62 Trisomy 16 Mouse Model of Alzheimer's Disease

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Aging individuals with Down's syndrome (DS) develop the neuropathologic hallmarks of Alzheimer's disease (AD) and a large proportion display evidence of decreasing cognitive abilities (1-3). In addition, other lines of research suggest an association between DS and AD: extra copies of 21st chromosome genetic material are found in nonfamilial AD (4); genetic polymorphisms in genes coded on the 21st chromosome have been found in families with familial AD (5); the gene for β-amyloid, one of the abnormally stored materials in AD, has been mapped to the 21st chromosome (6). Cerebral cortical dendritic abnormalities similar to those found in DS have been described in AD (7-9). These morphologic changes may be due to underlying abnormalities in neuronal microtubules (10). Furthermore, cytoskeletal abnormalities are common to both DS and AD. A microtubule-dependent event, lymphocyte capping in response to concanavalin A, has been shown to be defective in both DS and AD (11). Similar findings have been made in cultured DS and AD fibroblasts (12). These DS and AD cytoskeletal related findings may be due to abnormal phosphorylation, suggestive of a defect in post-translation modification (13). The highly phosphorylated 210-kDa neurofilament subunit is redistributed to aberrant locations in AD (13) and is precociously expressed in DS (14). Nonphosphorylated neurofilament proteins are markers for vulnerable cortical neurons in AD (15). The Alz-50 antigen which is specific for AD (16) has kinase activity and may be involved in abnormal cytoskeletal phosphorylation (17). In AD protein kinase C dependent phosphorylation is abnormal (18). Early stages of AD are typified by the loss of neurofilament-rich axonal systems (19).

The proposed cytoskeletal abnormalities in DS may be on the basis of the enzymes coded on the 21st chromosome and this may be related to the interferon α and β receptors which are coded by this chromosome (20). In DS, cellular responsiveness to interferon is exaggerated such that a given dose of interferon elicits not a 1.5-fold antiviral response but a 3- to 15-fold response (21). In the initiation of the antiviral state, interferon treatment decreases the rates of cell mitosis, locomotion, membrane ruffling and saltatory movements of intracellular granules (22,23). Fibroblasts treated with interferon contain three times the number of actin fibers when compared to untreated cells (23). Interferon treatment of normal cells produces defective

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lymphocyte capping following concanavalin A administration (23)—results which are similar to lymphocyte capping abnormalities in DS and AD (11). Interferon is present in the cerebrospinal fluid (24). In monkeys the CNS can produce interferon-dependent RNA following intrathecal administration of interferon (25). Neurons in vivo and in culture are sensitive to interferon, suggesting the possibility of functional interferon receptors in the CNS (26). In DS neuronal cytoskeletal changes may be due to enhanced responsiveness to interferon.

Murine trisomy 16 is an excellent model for DS (20,27). The human 21st chromosome and the mouse 16th chromosome both code for the free radical scavenging enzyme superoxide dismutase-1 (SOD-1), the purine biosynthetic enzyme phosphoribosylglycinamide synthetase (PRGS), the proto-oncogene ETS-2, interferon α and β receptors, and amyloid precursor protein (28). Phenotypic features of human DS and murine trisomy 16 are also similar including flat facies, shortened neck, ear abnormalities, congenital heart disease (endocardial cushion defects and aortic arch abnormalities), fetal edema, thymic hypoplasia and decreased T-lymphocyte and antibody responsiveness. Both conditions have high rates of fetal wastage. The electrical membrane properties of cultured dorsal root ganglion neurons from DS and trisomy 16 are similar (29,30). Brain development is likewise similar with decreased brain size, reduced neuronal numbers and decreased brain levels of catecholaminergic, cholinergic and serotonergic markers (20,31-33).

ELECTRON MICROSCOPIC OBSERVATIONS

Cytoskeletal EM observations in trisomy 16 revealed microtubular profiles which were more curved and coiled than in normals (34). The apparent numerical density and spacing of microtubules did not differ between normal and trisomy 16 mice as has been reported in AD (10). Paired helical filaments, one of the pathologic hallmarks of AD, were not observed in the trisomy 16 material. The ultrastructural microtubular differences in trisomy 16 may be related to reported cytoskeletal abnormalities in AD.

The EM also revealed increased cellular membrane fragility in trisomy 16 CNS neurons (34). During the processes of fixation, embedding and sectioning, trisomic neuronal membranes became fragmented and lost cell-to-cell apposition. CNS lipid abnormalities have been reported in DS. In particular, phosphatidylethanolamine content is decreased and the cholesterol/phospholipid ratio is increased (35,36). Significantly, in AD CNS neuronal membrane abnormalities have likewise been noted, including abnormalities in gangliosides (37-40). Possibly similar membrane lipid abnormalities occur in trisomy 16 which may account for the observed ultrastructural membrane fragility. Alternatively, anomalous expression of amyloid precursor protein (APP) may account for these membrane findings. In normal rodents, APP is widely expressed throughout the CNS as a cell surface receptor (41). APP is coded on the mouse 16th chromosome and may be anomalously expressed in trisomy 16. APP may play an important role in cell membrane integrity and in maintaining cell-to-cell contact (41,42).

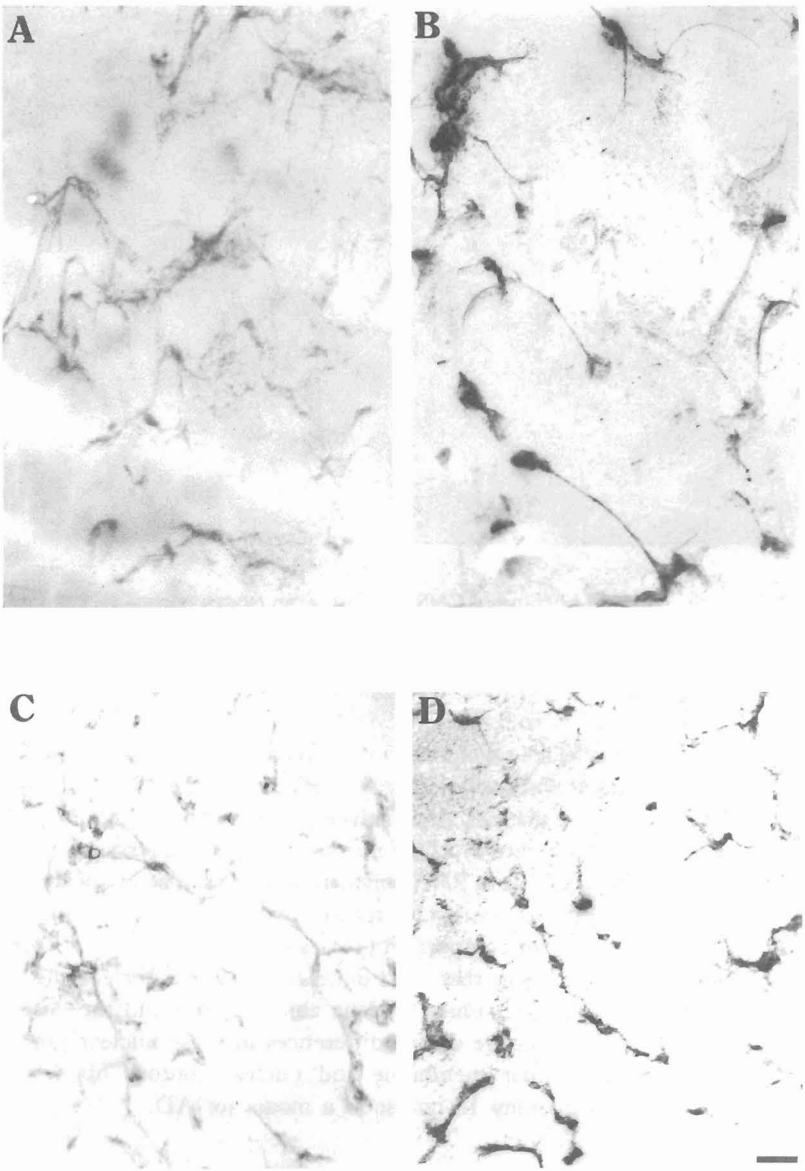

Figure 1. Photomicrographs of cultured CNS neurons taken from normal E17 mice (A and C) and trisomy 16 littermates (B and D), immunoperoxidase stained with a monoclonal antibody directed against the 210-kDa neurofilament subunit (mabN210, Dr Hawkes; A and B) and the 68-kDa neurofilament subunit (Boehringer Mannheim; C and D). There is greater 210-kDa subunit immunoreactivity in the trisomic culture (B) than in the normal culture (A), a result which confirms previously published results (32). There is likewise greater 68-kDa subunit immunoreactivity in trisomic cultures (D) as compared to controls (C). Scale bar 50 μm

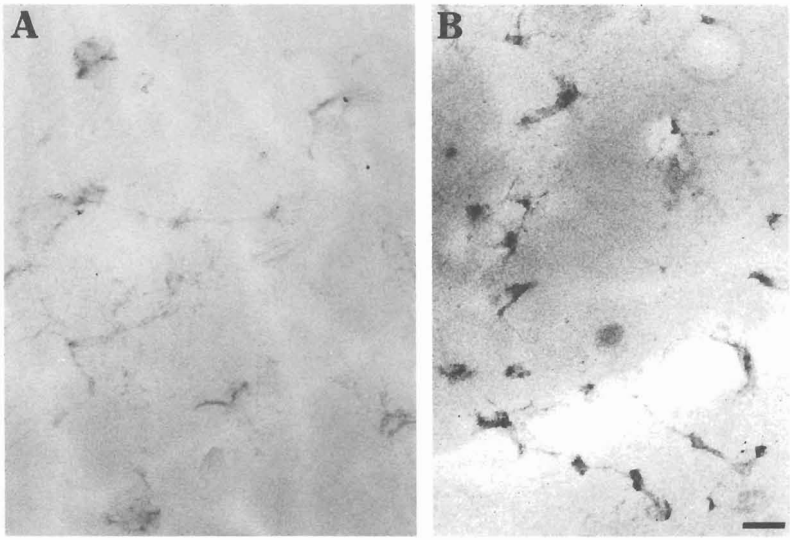

Figure 2. Photomicrographs of cultured CNS neurons taken from a normal E17 mouse (A) and a trisomy 16 littermate (B), immunoperoxidase stained with sera directed against microtubule-associated tau proteins (Sigma). There is greater immunoreactivity in the trisomic culture (B) than in the normal one (A). Scale bar 50 μm

Trisomy 16 neuronal nuclei are smaller and more irregular in size than normals (34). The trisomy 16 CNS nuclear observations may be a reflection of the additionally stored chromosomal material in each nucleus. Alternatively, abnormalities in chromatin and nuclear histones have been described in AD. For eukaryotic gene expression it is necessary for the genome to be accessible to RNA polymerase systems. The H1 histones can condense DNA and make it inaccessible for transcription (43). CNS chromatin in AD is in a much higher state of compaction (44), is less accessible to the enzyme micrococcal nuclease (45) and has increased H1 linker histones on dinucleosomes (46). Possibly, trisomy 16 nuclei have similar histone abnormalities and the observed nuclear morphologic differences are due to differences in these nuclear proteins.

The EM cytoskeletal, cellular membrane and nuclear contour observations strengthen the role of the trisomy 16 mouse as a model for AD.

CENTRAL NERVOUS SYSTEM NEURONAL CULTURES: EFFECTS OF INTERFERON

When a mixture of α and β mouse interferon was applied to normal cultured neurons, there was an increase in the immunohistochemical staining intensity of the 210-kDa neurofilament subunit in neuronal cell bodies (47). Significantly, there was a difference between untreated normal and trisomic CNS neurons: there was more 210-kDa neurofilament immunohistochemical expression in the trisomic cultures. When an inhibitor of the interferon mediated antiviral state, oxyphenbutazone,

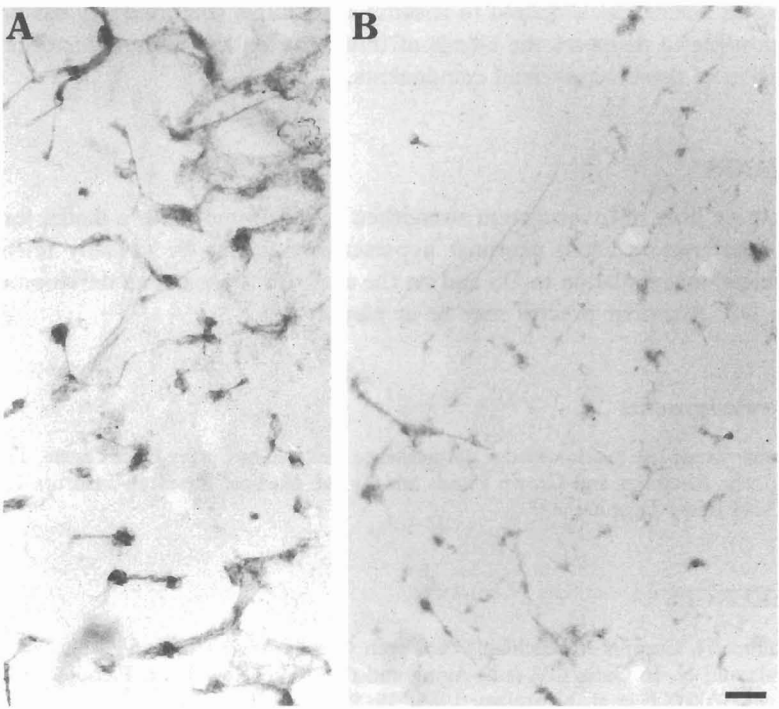

Figure 3. Photomicrographs of cultured CNS neurons taken from a normal E17 mouse (A) and a trisomy 16 littermate (B), immunoperoxidase stained with sera directed against tubulin (Sigma). There is greater immunoreactivity in the normal culture (A) than in the trisomic one (B). Scale bar 50 μm

without the presence of interferon, there was a significant reduction in the intensity of neurofilament immunohistochemical expression. It is postulated that the observed effects on trisomic neurons were due to the presence of interferon which is endogenously produced within the culture by concurrently growing fibroblasts.

These results have demonstrated that interferon has a regulatory effect on neuronal neurofilament expression. Also, a difference has been shown in neurofilament immunohistochemical expression between normal and trisomy 16 cultured CNS neurons. Finally, and possibly of greater importance, an interferon inhibitor has been shown to normalize trisomic CNS neurofilament expression.

These tissue culture investigations have been extended to other cytoskeletal components. As with the 210-kDa neurofilament subunit, the 68-kDa subunit has more intense immunohistochemical expression in trisomy 16 than in normals (Figure 1). A similar result was found with the immunohistochemical expression of the microtubule-associated tau proteins (Figure 2) (48). It should be noted that in AD tau proteins are abnormally distributed (49,50). Sera directed against the main constituent of microtubules, tubulin, produced the opposite effect with increased

staining in normal as compared to trisomy 16 cultures (Figure 3). It has not yet been possible to delineate the effects of interferon on the immunohistochemical expression of these cytoskeletal components.

SUMMARY

All of these lines of investigation strengthen the T-16 mouse as a model for AD. Also, interferon-mediated neuronal hypersensitivity may be causally related to cytoskeletal misregulation in DS and set the stage for the eventual development of AD in DS. A similar process may be at play in AD.

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