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DOWN'S SYNDROME
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Down's Syndrome and Cytoskeletal Abnormalities

Despite the prevalence of Down's syndrome (DS), 1.5 per 1,000 births [23] and high societal costs involved, the basic biologic cause of the mental deficiency seen in this condition is poorly understood [6]. It is only through an understanding of the basic cause of intellectual impairment in Down's syndrome that potential therapeutic interventions might be developed.

The published literature suggests that in Down's syndrome the neuronal cytoskeleton may be abnormal. A mechanism by which the triplicated chromosomal material in Down's syndrome might cause cytoskeletal abnormalities will be discussed. Should these hypotheses be correct, then pharmacologic manipulations normalizing cytoskeletal expression in Down's syndrome might be possible.

Cytoskeletal components in neurons are composed of three families of polymeric proteins: intermediate filaments (as neurofilaments), microtubles and microfilaments. These structural proteins are involved in elaboration and maintenance of axonal and dendritic ramifications, movement of intracellular molecules and organelles, and at the synaptic level may be involved in the process of learning [11,16,18]. Any abnormality of cytoskeletal components might lead to widespread neuronal malfunction and clinically to mental impairment.

Abnormalities in cytoskeletal expression may be common to a number of conditions associated with mental deficiency. For example, in hypothyroid experimental animals microtubule assembly is defective [10], there is a reduced number of microtubular profiles seen with electron microscopy [9], and neurofilament-antigen expression is delayed in selected axonal systems [26]. In phenylketonuria, a disorder of phenylalanine metabolism, there is an 8-fold increase in the concentration of phenylalanine at the carboxyl terminal of tubulin, a component of microtubules, suggesting that neuronal impairment may be on the basis of defective microtubules [33]. Also, in an electron microscopic study of cerebral cortical tissue taken from individuals with mental impairment microtubular disarray was noted [3,31].

In Down's syndrome, cerebral cortical tissue, when stained with Golgi techniques, has revealed abnormalities in dendritic arborization and in dendritic spine shape and distribution [21, 22, 36, 39]. Dendritic atrophy has also been noted in Down's syndrome cortical neurons during postnatal development [1]. These morphologic results suggest that there may be an abnomal underlying cytoskeleton. Quantitative neuropathologic studies have shown a decreased number of cortical neurons [34,41]. These results suggest slowed neurogenesis which maybe on the basis of defective cytoskeletal interactions during neuronal mitosis and migration.

Possibly the strongest line of evidence implicating cytoskeletal abnormalities in Down's syndrome is the universal appearance of the neuropathologic findings of Alzheimer's disease in elderly Down's syndrome individuals [20, 40]. Alzheimer's disease is typified by the accumulation of abnormal fibrillary material which shares antigenic determinants with neurofilaments and microtubules [11, 30]. It is tempting to speculate that in Down's syndrome the neuronal cytoskeleton might be regulated in a fashion different from normal, thus predisposing to the eventual development of Alzheimer's disease.

To investigate the possibility that neuronal cytoskeletal components might be regulated in a fashion different from normal, the author applied a monoclonal antibody (mab N210), which recognizes the 210 Kdalton neurofilament subunit [17], to autopsy-derived, formalin-fixed Down's syndrome and normal brain tissue sections. The specimens were obtained from individuals who died during the first few months of life. The results suggested precocious neurofilament antigen expression in Down's syndrome, early in life [27]. Also mab N210-staining profiles within axons seemed to have a larger caliber in Down's syndrome as compared to normals. These results suggested that indeed there is a difference in neurofilament antigen expression in Down's syndrome as compared to normals.

Microtubule assembly has been shown to be abnormal in brain tissue taken shortly after death from patients with Alzheimer's disease [15]. The neurofilament antigen expression results in Down's syndrome suggest a similar implication: aberrant regulation of normal cytoskeletal components may be predisposing factor in the development of Alzheimer's disease.

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Michael Grimaldi, exploring with joy, age one year (trisomy 21)

There is always a difficulty in using human autopsy-derived brain tissue for scientific investigation: death related events cannot be controlled for. This problem is particularly applicable to the above cited study where the Down's syndrome individuals died from complications related to heart failure, and the controls from entirely different causes. It is conceivable that the observed findings were due to the effects of either chronic hypoxia or cardiotropic medications. A different avenue to further these investigations is necessary.

Fortunately, there is a mouse model which is extremely analogous to trisomy 21: mouse trisomy 16 [7, 13, 32]. The human 21st chromosome and the mouse 16th chromosome both code for superoxide dismutase 1 (SOD-1), phosphoribosylglycinamide synthetase (PRGS) and interferon alpha and beta receptor. In both cases it is the distal part of the chromosome that codes for SOD-1 and PRGS. Phenotypic features of human Down's syndrome and murine trisomy 16 are also similar including flat facies, shortened neck, congenital heart disease (endocardial cushion defects and aortic arch abnormalities), thymic hypoplasia and decreased T-lymphocyte and antibody responsiveness. Both conditions have high rates of fetal wastage. Brain development is likewise similar with decreased brain size and reduced neuronal numbers [2, 7, 34, 37, 41].

In our laboratory, we have been breeding the trisomy 16 mouse and have been able to obtain intriguing results. Neurons taken from dorsal root ganglia (DRG) when grown in tissue culture and then reacted with mab N210 revealed that there is significantly greater mab N210-immunoreactivity in trisomy 16 DRG cell bodies than in normals [19]. These results are similar to those of neurofilament expression in human Down's syndrome [27].

If these results suggesting differences in neurofilament antigen expression in Down's syndrome and mouse trisomy 16 are indeed correct observations, then how may one explain their occurrence? The answer may lie in the genes coded on the human 21st and mouse 16th chromosomes.

The human 21st chromosome codes for SOD-1 and interferon alpha and beta receptors [7]. In Down's syndrome, cellular responsiveness to interferon is exaggerated such that a given dose of interferon elicits not a 1.5-fold response, but a 3 to 8-fold response. [6]. In the initiation of the antiviral state, interferon decreases the rates of cell mitosis, locomotion, membrane ruffling and staltatory movements of intracellular granules [24, 25]. Fibroblasts treated with interferon contain three times the number of actin fibers when compared to untreated cells. Calculated on the basis of number of action fibers per unit of surface area the increase is 82% [25]. In interferon treated cells fibronectin distributes in arrays of long filaments covering most portions of the cell surface [25]. Fetal and newborn human fibroblasts and mononuclear cells can produce adult levels of virus-induced interferon [4, 5] and interferon is present in the cerebrospinal fluid [12]. Thus Down's syndrome cytoskeletal changes may be due to enhanced responsiveness to interferon.

To investigate whether cytoskeletal components in neurons are sensitive to interferon, DRG neurons from normal adult and fetal mice, grown in tissue culture, were exposed to interferon [19]. With interferon treatment there was a significant increase in the incidence of DRG cell body staining with mab N210. The same effect took place when interferon was applied to trisomy 16 DRG cultures. These results support the hypothesis that enhanced interferon sensitivity in Down's syndrome might produce neuronal cytoskeletal changes which in turn might adversely affect neuronal growth and development, neuronal functioning, and may set the stage for the eventual development of Alzheimer's disease.

These sorts of basic neurobiologic observations are of interest, but are there any possible therapeutic implications? Most intriguingly, there may be.

If the interferon-mediated neuronal cytoskeletal sensitivity causes neuronal impairment in Down's syndrome, then appropriate investigatory medications would be ones that block interferon's effects.

SOD-1 plays an important role in establishing the interferon mediated antiviral state [28]. Diethyldithiocarbamate (DDC) chelates the divalent copper ion which is necessary for the catalytic activity of SOD-1. When given in vitro, DDC produces dose- and time-dependent inhibition of SOD-1 activity and, simultaneously, reduction of the antiviral response induced by exogenous interferon [29]. When given to mice in vivo, DDC is distributed widely and inhibits SOD-1 activity throughout all tissues including the brain [14]. Investigations of the possible ameliorative effects of DDC when applied to tissue culture or given to pregnant mice carrying trisomic 16 offspring could be undertaken.

Fatty acid cyclooxygenase likewise is involved in the interferon-mediated antiviral state [28]. Inhibition of the activity of cyclooxygenase with agents such as oxyphenylbutazone, indomethacin or phenylbutazone prevents the development of an interferon-mediated antiviral state [28]. Since the strongest agent blocking cyloox-genase activity is oxyphenylbutazone, it would be the natural choice to investigate.

Agents which have direct effect on the cytoskeleton could also be studied. Unlike calcium, colchicine or cold, which depolymerize microtubules, taxol stabilizes them [35, 36]. Since microtubular organization may be aberrant in Down's syndrome, it would be a reasonable research drug. It is an antimitotic agent and would have to be investigated carefully since it does produce growth suppression.

Agents which block normal cytoskeletal functioning, or disassociate the interactions between the components of the cytoskeleton, include substances such as colchicine, iminodipropionitrile, hexacarbons and acrylamide [11]. By transiently blocking one cytoskeletal component or one cytoskeletal interaction, it may be possible to induce a more normal subsequent cytoskeletal homostasis.

Thus, basic neurobiologic explanations are opening up avenues of potential therapeutic interventions in Down's syndrome. Much, much more research is necessary before any practical applications can be sought. One should remember that several decades ago cancer was incurable. With advances in medical and biologic research many cancer victims have their life expectancy prolonged, and are actually cured. Down's syndrome research is currently at the same stage that cancer research was decades ago. It is only through concerted laboratory effort that progress may be made.

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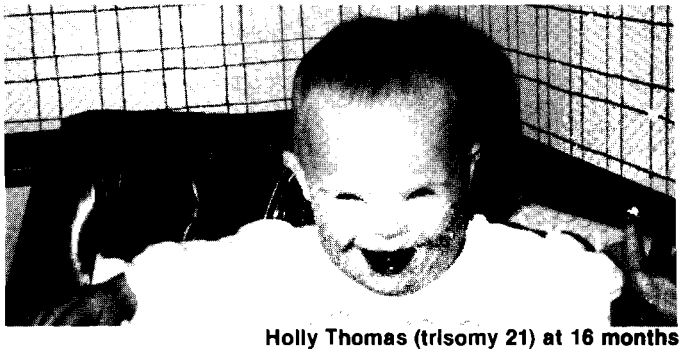
Editor's Note: This paper outlines an exciting new research approach to Down's syndrome. At this time, the therapeutic implications of the hypothesis proposed here are not yet tested and the efficacy and safety of the research drugs proposed here unknown.



Holly Thomas (trisomy 21) at 16 months