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Interference with Axonal Transport of Neurofilament: The Underlying Mechanism of Pathogenesis in Alzheimer's Disease, Amyotrophic Lateral Sclerosis, and Many Other Degenerations of the CNS

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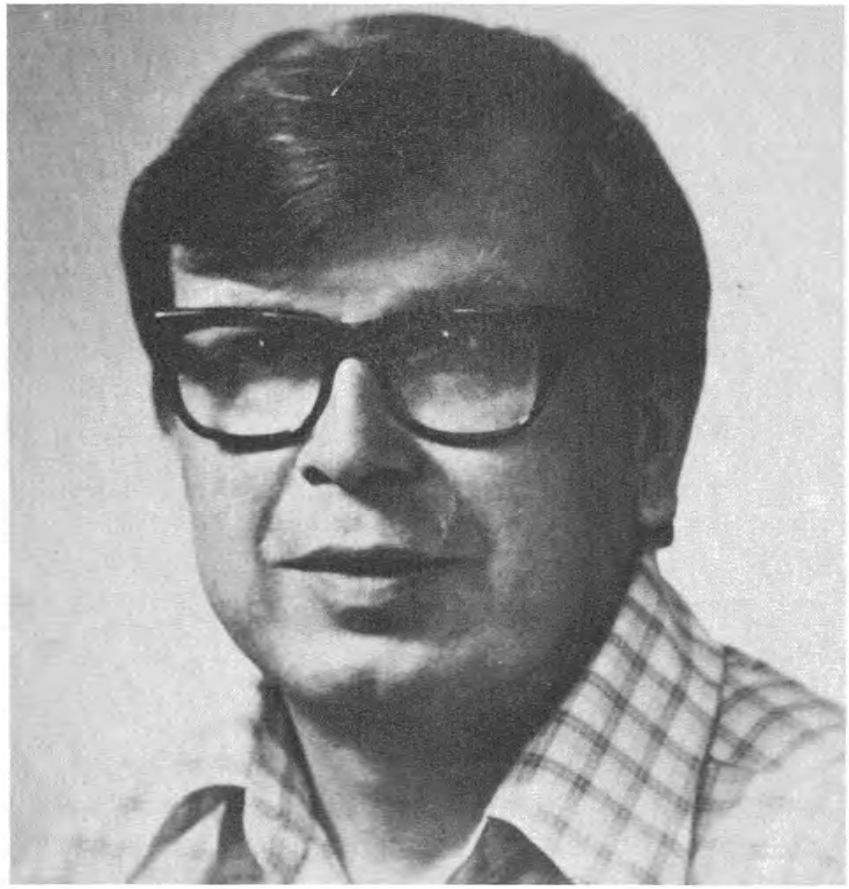

D. CARLETON GAJDUSEK

DANIEL CARLETON GAJDUSEK was born in September 1923 in Yonkers, New York. His father, who had emigrated from Slovakia in Austria-Hungary as a youth was married to the daughter of immigrants from Hungary. Dr. Gajdusek was very much influenced by the classical European cultural atmosphere in his home and what he describes as "the polyglot Eastern European immigrant communities" of Yonkers during his youth. He was drawn into science by an aunt, Irene D. Dobroscky, who worked as an insect virologist at the Boyce Thompson Institute of Plant Research in Yonkers. He became the precocious protege of the members of the institute, where he worked outside school hours, and had become a scientific investigator by age 10.

A graduate of the University of Rochester in biophysics, Dr. Gajdusek obtained the MD at Harvard in 1946. This was followed by a post-doctoral fellowship in physical chemistry at Cal Tech with Linus Pauling and Max Delbrück. While a medical student, he had been involved in the research in John Edsall's laboratory; while a pediatric resident at Boston's Children's Hospital he worked in John Enders's laboratory and at Babies Hospital at Columbia-Presbyterian with Dr. Michael Heidelberger, and at the Walter and Eliza Hall Institute in Melbourne with MacFarlane Burnet. He is clearly not only very energetic but extremely well connected. Delbrück, Enders and Burnet all became Nobel laureates and Pauling has been selected twice. Dr. Gajdusek has combined a strong fundamental background in basic science and virology with his interest in children and primitive cultures to make important discoveries about "slow" viruses. For these accomplishments he was awarded the Nobel prize in 1976.

The history of "Kuru" in the highlands of New Guinea is an engrossing story of the solution of a mysterious disorder among primitive people. Thought at first by some to represent a peculiar hereditary trait, Dr. Gajdusek demonstrated that it resulted from infection by a strange type of virus which has a very long incubation time and very unusual physical prperties. It has since been discovered that a number of diseases of sheep, mink and man result from similar agents.

One might conclude that Dr. Gajdusek is another example of a well known but rare human type: brilliant, energetic and highly focused. But he has another dimension, that of compassion. He is the father of at least 36 adopted children, children he has found in primitive cultures in New Guinea and elsewhere and is supporting in a variety of ways. At present he is the Chief of the Laboratory of Central Nervous System Studies of the National Institutes of Health. His laboratory is the world.

Interference with Axonal Transport of Neurofilament: The Underlying Mechanism of Pathogenesis in Alzheimer's Disease, Amyotrophic Lateral Sclerosis, and Many Other Degenerations of the CNS

The cytoskeletons of all cells contain three ultrastructurally distinct elements made of fibrous macramolecules: microtubules 24 nm in diameter, intermediate filaments 10 nm in diameter, and microfilaments about 5 nm in diameter and composed of polymerized actin. The neuronal intermediate filaments (10 nm) are antigenically distinct from intermediate filaments found in other cells even in the CNS, and are found in restricted stable parts of the perikaryon and in axons, while microtubules (24nm) predominate in the perikaryon and in dendritic processes and are thought to stabilize the complex shape of neurons (47,63,64,66,90). The neurofilaments, while accepted as a structural support system, have long been thought to be involved in axonal transport.

The "classical" neurofibrils of Beilschowsky (1902) and of Ramon y Cajal (1903) are silver staining neuronal intermediate filaments since only they are argyrophilic. The paired helical filaments (PHF) of neurofibrillary tangles (NFT) and of the neuritic (and senile) plaques of Alzheimer's disease and senile dementia of the Alzheimer type, and Pick's bodies of Pick's disease are composed of reduplicated or fused neurofilament-like fibrils and, although quantitatively increased and unique in configuration, they are immunologically and chemically indistinguishable from neurofilaments.

The neuronal neurofilament (10 nm) extends from the axonal process down the whole length of the axon; it is composed of three proteins of 200,000, 150,000, and 70,000 dalton molecular weights, respectively, and is usually associated with an additional 62,000 dalton protein. The synchronous movement of these three component proteins in slow axoplasmic transport has been demonstrated (53). This molecular complex is not a static cytoskeletal structure, but a moving fiber, perhaps itself responsible for the slow component of axonal transport of lysosomes, enzymes, and transmitter molecules to the presynaptic terminals.

We now have evidence that suggests that interference with the transport of this 10 nm neurofilament complex may be responsible for formation of paired helical filaments (PHF) in the neurofibrillary tangles and the neuritic plaques which characterize Alzheimer's disease. Furthermore, there are indications that amyloid deposits in the nervous system, particularly the amyloid plaques of Alzheimer's disease and the larger, more regular amyloid plaques of kuru, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler syndrome may be derived from yet further degeneration of the protein triad from which 10 nm neurofilaments are formed (38).

Thus, interference with axonal transport of neurofilament may be a basic mechanism of pathogenesis which may lead to 1) pooling of the neurofilament in the perikaryon and lysis of the neuron as in ALS and other motor neuron diseases or 2) neurofibrillary

tangles and neuritic plaque formation with neurofilament modified to form paired helical filaments and finally, 3) amyloid plaque formation, a further degradation of the same neurofilament, in Alzheimer's disease and many other CNS degenerations.

Amyloid plaques of Alzheimer's disease and senile dementia of the Alzheimer type, of Down's syndrome, Pick's disease, progressive supranuclear palsy and pugilistic encephalopathy as well as those of kuru, Creutzfeldt-Jakob disease and scrapie are formed extracellularly near degenerated or degenerating axons. The amyloid is composed of long neurofilament-like fibrils in two-stranded fibers similar to but morphologically distinguishable from the PHF and NFTs. The salient features of the pathology of AD or SDAT are the appearance of NFT, neuritic plaques, and amyloid plaques along with a granulovacuolar degeneration in neurons with resulting neuronal loss and gliosis. The first three pathological changes may be a consequence of impairment of axonal transport of neurofilament which may have many different causes, such as trauma, genetic defect, toxin or deficiency, slow virus infections, or aluminum deposition as in dialysis dementia. We already know that the compoent proteins of normal neurofilament share many epitopes with the PHF of NFTs and neuritic plaques (2,96). The partial amino acid sequence of the 4,000 dalton subunit protein of the amyloid plaque of AD and Down's syndrome has recently been determined (48,68), and we must await expectantly the sequence of similarly solubilized subunit proteins of PHF, which we would expect to share homology with amyloid.

Our work on the etiology of kuru and on the cause of amyotrophic lateral sclerosis (ALS) and parkinsonism with dementia (PD) with the early appearance of neurofibrillary tangles (NFT) in the populations in high incidence foci in the Western Pacific has led us to these realizations. Our further work on slow virus infections of the CNS has led to the discovery that these and many other nonviral CNS degenerations lead to the appearance of autoantibodies in the serum of patients specific for the component proteins of neurofilament and not to any other CNS antigens. The cumulative sequence of observations from the study of kuru, Creutzfeldt-Jakob disease and scrapie and their viruses, and of the high incidence foci in the Western Pacific of ALS, PD and the early appearance of NFTs in the populations which have led us to these hypotheses will be reviewed below:

Intraneuronal hydroxyapatite deposits containing nonessential metals lead to early appearance of NFTs and high incidence of amyotrophic lateral sclerosis (ALS) and parkinsonism dementia (PD).

Epidemiological study of the high incidence foci in the Western Pacific of ALS and PD and the early appearance of NFTs in the populations (1,19) has indicated a common etiology and pathogenesis for all three phenomena. The covariation of these three apparently unrelated but associated neuropathological phenomena in the high incidence foci on Guam and the Kii Peninsula of Japan and in West New Guinea (where we do not yet know about neurofibrillary tangles in the population) has indicated that an environmental factor underlies all three phenomena (32-34,39,43,45). We would like to have a single mechanism of pathogenesis for neuronal degeneration

in the three disease which neurologists do not ordinarily consider to be related: ALS (motor neuron disease), PD, and Alzheimer's disease (or senile dementia of the Alzheimer type).

We are forced to this conclusion by the close covariation of the three diseases in place and in time. In Saipan, Tinian and the Northern Marianas Islands, the Chamorro people do not experience them as they do on Guam and Rota (98). The Auyu and Jakai suffer from ALS and PD only when they live along certain river drainages (32-34,39); the Japanese only in a few isolated valleys (92). As ALS recedes so does PD on GUAM (43), on the Kii Peninsula of Japan (92), and in West New Guinea (39).

Environmental deficiencies of calcium and magnesium in people who have no outside source of these elements have been the only unusual environmental factors discovered which are the same in all three high incidence foci (44,95-109). The disappearance of these diseases after a lag of over a decade in all three foci coincident with increased access to calcium from the outside further confirms the role of calcium deficiency and the induced secondary hyperparathyroidism in pathogenesis (39,98,100-103). It has been verified by environmental analyses of soil and water (44), presence of osteoporosis in the affected populations (78,79), and the discovery of calcium and other metals in soft tissues (56,110,111,113-117), particularly neurons (41,42,55,77) of the patients. The Raman spectra of the deposits (107) and their stability and immobility (41,42,77) confirm the specificity of these deposits in the form of stable hydroxyapatites (107), and indicate they are not formed postmortem and their presence only in neurons which bear NFTs strongly suggests their pathogenic role in the neuronal damage (41,42,55,77).

In these three foci of high incidence ALS and PD the etiology of the associated NFTs has been discovered and the mechanism of pathogenesis demonstrated from the following evidence:

1. epidemiology of occurrence and disappearance of the disease in time and place (33,43,92);
2. environmental analyses of soil and water led to and substantiated Yase's hypothesis (103);
3. calcium and other metal analyses of tissues, especially brain, confirm the hypothesis (41,42,77,110,111,113-117);
4. access to calcium has resulted, after an appropriate delay, in slow cessation of the appearance of new cases of the phenomena in certain villages in all three foci (33,43,92,99), in a way similar to that which occurs as access to iodine eliminates the problem in an area of endemic goiter and cretinism.

With the cause of ALS and PD and the early appearance of NFTs in these high incidence foci essentially solved, interesting and important problems of individual variation of response remain, as they do also for explaining which individual gets goiter, deaf mutism, mental retardation, or full cretinism in an iodine deficient area, or who gets general paresis or tabes dorsalis from untreated infection with *Treponema pallidum*.

Further evidence in interference with neurofilament transport as a basic mechanism of pathogenesis.

The brilliant use by Asao Hirano of his good fortune in obtaining an early autopsy on a case of early ALS has suddenly given us the basic pathological mechanism we have been seeking: he demonstrated swollen motor neurons in the spinal cord in a patient with pure ALS in New York, and found the neuronal perikaryon filled with a piled-up mass of densely-packed neurofilaments (50-52,57) which proved to be the 10 nm neurofilament by immunospecificity (40). The chromatolytic changes in the motor neurons resembled those seen after axtomy, and corpora amylacea-like structures surrounded by pooled neurofilament appeared within myelinated axons (57).

We have been searching for a basic pathogenesis that would account for the association of all three phenomena: NFT, ALS and PD. We may now suggest a basic pathogenetic pathway common to many different diseases and insults to the CNS: namely, interference with axonal transport of 10 nm neurofilament down the axon, without interruption of its synthesis and assembly in the perikaryon. Such interference leads to deformation of the neurofilament into paired helical filaments and neurofibrillary tangle formation and, when it occurs at different rates, to neuritic plaque formation or to amyloid formation and its deposition into plaques.

Such a mechanism may operate in pugilistic encephalopathy, in Alzheimer's and Pick's diseases, progressive supranuclear palsy, in congenital genetic defects of Down's syndrome and Werdig Hoffman disease, in hydroxyapatite deposition as in neurons in Guamanian ALS and PD, in metal intoxication as in dialysis dementia, in intoxication of rats with $AlCl_3$ or β,β'-iminodipropionitrile (IDPN) (21,22) or in early motor neuron disease (Hirano's case) (50-52,57). Precisely which specific neurons are affected depends on host factors we cannot yet discern (no more than we can in tertiary syphilis or in CNS lesion from congenital iodine deficiency), but in neurons with large demands on axonal transport (such as motor neurons) sudden ballooning and death with piled-up neurofilaments may ensue, while in neurons with less voluminous axonal transport (such as the hippocampal neurons) NFTs develop slowly (20-23,50-52,57).

Uniquely specific antineurofilament autoantibodies appear in many degenerations of gray matter.

The strange appearance of a specific autoantibody to 10 nm axonal neurofilament in scrapie, kuru, and Creutzfeld-Jakob disease (3-6,93), and in a significant but lesser portion of ALS, PD, Alzheimer's disease, Parkinson's disease, and other degenerations of the CNS (4,5,25,93), has led to the focus of our attention on the three proteins, having molecular weights of 200,000, 150,000, and 70,000 daltons (5,96,97), which comprise the 10 nm neurofilament, and the 62,000 dalton neurofilament-associated protein, especially since autoantibodies against any of the thousands of other brain antigens are not formed in these diseases. The live neuron cultures used as substrate to detect these autoantibodies (3,93-95), readily detect other antibodies specifically directed against other brain proteins, as indicated by the use of these cultures for detection of monoclonal antibodies from hybridomas (28). Why, then, is it specifically the neurofilament which excites autoantibody production and not other brain proteins?

The autoantibody, which appears in Creutzfeldt-Jakob disease and in other neurodegenerative diseases against normal 10 nm neurofilament, also reacts with paired helical filaments in NFTs of Alzheimer's disease (96), with the piled-up neurofilaments of IDPN-poisoned rats (5,48), and with the piled-up neurofilaments in motor neurons of the spinal cord in Hirano's patients with early ALS (40). Furthermore, polyclonal antibody prepared in laboratory animals to normal 10 nm neurofilament also reacts with the NFTs of Alzheimer's disease (96) as well as with the piled-up accumulations of 10 nm neurofilaments in brain cells of rats poisoned with $AlCl_3$ (24) or IDPN (5,48). Monoclonal antibodies to neurofilament likewise show that normal neurofilaments and PHF share antigenic sites (2).

The PHF in NFTs of Alzheimer's disease and senility, like those found in pugilistic encephalopathy, Down's syndrome, or certain slow virus infections, are nevertheless distinguishable ultrastructurally from normal 10 nm neurofilaments (58). Thus, normal 10 nm neurofilament and paired helical filaments share antigenic sites and probably arise from the same source; and amyloid plaques may well be a further accumulation of packed neurofilament degraded into amyloid fibrils (7,12-18,29,30). This unifying hypothesis gains further support from a recent report by Rasool and Selkoe of immunologic similarity between the Pick's bodies in Pick's disease and the NFTs of Alzheimer's disease (38,86). Reports that antigenic sites on microtubules are also shared by NFT (49,112) suggest that microtubule components may be entrapped in the neurofilament derived PHF accumulations that comprise NFTs, particularly epitopes of microtubule-associated protein 2 (61). Cross-linkages betwen neurofilaments and microtubules have already been demonstrated (26,27,65,91,112).

Interference with transport of 10 nm neurofilament down the axon may lead to its piling-up and destroy motor neurons, or to paired helical filament formation in NFTs in more slowly metabolizing neurons. It is tempting to assume that in neurons with high transport requirements (i.e., motor neurons) an accumulation of neurofilament in the perikaryon, proximal to the axonal process, causes cell death as in Hirano's cases of ALS (50-52,57), whereas in a neuron requiring less axonal transport, paired helical filaments develop to form NFTs or neuritic plaques, or even amyloid plaques (7,12-18,35-37,54,66). In the case of both kinds of neurons, lysis would release an extraordinary amount of the piled-up component proteins of neurofilaments, and thus stimulate specific autoantibody response in prespecified B cells (3-6,25,93). The autoantibodies in individual subjects show differing extraordinary specificity for one or more of the component proteins of the neurofilament, most usually for the 200,000 protein (96); scrapied sheep react with only the 62,000 dalton neurofilament associated protein (97).

Filamentous unconventional viruses resembling PHF and amyloid cause scrapie-kuru-CJD.

The nonantigenic, replicating unconventional slow viruses causing scrapie, kuru and CJD evoke amyloid deposition in the brain of many, but not, all, patients and some of the species or breeds of experimentally infected animals (7,31,35-37,60,67,83). These viruses are unusually resistant to physical and chemical inactivation (31,35-37, 69-72,87) and appear to lack a nucleic acid (31,35-37,80, 87) or a non-host protein antigen (31,35-37,59,69). Merz *et al.* (72-76) have identified by electron microscopy of density gradient sedimented brain preparations a scrapie associated fibril (SAF) in all of the patients and affected animals, but not in other diseases (72,73,76). These SAFs are 2- or 4-stranded amyloid-like fibers about 100 nm long, which increase in quantity with virus titer (72,74). They may be the infectious agents (72,74). They stain with Congo red and show birefringence, as do amyloid fibers which they resemble ultrastructurally (81,84). They are also very similar to paired helical filaments of NFTs, yet they can be distinguished morphologically from both paired helical filaments and amyloid fibers by the degree of coiling and the regularity of their periodic narrowings (72,76). The ultrastructure of paired helical filaments, normal neurofilaments and amyloid fibrils with each strand divided into two protofilaments of 2-6 nm diameter per strand further suggests their common origin. Normal neurofilaments can alter their size and shape *in vitro* with change in the ionic structure of the medium in which they are suspended (62,88) and their solubility may alter with their configuration (89). The scrapie associated protein of Prusiner (11,80-84) of under 30,000 daltons molecular weight may aggregate to form these infectious SAFs (11,84,85); Prusiner calls these SAFs his "prion rods" (80,81). Antibodies to this protein cross react with amyloid plaques in the brains of scrapie infected hamsters (8-10,81). Certain monoclonal mouse hybridoma antibodies react with neurofibrillary tangles, paired helical filaments and SAFs (unpublished data of this laboratory and Patricia Merz, Staten Island, NY.).

Thus, we now have in these unconventional viruses a possible link between normal 10 nm neurofilament and amyloid, and it appears that the SAFs are filamentous viruses which bring to mind filamentous phage fd and filamentous plant viruses which are of about the same diameters (35-37,87). However, evidence is mounting that these subviral pathogens really may be composed only of host-specified protein and contain no nucleic acid. Our neurofilament-like unconventional viruses apparently interfere with slow axonal transport of neurofilament down the axon and occasionally lead to amyloid accumulations. These viruses seem to be parasites of this slow axonal transport mechanism, themselves incorporating neurofilament proteins into their structure. If the viral filamentous protein does not share sequences with the normal neurofilament proteins and amyloid, it may well be a host-specified protein from derepressed host genes—as for example, a fetal form or precursor of neurofilament. Otherwise, we would be forced to assume that it acts like a "decoy duck", interfering with normal function by virtue of structural homology to the normal neurofilament.

These hypotheses are but a framework of thought. They do however reflect a synthesis of much of our recent data and clearly indicate numerous new lines of inquiry which may either validate or refute these predictions.

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