Down's syndrome

Precocious neurofilament antigen expression

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SUMMARY

Neuronal cytoskeletal abnormalities may be a common factor in the neurobiologic causes of diverse forms of mental deficiency including Down's syndrome (DS). MabN210 which recognizes the 210 kDa subunit of neurofilaments was applied to sections of autopsy-derived DS and control central nervous system tissue. The findings included precocious and possibly aberrant neurofilament antigen expression during the first few months of life in DS cerebellar basket cell axons. Staining of central white matter tracts revealed an increased caliber of immunoreactive axons suggesting a widespread abnormality in mabN210 antigen expression in DS neurons. This abnormal regulation of normal neurofilament antigenic epitopes may be causally related to the development of Alzheimer's disease in DS.

Key words: Down's syndrome; Monoclonal antibodies; Neurofilaments

INTRODUCTION

Down's syndrome (DS) is the most common identified cause of mental retardation with an incidence of 1.5 per 1000 births (Menkes 1979). Even with the trisomic 21st chromosome as a marker, it is not at all clearly understood how this additional genetic material causes neurologic impairment (Coyle et al. 1986).
Most cells, including neurons, contain three major polymeric protein systems: microtubules, intermediate filaments, and microfilaments, as well as interconnections that link these systems together. These cytoskeletal components play important roles in determining and maintaining cell shape, allowing cell motility and moving organelles and molecules within the cytoplasm and axoplasm. They may also be involved at the synaptic level in the process of learning (reviewed by Goldman and Yen 1986; Lazarides 1980; Lynch and Baudry 1984). Abnormalities in the neuronal cytoskeleton may be factors in the neurobiologic cause of the mental deficiency seen in DS.

Using autopsy-derived tissue and Golgi impregnation techniques, DS cerebral cortical neurons have been shown to have abnormalities in dendritic arborization and in dendritic spine shape and distribution (Marin-Padilla 1972, 1976; Suetsugu and Meharaein 1980; Takashima et al. 1981). Similar findings have been reported in other chromosomal abnormalities (Patau syndrome; Marin-Padilla 1972, 1974) and in mental retardation of unknown etiology or associated with infantile spasms (Huttenlocher 1974; Purpura 1974). These results suggest an underlying cytoskeletal abnormality in conditions associated with mental retardation, including DS. This possibility has been further substantiated in an EM study of cerebral cortical tissue from five children with unspecified mental deficiency. Neuronal microtubules were found to be in disarray (Bodick et al. 1982; Purpura et al. 1982). Recent human neuropathologic results have shown a decreased number of cortical neurons in DS (Ross et al. 1984; Wisniewski et al. 1984). These results in conjunction with those from the murine trisomy 16 model of DS (Blue et al. 1984; Singer et al. 1984) suggest slowed neurogenesis which may be on the basis of abnormal cytoskeletal interactions.

Another line of evidence strongly suggests an underlying cytoskeletal abnormality in DS: the association of DS with Alzheimer's disease. DS individuals with aging develop dementia and the neuropathologic findings of Alzheimer's disease (Malamud 1972; Wisniewski et al. 1985). Alzheimer's disease is characterized by the accumulation of paired helical filaments which share antigenic determinants with microtubules and neurofilaments (reviewed by Goldman et al. 1986; Price et al. 1986). It is tempting to speculate that DS neuronal cytoskeletal components or their expression are intrinsically different from normals, thus predisposing DS individuals to the development of Alzheimer's disease.

DS has not been previously investigated specifically with an eye to cytoskeletal abnormalities. An anti-neurofilament monoclonal antibody (mab) was applied to DS and control autopsy-derived human central nervous system (CNS) tissue. Neurofilaments are composed of three subunits of different molecular weights: 68 kDa (K), 150 K and 210 K (Liem et al. 1978). The mab used was mabN210, which recognizes the 210 K subunit (Leclerc et al. 1985; Plioplys et al. 1986).

MATERIALS AND METHODS

The production and characterization of mabN210 has been described previously (Leclerc et al. 1985). Central nervous system (CNS) specimens were obtained at the time of autopsy from six neurologically normal adults. Autopsies were performed within...
24 h of the time of death. Routine neuropathologic investigations, both grossly and microscopically, did not reveal any other findings. At the time of autopsy, CNS samples were placed in ice-cold 4% paraformaldehyde in phosphate buffer (0.1 M phosphate buffer, pH 7.4) for a period of 24 h. Tissue was stored subsequently in phosphate-buffered saline (PBS) containing $10^{-5}$ M sodium azide.

CNS specimens were obtained from two children with DS, a female of 5 months and a male of 9 months, and two controls, a male of 6 months and a female of 8 months. The causes of death were a motor vehicle accident and presumed aspiration in the controls, and severe viral pneumonitis (RSV) complicating progressive cardiac failure due to AV canals in the DS cases. Autopsies were performed 18 and 23 h after death in the DS cases, and 20 and 27 h in the controls. The entire brains were fixed by immersion in 10% formalin. After periods of fixation of 2 and 5 months in the DS cases, and 3 and 4 months in the controls, CNS specimens were taken and rinsed extensively in PBS and stored in PBS containing $10^{-5}$ M sodium azide. Neurofilament antigen expression survives prolonged formalin fixation (Majocha 1985; Plioplys, unpublished observations).

Tissue sections were cut coronally at 75 µm using a freezing stage microtome. To detect specific immunoreactivity, sections were incubated overnight in the monoclonal antibody. In all examples shown here, mabN210 was used diluted 1:8 into 10% normal horse serum (NHS) in PBS. To detect specific antibody binding, sections were incubated for 2 h in rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase diluted 1:100 in 10% NHS (Dako Inc.). Antibody binding was revealed using 0.5 mg/ml 4-chloro-1-naphthol--0.1% (v/v) hydrogen peroxide (Hawkes et al. 1982). The sections were washed for 15 min each of two changes of PBS between the incubations. Sections in which the primary antibody was omitted gave no staining.

Brain samples were taken from the above described normal adults at the time of autopsy and frozen at $-80^\circ$C until used. This tissue was used to prepare Western blots using standard techniques (Towbin et al. 1979). Homogenates of frontal cortex, caudate, cerebellum and central white matter were homogenized and boiled for 2 min in 2.5% sodium dodecyl sulfate (SDS; w/v), 7% 2-mercaptoethanol (v/v) in TBS (50 mM Tris-HCl, 200 mM NaCl, pH 7.4) and the proteins separated as a curtain by polyacrylamide gel electrophoresis (PAGE) through a 5–17% acrylamide gradient. The gel loading was 10 µg protein/mm track width. To detect specific antibody binding 3 mm wide strips from the blot were first incubated 30 min in 10% NHS in PBS to block non-specific binding sites, then overnight in mabN210. After two 15 min washes in PBS, the blot strips were incubated for 2 h in horseradish peroxidase conjugated rabbit anti-mouse IgG diluted 1:100 in 10% NHS. Antibody binding was detected by washing the blot twice for 15 min in PBS and then for 15 min in 0.5 mg/ml 4-chloro-1-naphthol--0.01% (v/v) hydrogen peroxide. The apparent molecular weights of antigenic polypeptide bands in kDa (K) were estimated from prestained molecular weight standards (B.R.L. Inc.) which were blotted concomitantly. Control blots in which the serum sample was replaced by 10% NHS revealed no bands. MabN210 always recognized a solitary immunoreactive band at 210 K (not illustrated).
Fig. 1. Coronal sections of autopsy-derived normal, adult, human cerebellar vermis immunoperoxidase stained with mabN210. A, B and C were taken from three different cases. Purkinje cell bodies are surrounded by an exuberance of neurofilament rich basket cell axons. Purkinje cell bodies themselves are not stained, several of which are indicated by arrowheads. The scale bar indicates 50 μm.
The most impressive results concerned cerebellar basket cell neurofilament expression. In six adults cerebellar basket cell axonal staining with mabN210 is essentially identical (three cases are illustrated in Fig. 1). This indicates that death-related events and formalin fixation do not produce significant inter-individual differences in mabN210-immunoreactivity.

There was a significant difference in cerebellar basket cell neurofilament expression when the DS cases were compared to the controls (Fig. 2). The DS basket cell axons, which ramify around Purkinje cell bodies, have much greater neurofilament expression than the controls. This difference is not explained by premorbid events and delay in time from death to autopsy. Similar results were obtained in the tissue sections taken from the cerebellar vermis and cerebellar hemispheres. In rodent species cerebellar development and neurofilament expression is largely a post-natal event (Gravel et al. 1985; Leclerc et al. 1985). The control results (Fig. 2) are similar in appearance to the

![Figure 2](image)

Fig. 2. Coronal sections of autopsy-derived, human cerebellar vermis immunoperoxidase stained with mabN210. A and C are from two cases with DS of ages 5 and 9 months. B and D are from two controls of ages 6 and 8 months. In A and C the Purkinje cell bodies (arrowheads) are surrounded by an exuberance of neurofilament rich basket cell axons. In B and D the Purkinje cells are outlined by relatively little neurofilament staining. The scale bar indicates 50 μm.
expression of phosphorylated neurofilament epitopes in young post-natal rodents (Leclerc et al. 1985; Marc et al. 1986). This suggests that in DS cerebellar basket cell axons, neurofilaments are precociously expressed in a pattern more similar to the adult (Fig. 1).

These observations were extended to other CNS axonal systems. Within the corpus callosum and internal capsule, stained axons appear to have a larger caliber and stain more intensely in DS than in controls (Fig. 3). Similar findings were made in the dorsal columns and lateral corticospinal tract in the cervical spinal cord (Fig. 4) and in cerebellar white matter tracts (not illustrated). The noted difference in caliber of stained axons has not yet been quantitated.

Fig. 3. Coronal sections of autopsy-derived, human corpus callosum (A and B) and internal capsule (C and D) immunoperoxidase stained with mabN210. A and C are from a case with DS, B and D are from a control. In A and C the stained axons appear to have a larger caliber than in B and D. The scale bar indicates 50 μm.
DISCUSSION

The results suggest that there is a precocious and possibly aberrant expression of the 210 K neurofilament subunit in individuals with DS. These results should be interpreted with caution. The number of cases is small – only two DS cases and two controls for comparison. Also, even though there were no significant differences in death-related events and time from death to autopsy, there was a difference in premorbid medical events. Both DS cases had progressive cardiac failure secondary to AV canals requiring cardiotropic medications. The effects of chronic heart failure upon neurofilament maturation have not been reported.
The results are extremely intriguing with the following implications:

1) precocious and possibly aberrant neurofilament expression is present in axons of DS individuals within the first few months of life;

2) this anomalous neurofilament expression may be associated with the eventual development of Alzheimer’s disease in DS individuals;

3) Alzheimer’s disease itself may be a congenital disease in which aberrant cytoskeletal components may be present early in life predating by decades the symptomatic phase of the illness;

4) anomalous neurofilament expression may have implications for cytoskeletal integrity and axoplasmic transport, possibly in part accounting for the neuronal malfunction in DS.

Recently it has been shown that microtubule assembly is abnormal in CNS tissue taken shortly after death from patients with Alzheimer’s disease (Iqbal et al. 1986). Our results concerning neurofilament expression in DS suggest a similar implication: aberrant expression of normal cytoskeletal components may be a predisposing factor in the development of Alzheimer’s disease.

Cytoskeletal abnormalities in DS may be on the basis of the enzymes coded on the 21st chromosome. This chromosome codes for SOD-1 and interferon alpha and beta receptor (Epstein et al. 1985). In DS, cellular responsiveness to interferon is exaggerated such that a given dose of interferon elicits not a 1.5-fold response but a 3–8-fold response (Epstein and Epstein 1980). In the initiation of the antiviral state, interferon treatment markedly decreases the rates of cell mitosis and locomotion as well as membrane ruffling and staltatory movements of intracellular granules (Pfeffer et al. 1979, 1980). Interferon-treated fibroblasts contain three times the number of actin fibers when compared to untreated cells. Calculated on the basis of numbers of actin fibers per unit of surface area the increase is 82% (Pfeffer et al. 1980). In interferon-treated cells fibronectin distributes in arrays of long filaments covering most portions of the cell surface (Pfeffer et al. 1980). Fetal and newborn human fibroblasts and mononuclear cells can produce adult levels of virus-induced interferon (Cantell et al. 1968; Carter et al. 1971) and interferon is present in the cerebrospinal fluid (Gresser et al. 1964). Thus cytoskeletal changes may be due to enhanced responsiveness to interferon in DS.

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REFERENCES


