Blood and cerebrospinal fluid lactate concentrations were measured only at the time of mitochondrial assessment and were not retrospectively available. Nuclear magnetic resonance imaging at age 16 months showed large necrotic lesions of the white

	Enzyme activities (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )				Activity ratios					Pyruvate oxidation (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )	Succinate oxidation (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )
	C1	CII	CIII	CIV	CIV/C1	CIV/CII	CIV/CIII	CIII/C1	CIII/CII		
<b>Patient 1 (age 1 year)</b>											
Liver homogenate	12	225	210	270	22.5	1.2	1.3	17.5	0.9		
Skeletal-muscle mitochondria	37	114	702	556	15.0	4.9	0.8	19.9	6.2	12	27
<b>Patient 2 (age 5 months)</b>											
Liver homogenate	20	119	168	46	48	0.6	0.6	8.4	1.4		
Skeletal-muscle mitochondria	100	174	1190	1396	14.0	8.0	1.2	11.9	6.8	14	46
<b>Patient 3 (age 1 year 5 months)</b>											
Cardiac muscle homogenate	77	243	1418	1397	18.1	5.7	1.0	18.4	5.8		
Skeletal-muscle mitochondria	23	175	1385	1123	48.8	6.4	0.8	60.2	7.9	9	69
<b>Patient 4 (age 2 years 4 months)</b>											
Skeletal-muscle mitochondria	66	172	1748	947	14.3	5.5	0.5	26.4	10.2	23	46
<b>Patient 5 (age 3 years 4 months)</b>											
Skeletal-muscle mitochondria	46	223	1251	1158	25.1	5.2	0.9	27.1	5.6	18	58
<b>Patient 6 (age 1 year 1 month)</b>											
Skeletal-muscle homogenate	9	13	82	56	6.2	4.3	0.7	6.2	6.3	..	..
<b>Patient 7 (age 6 months)</b>											
Skeletal-muscle homogenate	22	29	197	127	5.8	4.3	0.6	8.9	6.7	..	..
<b>Patient 8 (age 2 years)</b>											
Skeletal-muscle homogenate	30	35	260	205	6.8	5.8	0.8	8.6	7.4	..	..

C=Complex.

**Table 2: Respiratory-chain enzyme investigations in isolated skeletal-muscle mitochondria, tissue homogenates, and circulating lymphocytes from patients**

	Enzyme activities (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )				Activity ratios					Pyruvate oxidation (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )	Succinate oxidation (nmol min <sup>-1</sup> mg protein <sup>-1</sup> )
	CI	CII	CIII	CIV	CIV/CI	CIV/CII	CIV/CIII	CIII/CI	CIII/CII		
<b>Liver homogenate</b>											
Normal values	10-40	69-276	89-334	89-353	9.2 (1.1)	1.5 (0.2)	1.2 (0.2)	8.3 (1.0)	1.3 (0.2)	..	..
n	128	163	153	163	60	60	85	56	60		
<b>Skeletal-muscle mitochondria</b>											
Normal values	47-182	69-268	421-1654	607-2419	10.9 (1.0)	8.4 (0.7)	1.2 (0.2)	9.7 (0.9)	8.4 (0.8)	27-94	46-183
n	117	141	210	285	56	80	99	48	83	182	272
<b>Skeletal-muscle homogenate</b>											
Normal values	8-32	18-70	67-268	86-342	10.6 (0.9)	5.4 (0.4)	1.3 (0.2)	8.8 (1.0)	5.5 (0.7)	..	..
n	131	173	166	346	57	62	79	50	62		
<b>Cardiac-muscle homogenate</b>											
Normal values	25-81	43-188	158-745	242-1105	9.2 (1.7)	5.5 (1.0)	1.3 (0.3)	6.9 (0.9)	3.4 (0.5)	..	..
n	12	19	27	87	9	15	10	7	9		
<b>Lymphocytes</b>											
Normal values	..	15-40	55-150	67-205	..	6.3 (0.4)	1.4 (0.2)	..	5.8 (0.5)	2.5-8.3	4.9-15.0
n		193	205	205		138	162		113	79	194

C=Complex. Non-normally distributed activities given as median (range), normally distributed activities given as mean (SD)

Table 3: Respiratory-chain enzyme investigations in isolated skeletal-muscle mitochondria, tissue homogenates, and circulating lymphocytes from controls

matter and cortical grey matter. At age 3.5 years the child had severe sequelae and microcephaly.

Patient six was symptom-free until age 14 months, but persistent biochemical abnormalities were seen on standard follow-up of the epidemiological survey (which included lactate assays). The child had had high concentrations of blood lactate (4 mmol/L), hepatic aspartate aminotransferase (50 IU/L), and pancreatic lipase (200 IU/L [ $<150$  IU/L]) since birth that persisted until age 14 months. Cerebrospinal fluid lactate was normal. These biological abnormalities led to specific mitochondrial investigation, including cerebral nuclear magnetic resonance imaging that showed delayed myelinisation, which is difficult to interpret at that age.

Patient seven was symptom-free until age 4 months, at which time he became hypotonic with apnoea. The child regained normal breathing and consciousness after resuscitation, with no apparent sequelae. There were no biological abnormalities during routine biological follow-up, but blood lactate concentrations (routinely assayed in this institution) were continuously high ( $>4$  mmol/L) from the first test at 4 weeks to 7 months. Cerebral nuclear magnetic resonance imaging was normal. Near-miss syndromes and lactataemia justified mitochondrial investigations.

The eighth child was symptom-free. Persistent hepatic and pancreatic abnormalities (alanine aminotransferase 80 IU/L and lipase 180 IU/L) were seen from birth in the routine prospective biological follow-up. Blood lactate concentrations that were systematically added to the normal screening in the institution were normal, as were cerebrospinal fluid concentrations. At age 20 months, biological abnormalities persisted unchanged; a specific mitochondrial investigation was therefore done, including electroretinography, which was abnormal, and cerebral nuclear magnetic resonance imaging that showed abnormalities of the periventricular white matter.

No child was infected with HIV-1, and all were HIV-1 seronegative at age 15 months, or at death before this age for patients one and two. For all children, repeated tests for HIV-1 by PCR and by culture were negative.

#### Status and perinatal antiretroviral treatment of mothers

All mothers were HIV-1 infected through the heterosexual route, and none was a drug user. Four women were of African origin, three were European, and one was from

North Africa. One woman (mother of patient one) was coinfecting with hepatitis C virus. The only medication taken by the mothers other than the antiretroviral drugs were iron and vitamins, except for the mother of patient eight who was receiving co-trimoxazole (trimethoprim/sulphamethoxazole five/one). All pregnancies were uneventful: seven children were born at full term and patient two was born at 34 weeks' gestation; all were vaginal deliveries. Birth characteristics were normal. Two women (mothers of patients two and eight) were taking combined zidovudine and didanosine when they conceived; didanosine was stopped after 5 weeks and 6 weeks of amenorrhoea, respectively, and zidovudine was continued throughout pregnancy. One of these two women (mother of patient eight) was given lamivudine from 7 weeks' gestation. Antiretroviral treatment was started during pregnancy in five women; three were given zidovudine and lamivudine, started simultaneously at 32 weeks' gestation. In one woman, the treatment (intravenous zidovudine) was started during labour.

All children were treated after birth with zidovudine alone or with zidovudine and lamivudine. Treatment continued for 6 weeks in four children and was stopped prematurely because of haematological or biochemical intolerance in four children. Mean prenatal exposure (for zidovudine and lamivudine) was 17.2 weeks (range 0-40) and mean postnatal exposure was 5.2 (2-6) weeks. The zidovudine dose was 500 mg per day for mothers and 8 mg/kg daily for children. The lamivudine dose was 300 mg per day for mothers and 4 mg/kg daily for children. Nine blood samples that were collected and stored frozen as part of the trial (mothers and patients one, two, and six) were available for assaying zidovudine and lamivudine retrospectively. Although the time lag between administration of the drugs and collection of the samples was not known, all values, whether mother or child, were within the normal therapeutic ranges.

#### Mitochondrial studies

Muscle histology, including histoenzymology and electron microscopy, was normal or near normal in six children. Muscle from child two had limited red-ragged fibres and excessively numerous and deformed mitochondria on electron microscopy. For the only patient who had muscle weakness and high serum creatine phosphokinase

concentrations (patient three), Gomori staining showed numerous fibres with subsarcolemma fuschinophilic aggregates, and oxidative activity staining showed co-negative mitochondrial aggregates and fibres consistent with mitochondrial dysfunction. No electron microscopic observations were made for this child.

Skeletal-muscle mitochondria isolated from patient one at age 12 months showed severe defects of complex I and complex IV, which led to skewed ratios between respiratory-chain complex activities (table 2). In parallel, the oxidation of pyruvate and succinate in isolated mitochondria was low. In the liver, complex IV/complex I, and complex III/complex I ratios were high, which suggests a partial defect of complex I. Study of circulating lymphocytes showed a low malate+pyruvate oxidation, consistent with a deficiency in complex I (data not shown). Investigation of respiratory-chain activities in liver from patient two at age 11 months showed low complex IV activity compared with other respiratory-chain complexes, although the residual activity of complex IV was in the low values of the control range (table 3). In mitochondria isolated from skeletal muscle, no decrease in activity of complex IV (residual activity or activity ratio) was seen. Malate+pyruvate oxidation was, however, low. The enzymatic activities of mitochondria in cultured fibroblasts from children one and two were normal.

Analysis of mitochondrial-enzyme activities for patients three, four, and five gave similar findings. For example, enzyme investigations done in cardiac muscle from patient three showed increased activities of citrate synthase (data not shown) and all respiratory-chain complexes, except complex I. This finding is suggestive of an accumulation of complex-I-defective mitochondria. The enzyme defect was also seen in isolated mitochondria from skeletal muscle, which showed a decline in complex I activity and malate+pyruvate oxidation.

In the three symptom-free children, enzyme investigations with skeletal-muscle homogenate showed a similar pattern, characterised by a lower complex IV and citrate synthetase ratio than that of controls. This finding suggests a partial complex IV deficiency. However, some of the enzyme-activity ratios between the different respiratory-chain complexes, such as that for complex IV/complex II, which were expected to be altered, were normal. Partial defects may therefore have been present in these three patients that did not lead to an overall imbalance in the respiratory chain.

Because nucleoside analogues, especially zidovudine, can potentially cause myopathy associated with profound depletion of mtDNA in HIV-1-infected patients, we looked at the mtDNA content of enzyme-deficient tissues in patients one, two, and six. There was no substantial decrease in mtDNA (all 80–110% of that in controls). Depletion of mtDNA was, therefore, ruled out at the time of taking samples, which was long after drug exposure. Similarly, we failed to find large deletions or duplications of the mtDNA in any patient. Finally, no mutation currently encountered in characterised mitochondrial diseases was found in these patients (data not shown).

## Discussion

The clinical and biological symptoms of these eight children, and especially the persistently increased blood lactate concentrations in five of them, are compatible with mitochondrial respiratory-chain dysfunction. This diagnosis is supported by specific tissue and cell

mitochondrial analyses. Caution is required in interpretation of the enzymology results because of the variations in control populations. We took only values substantially different from normal to be informative. We used ratios of activity between different complexes to lessen the distortion seen with a proliferation of abnormal mitochondria.<sup>13,14</sup> The five most severely affected children showed complete respiratory-chain dysfunction, and the three remaining children, with only biological abnormalities had milder or partial enzyme deficiencies. The abnormal enzymatic profile does not show the causal mechanism and the mtDNA contained no deletions, duplications, or mutations associated with constitutional mitochondrial encephalopathy.<sup>15,17,18</sup> However, the genetic bases of most mitochondrial diseases have not yet been identified and the inability to detect mutations or deletions of mtDNA does not rule out a genetic origin.

Antiretroviral nucleoside analogues are toxic to mitochondria in HIV-1-infected adults and children, mainly through inhibition of polymerase, the enzyme that causes duplication of mtDNA. Currently used molecules, members of the 2',3'-dideoxy analogue family, lack the hydroxyl radical in the 3' position, and are incorporated into DNA but prevent elongation of the DNA strand. Clinical and animal studies confirm this effect.<sup>19,20</sup> In adults, the main histological findings are red-ragged fibres and abnormal mitochondria on electron microscopy.<sup>21</sup> These findings are commonly associated with depletion of mtDNA. Only one child showed similar histological characteristics and no child had substantial depletion of mtDNA. In adults, however, these features correspond to observations during treatment, whereas our findings were present several months after treatment had stopped. Depletion of mtDNA may disappear after proliferation of abnormal mitochondria, as happens in genetic diseases. Furthermore, red-ragged fibres associated with constitutional mitochondrial diseases are less frequent in children than in adults. Other than anaemia, the physiopathology of which may be multifactorial,<sup>22</sup> no toxic effects in mitochondria have been identified with certainty in children or fetuses exposed to nucleoside analogues in utero. One report of severe transient lactic acidosis in a neonate during treatment suggested transient mitochondrial dysfunction, although there was no formal proof.<sup>23</sup>

Poirier and colleagues<sup>24</sup> at the National Cancer Institute showed incorporation of zidovudine into mitochondrial DNA in fetuses of the monkey *Erythrocebus patas* after exposure through the placenta. With the same animal model, Gershenson and colleagues<sup>25</sup> showed dysfunction of the mitochondrial enzymes and mitochondrial damage on electron microscopy in skeletal and cardiac muscle of fetuses after treatment for periods of time and with doses similar to those used for pregnant women. The experimental model does not show whether the toxic effects are reversible after birth. Also, it gives no insight into the possible clinical impact of this type of dysfunction throughout a tissue, especially a long time after the drug is stopped. The myopathy associated with zidovudine is generally reversible, but more information is needed about symptoms or mitochondrial functions in the long term after the withdrawal of the drug. As with other drug-induced toxic effects in mitochondria, these lasting abnormalities may be associated with or show a symptomless constitutional dysfunction.<sup>26,27</sup>

The prevalence in French children of constitutional mitochondrial disease is not clear, but between one per

5000 and one per 20 000 children is generally accepted (A Munnich personal communication). This estimate covers all mitochondrial diseases. A continuing study of the incidence of neurological mitochondrial diseases in the UK that correspond to those seen in patients one, two, four, and five, has identified only 21 cases in 20 months in about 12 million children younger than 16 years (C Verity, A Nicoll, personal communication and reference 28). Even if this UK study had underestimated the number, the observation of several cases in a population of about 1700 exposed children in our network strongly suggests an acquired mitochondrial dysfunction in these non-HIV-1-infected children born to infected mothers. Retrospective and prospective assessments of mitochondrial dysfunction are therefore needed in all children born to HIV-1-seropositive mothers, whether or not they were exposed to antiretroviral agents.

The symptoms in the children in our study were not specific, and may therefore have not been identified as toxic effects of treatment. In symptom-free children who had only persistent biological abnormalities, the persistent lactic acidosis and the anomalies of myelinisation and electroretinographic findings—the long-term progression of which is unknown—were detected by specific diagnostic procedures. Prospective studies designed to investigate this effect are essential. The first data from the long-term follow-up of children exposed to zidovudine (protocol ACTG219) have been published. Three of the 107 children had unexplained symptoms of the heart and eye, which could be related to mitochondrial dysfunction and reinforces the need for specific enzymatic investigation.<sup>10</sup>

We are aware that the suggestion that antiretroviral drugs are toxic raises delicate issues. Prophylaxis of mother-to-child transmission of HIV-1 infection has saved thousands of children from death. The implementation of prophylactic-treatment programmes requires much multidisciplinary effort, and any action that impedes this approach to prevention could lead to a substantial step backwards. We believe our observations are however sufficiently significant to be shared. It is too early to do a risk/benefit analysis. Our view is that the current recommendations for zidovudine monotherapy prophylaxis should be maintained. We believe that combinations of molecules that could have cumulative toxic effects on the same cellular target should be avoided. Other nucleoside or combinations of nucleosides may have similar toxic effects. Pregnant women should be informed of the potential effects associated with these treatments during pregnancy.

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