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Nuclear and mitochondrial changes of muscle fibers in AIDS after treatment with high doses of zidovudine*

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Summary. Zidovudine (formerly azidothymidine) is a potent inhibitor of the human immunodeficiency virus (HIV) reverse transcriptase and represents the first approved drug showing clinical efficacy in HIV-associated diseases. However, considerable toxicity causing macrocytic anemia, neutropenia, and myopathy has been reported, with severe mitochondrial alterations as a special feature of this myopathy. The mitochondrial changes are consistent with the fact that zidovudine acts as an inhibitor of the mitochondrial gamma-polymerase. Electron microscopically, we could confirm the presence of severely altered mitochondria in a 32-year-old male, who developed a necrotizing myopathy after daily administration of 1,000 mg zidovudine over a period of 15 months. In addition, there were even more severe nuclear changes that, for the most part, have not been documented electron microscopically in HIV-related myopathy either with or without zidovudine treatment, especially in non-necrotic and non-regenerating fibers. Since various in vitro studies have shown interference of zidovudine with nuclear DNA metabolism even in human cell lines, we assume that the nuclear changes that we observed are at least in part related to zidovudine treatment.

Key words: Zidovudine – Myopathy – Nucleus – Mitochondria – AIDS

Zidovudine [1-beta-D-(3′-azido-2′,3′-dideoxy)ribofuranosylthymine], formerly termed azidothymidine (AZT), is one of the first drugs reported to have a potent in vitro activity against HIV [4, 24, 47]. It acts as an effective, highly selective inhibitor of the HIV-reverse transcriptase [10, 22, 33, 46, 59]. Zidovudine, after phosphorylation by cellular enzymes [59], can be incorporated into viral DNA by a reverse transcriptase, which leads to premature termination of the elongating viral DNA chain, since there is no attachment site for the formation of the next 3′,5′-phosphodiester bond [8]. Zidovudine has in fact been shown to reduce significantly HIV-related morbidity and mortality [15, 19, 20, 73]. However, despite the clinical efficacy of this agent, its use is frequently limited by its toxicity, which often included myelosuppressive effects (e.g. [15, 19, 29, 40, 49, 73].) Before 1986, when Zidovudine became introduced into the treatment of AIDS, HIV-associated myopathy had been considered as a relatively rare complication of HIV infection [12, 60]. During the past 4 years an increasing number of patients receiving long-term zidovudine therapy developed a myopathic syndrome, which generally improved when zidovudine was discontinued [5, 13, 26, 28, 31, 37, 44, 45, 49]. Until 1989, the pathological findings reported to occur in muscle biopsy specimens appeared to be rather nonspecific and did not allow a clear distinction between zidovudine-induced myopathy and that caused by HIV and other opportunistic infectious agents. Thereafter Dalakas et al. [13], Panegyres et al. [45] and Mhiri et al. [41] reported severe mitochondrial abnormalities exclusively in zidovudine-treated subjects but not in non-zidovudine-treated patients suffering from AIDS-associated myopathy. These findings are consistent with the ability of zidovudine to inhibit gamma-polymerase in therapeutic concentrations of 1–10 μM [46, 54]. This enzyme is found exclusively in the mitochondrial matrix where it induces mtDNA replication [7, 53].

We report a case of myopathy in an HIV-infected patient treated with high dosages of zidovudine for 15 months. The muscle biopsy revealed striking nuclear alterations in addition to mitochondrial changes.

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Case report

A 32-year-old homosexual male had unilateral, thalidomide-induced phokomelia and was known to be HIV positive by enzyme-linked immunosorbent assay since 1985. He was admitted to Klinik Mara II of the von Bodelschwinghsche Anstalten (Bielefeld, Germany) in February 1990, presenting with lower limb myalgias, edemas, difficulty in walking, and reduced general condition.

He had a past history of perianal herpes, severe pneumocystis carinii pneumonia and soor esophagitis, all in December 1988, which had been successfully treated with acyclovir, co-trimoxazol and ketoconazol, respectively. For prophylactic reasons, treatment with acyclovir and co-trimoxazol was maintained. Zidovudine therapy with daily doses of 1,000 mg had been initiated 15 months before muscle biopsy. About 3 months after onset of treatment, there had been recurring phases of muscle weakness and myalgia. The T4-cell count in December 1988 was 85/μl (7%), a control in 1989 revealed 286/μl (14%).

On admission, in February 1990, CPK and LDH levels were elevated (1,238 U/l and 1,469 U/l, respectively), and the EMG showed myopathic as well as neurogenic changes, reduced conduction velocity indicating polyneuropathy. General clinical examination and sonography revealed pericardial effusion as well as hepatomegaly, which was accompanied by elevated levels of transaminases (GOT 130 U/l, GPT 55 U/l). Laboratory investigations showed pronounced leukopenia (1,200/μl) and macrocytic anemia with Hb levels of about 7 g/100 ml. A skin and a muscle biopsy was taken from the site of the right M. vastus lateralis 10 days after admission. Blood transfusion and withdrawal of zidovudine and co-trimoxazol treatment rapidly led to improvement of his general condition and to an increase of the blood cell counts. Recurrent fever attacks disappeared upon antibiotic therapy.

About 1 month after muscle biopsy he rapidly developed pneumonia finally leading to respiratory insufficiency and death in April 1990.

Materials and methods

The muscle specimen was divided into three parts. One portion with the skin specimen was fixed in formalin, embedded in paraffin, and stained with H&E, elastica van Gieson and PAS. Frozen sections from the muscle specimen, fixed in formalin, were stained with Sudan III and Sudan black B. Another portion was rapidly frozen in liquid nitrogen and cryostat sections were stained with H&E, Gomori trichrome, for ATPase (at pH 9.4, 4.6, and 4.2) and NADH. The third portion of the muscle specimen, fixed with 6% glutaraldehyde in 0.4 M phosphate buffer, was used for semithin and ultrathin sections applying standard methods [52]. Ultrathin sections were examined with a Philips 400T electron microscope.

Results

Biopsy findings

Muscle biopsy. Light microscopically, marked variation of fiber size was observed (Fig. 1) with numerous small, angulated fibers, preferentially involving histochemical type 2 fibers. Atrophic fibers were often arranged in small groups, with slight or no endomysial fibrosis, indicating rather acute, neurogenic atrophy. Fiber type grouping was not apparent. Fibers of subtype 2C were moderately increased. As the most prominent feature, we observed numerous necrotic fibers in an irregular, disseminated pattern. Necrosis was in a rather early stage with only rare myophagic reactions. Nuclear centralization was infrequent and occurred only in some basophilic, regenerating fibers. Many of these nuclei showed enlarged nucleoli. In frozen sections as well as in semithin sections, a marked increase of lipid droplets was noted, especially in the severely altered or atrophic fibers. Others exhibited subsarcolemmal or intermyofibrillar glycogen accumulation or cytoplasmic bodies in different stages of development. Some severely damaged muscle fibers showed marked disorganization of myofibrils or disappearance of sarcomeres. Significant inflammatory infiltrates were not observed, although there were isolated perivascular lymphocytes. Nerve fascicles were not present in the semithin sections of the muscle specimen.

Electron microscopic examination of the muscle specimen revealed striking nuclear and mitochondrial changes. The nuclear changes comprised the nuclear matrix (Fig. 2) and envelope (Fig. 3). Some nuclei were

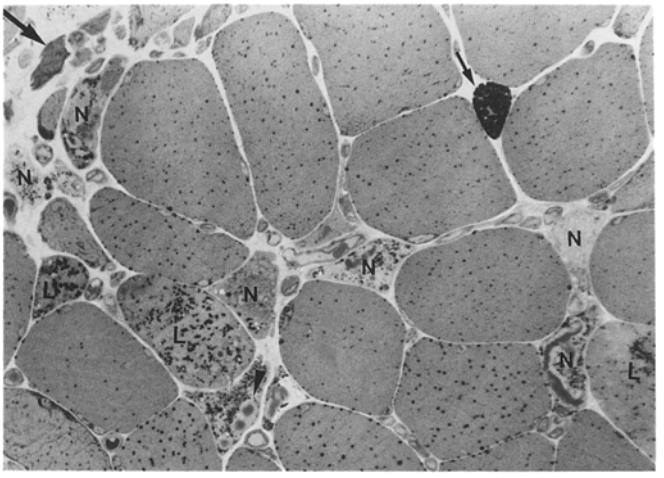
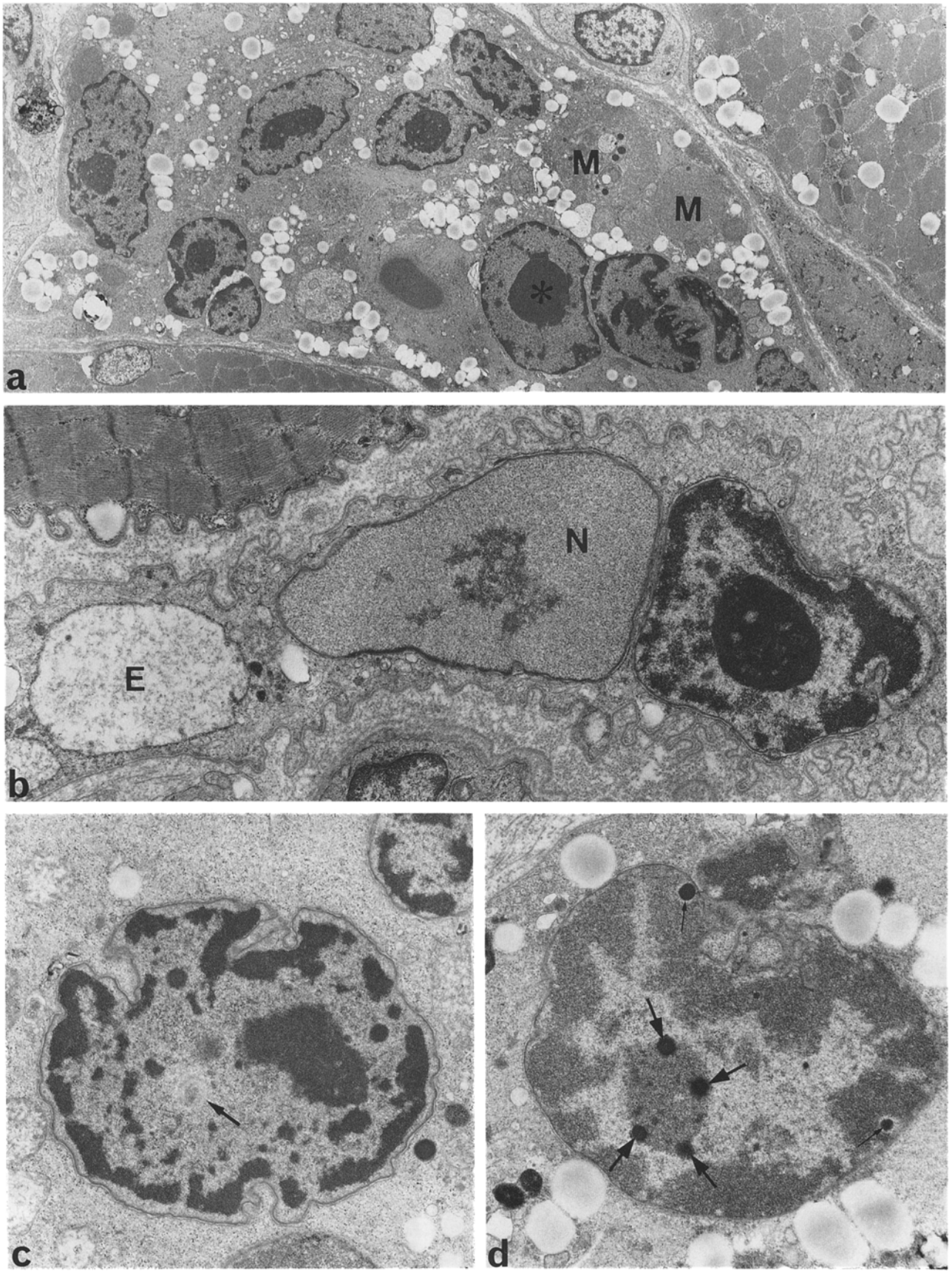


Fig. 1. Semithin section from the vastus lateralis muscle, stained with paraphenylendiamine, showing necrotic (*N*) and grouped atrophic fibers (*large arrow*). Numerous fibers exhibit marked lipid accumulation (*L*), the *small arrow* indicating a completely atrophic fiber filled with large osmiophilic lipid droplets. Another atrophic fiber contains a group of cytoplasmic bodies (*arrowhead*). × 312

Fig. 2a–d. Nuclear changes within the muscle fibers. **a** Accumulation of nuclei with enlarged nucleoli, enlarged mitochondria (*M*), and numerous lipid droplets in an atrophic muscle fiber. The nucleolus marked by an *asterisk* appears partly segregated, the filamentous component (as seen at higher magnification) forming irregular, cap-like masses around the granular component. **b** Homogeneous dispersion of nuclear chromatin (*N*) and segregation of heterochromatin in the *right nucleus* containing an enlarged nucleolus within another severely atrophic muscle fiber; distended ergastoplasma (*E*) is shown on the *left*. **c** Advanced separation of peripherally segregated heterochromatin from the nuclear envelope. A spherical filamentous inclusion body is indicated by an *arrow*. **d** Considerably enlarged perichromatin granules (giant perichromatin granules, indicated by *thin arrows*) and other globoid, electron-dense inclusions (*thick arrows*) in a nucleus, which is surrounded by lipid droplets and other, osmiophilic inclusions in an atrophic fiber. **a** × 3,300; **b** × 9,800; **c** × 11,200; **d** × 9,900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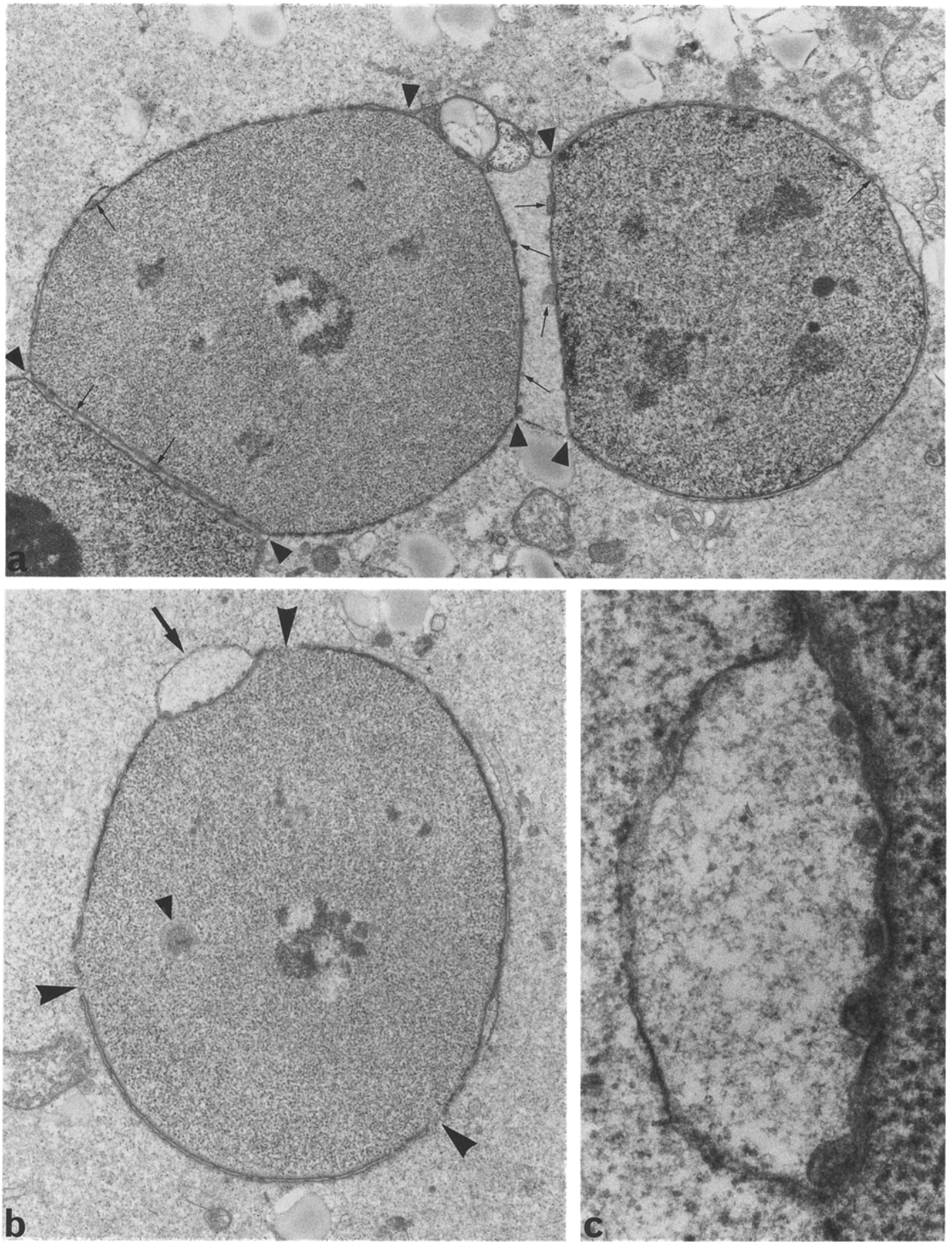
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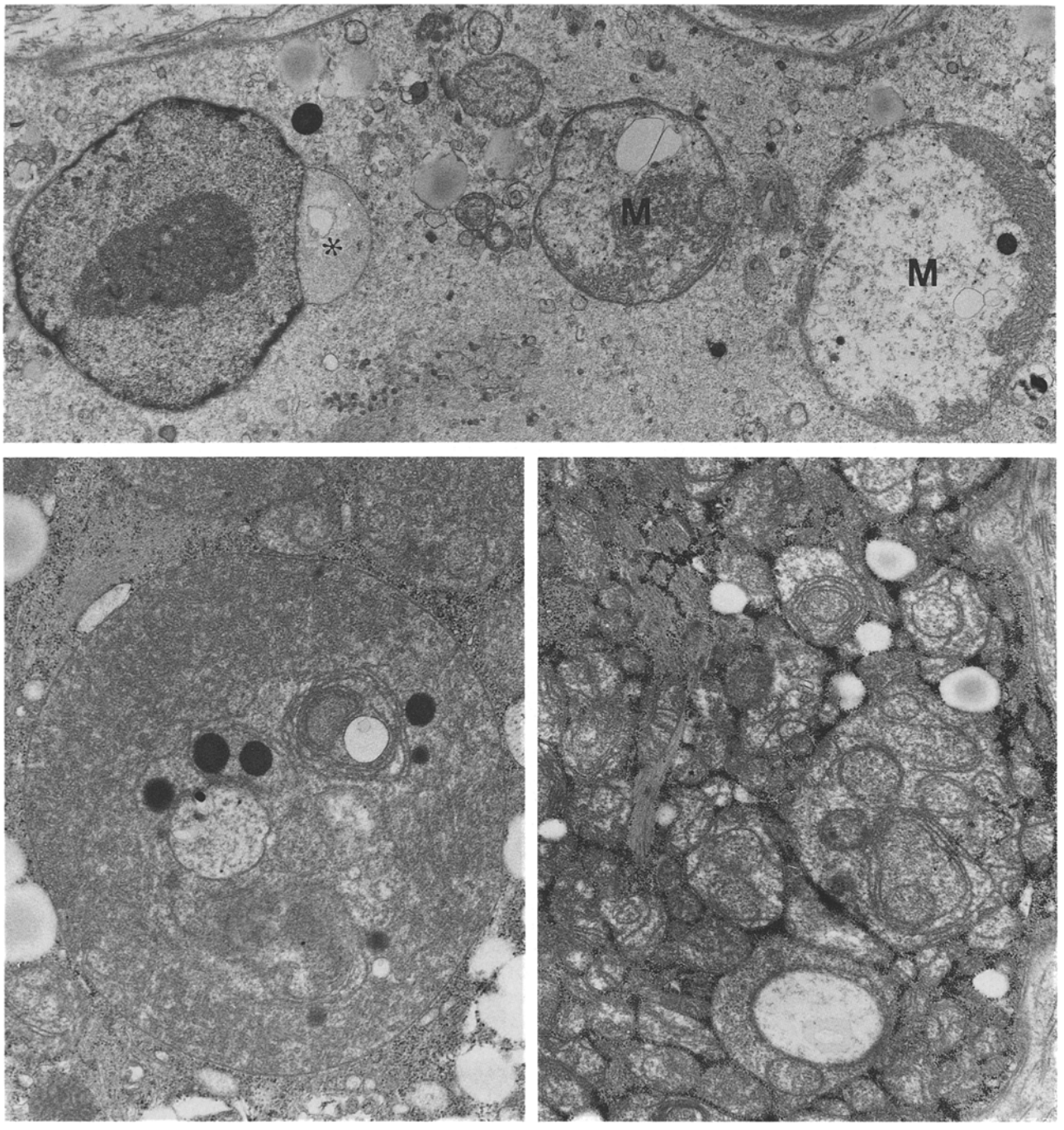

Fig. 4. **a** Considerably enlarged mitochondria (*M*) with focal clearing and vacuolization of the mitochondrial matrix. A nucleus with an enlarged nucleolus and bleb formation of the outer nuclear envelope is shown on the *left* (*asterisk*). **b** Large, osmiophilic, globoid inclusions in an enlarged mitochondrion with tubular and vesicular proliferation, and atypical orientation of cristae. **c** Subsarcolemmal accumulation of enlarged mitochondria exhibiting various abnormally arranged cristae. **a** × 8,400; **b** × 14,400; **c** × 9,400

Fig. 3a–c. Changes of the outer nuclear envelope in muscle fibers. **a** There is a common outer nuclear envelope connecting neighboring nuclei (*triangles*). Inclusions of variable size (*arrows*) occur in the distended space between the outer and the inner membrane of the interconnected nuclei. The chromatin in one of the nuclei is rather evenly distributed and the nucleolar substance segregated, whereas a compact nucleolus is seen in the *lower left corner.* **b** Focal protrusion (*arrow*) and defects (*arrowheads*) of the outer nuclear envelope. The chromatin of this nucleus appears homogeneously dispersed and the nucleolus segregated. A spherical filamentous inclusion is also seen (*triangle*). **c** Higher magnification of the bleb shown in **b** reveals inclusions of variable shape and size (50–160 nm). **a** × 11,200; **b** × 13,400; **c** × 68,900

characterized by an increase and peripheral clumping of the heterochromatin, which appeared to be separated from the nuclear envelope (Fig. 2b, c). Others showed more or less homogeneous, fine granulation of the karyoplasm (Figs. 2b; 3a, b). Several nuclei contained unusually numerous perichromatin granules, some of them being markedly enlarged up to ca. 240 nm in diameter (giant perichromatin granules), as well as other, globoid, electron-dense inclusions with diameters up to ca. 300 nm (Fig. 2d). Nucleolar changes comprised considerable enlargement in non-regenerating fibers, sometimes up to 3.7 μm (Figs. 2a, b; 4a), as well as nucleolar segregation, the dense filamentous component forming crescentic or cap-like masses covering the centrally located granular component (Fig. 2a). The nuclear envelope showed bleb-like protrusions of the outer membrane (Figs. 3b, 4a), fusion of adjacent outer nuclear envelopes (Fig. 3a), and formation of confronting cisternae (not illustrated). Some of the blebs contained several inclusions, closely apposed to and indenting the inner nuclear membrane (Fig. 3c). These inclusions were of unequal size (from about 50 nm up to 160 nm in diameter) and contained a core of electron-dense material. The inclusions between outer fused nuclear envelopes as shown in Fig. 3a appeared, in part, more electron lucent and reached diameters of more than 450 nm.

The mitochondrial changes comprised mitochondrial proliferation (Fig. 4c) and enlargement with diameters up to about 6 μm (Fig. 4b). Many mitochondria showed vacuolization or focal swelling of the matrix (Fig. 4a), others had numerous, often distorted and concentrically arranged cristae (Fig. 4b, c). The inner mitochondrial compartment sometimes contained large globoid osmiophilic inclusions (Fig. 4a, b) approaching diameters of up to 350 nm.

Endothelial cells of endomysial capillaries also showed various alterations such as swelling and vacuolization of the cytoplasm, membraneous cytoplasmic deposits, and enlargement of mitochondria. Some endothelial cells contained unusually numerous Weibel Palade bodies.

Skin bioposy. The skin specimen contained sparse perivascular lymphocytes and histiocytes, which occurred predominantly around small vessels of the papillary layer of the dermis. Secretory tubules of the eccrine glands showed ectasis as well as dyschylia and contained PAS-positive secretory granules. The epidermis was of normal appearance.

Autopsy findings

Neuropathological examination revealed disseminated micronodular encephalomyelitis and leptomeningitis without light microscopically or immunohistochemically detectable microorganisms. In addition, metastatic granulocytic focal encephalomyelitis caused by gram-positive cocci and multiple small subarachnoid hemorrhages were apparent. Focal leptomeningeal glioneuronal heterotopia was observed at the frontal cortex. Dystopic neurons were noted in the hippocampus, the thoracic cord, and in thoracic roots. The upper cervical cord and the thoracic cord exhibited slight dysplasia including hydromyelia associated with phocomelia of the right arm. Slight cerebral atrophy, few recent and older ischemic lesions, as well as discrete vacuolar leukencephalopathy of the brainstem and nearly diffuse vacuolar myelopathy were found.

The peripheral nervous system showed a moderate demyelinating sensory and motor polyneuropathy. Light microscopical findings in muscle tissue were similar to those found in the muscle biopsy described above.

Discussion

The muscle biopsy taken 15 months after onset of treatment with high daily doses of 1,000 mg in a 32-year-old male patient with AIDS revealed severe necrotizing myopathy combined with neurogenic muscle atrophy. In addition to well-known mitochondrial changes, there were unusual nuclear alterations in degenerating as well as in non-necrotic and non-regenerating fibers. Similar changes have, to our knowledge, not been reported previously in AIDS with or without zidovudine-induced myopathy.

All of the light microscopic changes seen in the present patient's muscle biopsy have, among others, been previously reported in both, non-zidovudine-treated patients with AIDS [13, 23, 26, 30, 44, 45, 60, 69, 72], and zidovudine-treated patients [13, 41]. Also neurogenic atrophy is commonly seen in patients with AIDS, since peripheral neuropathy occurs as the most common neuromuscular disorder accompanying HIV infection [36, 56].

Mitochondrial alterations in AIDS patients can well be attributed to toxic side effects of zidovudine treatment (see introduction). The mitochondrial changes observed in our patient resembled, in general, those documented by Panegyres et al. [45] and Dalakas et al. [13].

Accumulations of lipid droplets and glycogen as observed in the present patient's muscle biopsy are nonspecific findings which, however, may also be related to zidovudine, since they have been reported only in treated, but not in non-treated patients [45, 69].

Several nuclear changes in AIDS-related myopathy have been described [45] in non-treated patients, but not in those, who had received zidovudine. However, the changes reported, although not illustrated by these authors (nucleolar enlargement, peripheral clumping of chromatin, separation of peripheral chromatin from the surrounding envelope, coalescence of nuclear membranes between dark, shrunken nuclei), comprise only part of the alterations observed in the present patient's muscle biopsy. Some of them, in contrast to those in our case, occurred only in regenerating fibers. Additional changes that we observed have been described elsewhere in the literature (see below) but in conditions that do not apply to the present patient. Thus, zidovudine-

related nuclear changes have to be taken into consideration.

Although in vitro studies using isolated enzymes and substrates revealed that alpha-polymerase, occurring in nuclei only, is rather insensitive to zidovudine in therapeutical concentrations ($IC_{50} > 200$ μM) [22, 39], several investigations using cell cultures, e.g., of human lymphocytes or myeloid and erythroid precursor cells, showed significant incorporation of zidovudine into nuclear DNA during replication leading to reduction of DNA synthesis and reduced proliferative activity in a dose-dependent manner ([1, 2, 57, 74, 75]; $IC_{50} = 4$ μM). Also DNA repair is impaired by zidovudine [42, 50], due to inhibition of the more sensitive nuclear beta-polymerase [22, 39, 46, 59]. Therefore, some of the nuclear changes may be caused by inhibition of alpha- and beta-polymerase due to zidovudine, at least in regenerating fibers during the S-phase.

Nuclear DNA metabolism is also impaired in postmitotic and G_0 cells by intracellularily enriched [1] zidovudine due to (a) inhibition of thymidylate kinase [2, 22], which leads to depletion of nucleotid triphosphate pools [6, 58], including ATP as a long-term effect [1], and to reduced induction of cytidine diphosphate reductase [64], and (b) inhibited transcription of special genes [67].

Inhibition of transcription morphologically can lead to nucleolar segregation [27, 48], which in our case could be an effect of zidovudine treatment. Panegyres et al. [45] did not note nucleolar segregation among the nuclear changes within the non-treated patients. Nucleolar segregation, on the other hand, has been reported in several infectious diseases (HSV [55, 61]; mycoplasma [34]; Coxsackie virus [68]), none of which were apparent at the time when the muscle biopsy from the present patient was taken.

In addition, the increase in number and size of perichromatin granules, especially the occurrence of giant perichromatin granules, obviously represent a feature of metabolically depressed cells [27] and, therefore, may also be induced by zidovudine in our case. Other globoid intranuclear inclusions similar to those shown in Fig. 2d, which unlike perichromatin granules are not surrounded by an electron-lucent halo, have been reported in some cases of dermatomyositis [3, 9], polymyositis [17, 63], and in human epidermis during keratinization [66]. Their significance is not understood in muscle cell nuclei, but since their number was found to be increased in epidermis cells after treatment with actinomycin-D and puromycin [21], they may also be a sign of inhibited RNA and protein synthesis.

Fusion of outer nuclear membranes of neighboring nuclei, previously reported as a rare finding in myotonic dystrophy [51] and virus-induced proliferation of the nuclear envelope [27], has been described by Panegyres et al. [46] "between dark, shrunken nuclei", obviously within atrophic fibers. We suppose that in our case this fusion is the consequence of confronting bleb formation of adjacent nuclei. Blebs formed solely by the outer membrane as we observed have been reported to occur in undifferentiated or fetal, differentiating cells [16, 71] and, thus, may be a feature of normal development. In adult normal or alterated human muscle fibers, such large bleb formations as in the present case have, to our knowledge, not been documented before. Therefore, we suggest that this change might also be caused by zidovudine.

The blebs and the electron-lucent spaces between adjacent nuclei that were enclosed by a common outer nuclear membrane, repeatedly contained inclusions, which were reminiscent of HIV particles. Although numerous cases of human viral myositis have been reported, very few attempts using different techniques to demonstrate viral antigen within myofibers have been successful [25, 43, 70]. The inclusions observed in our case were to a certain extent unlike virus particles (a) because of their localization and (b) since they varied more in shape and size (50 to 450 nm) than it is known from HIV particles (100–140 nm [35]). Whether they represent altered virus particles or transverse sections through finger-like protrusions of the inner envelope into the bleb formations remains unsettled.

Since myopathies have been reported to occur following a variety of drugs (e.g. [11, 32, 38, 62, 65]), the toxic effects of the other drugs administered to our patient for about 15 months (acyclovir, co-trimoxazol) must be discussed. However, no myopathic and especially no nuclear changes have, to our knowledge, been reported after application of one of these drugs. Furthermore, studies on a combined therapy with zidovudine and acyclovir have revealed no significant interactions between these two drugs [15, 18].

In conclusion, although all nuclear changes we observed in the case presented here have previously been demonstrated in a variety of different conditions, the present case is the first one, in which various nuclear changes occurred in the muscle biopsy of a HIV-infected patient receiving high-dose, long-term zidovudine treatment and in which the other conditions were not present. In accordance with several cell culture studies, zidovudine-related toxic effects should be taken into account in causing impairment of mitochondrial and nuclear DNA/RNA metabolism, leading to mitochondrial and nuclear alterations as a morphological substrate of zidovudine myopathy in AIDS.

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Note added in proof. Partial cytochrome c oxidase deficiency in patients with zidovudine deficiency has recently been reported by Chariot and Gherardi (1991; Neuromusc Dis 1:357–363). This extends present and previous fine structural observations on muscle mitochondria.