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Commentary

Niemann-Pick Type C Disease and Alzheimer's Disease

The APP-Endosome Connection Fattens Up

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Niemann-Pick Type C (NPC) is an inherited neurodegenerative disease of childhood and adolescence that develops from a failure of cholesterol trafficking within the endosomal-lysosomal pathway. Although NPC differs in major respects from Alzheimer's disease (AD), intriguing parallels exist in the cellular pathology of these two diseases, including neurofibrillary tangle formation, prominent lysosome system dysfunction, and influences of apolipoprotein E ϵ4 genotype. Added to these similarities are new findings that some neuronal populations develop abnormalities of endosomes resembling those seen at the earliest stages of AD and also accumulate β-cleaved amyloid precursor protein (APP) and Aβ peptides within endosomes. In this commentary, the common features of endosome dysfunction are reviewed. Emerging evidence that endosome dysfunction may lead to β-amyloidogenic APP processing or neurodegeneration by several different means is discussed. *(Am J Pathol 2004, 164:757–761)*

Niemann-Pick type C disease (NPC), an inherited lysosomal storage disorder, has long intrigued investigators of Alzheimer's disease (AD) as one of a very few disorders in which neurofibrillary tangles (NFT) robustly form in the brain in the absence of tau mutations or β-amyloid deposition.[1,2] That the primary deficit in NPC involves dysfunction of cholesterol trafficking adds to the intrigue, given the mounting evidence that high cholesterol is a risk factor in AD[3] and that the ϵ4 isoform of apolipoprotein E (ApoE), a protein carrier for cholesterol, promotes disease development in both disorders. With additional reports now linking changes in cholesterol homeostasis to altered APP processing and Aβ generation,[4–11] the pathobiology of NPC has become a potentially rich lode from which to mine clues about AD pathogenesis.

The studies reported by Jin et al[12] in this issue of *The American Journal of Pathology* tie the cellular mechanisms operating in NPC and early stage AD even more tightly by showing that amyloidogenic processing of APP in NPC neurons localizes to early endosomes, a compartment of the endocytic pathway that develops the earliest pathological changes yet known in AD[13,14] and is implicated in amyloidogenesis in sporadic forms of AD and in Down's syndrome (DS).[14,15] The striking parallels between the endosomal alterations in both diseases and the high disease-specificity of these alterations invite a deeper analysis into how a primary endosomal-lysosomal disorder can inform us about the origins of Alzheimer's disease, even when clinical and pathological manifestations of the two diseases differ.

The Niemann-Pick syndrome arises from inherited defects that cause either a cellular accumulation of cholesterol, as in the type C form of Niemann-Pick disease, or of sphingomyelin, in the case of Niemann-Pick types A and B. Depending in part on the age of onset, NPC can present initially as a systemic disease, featuring prominent hepatosplenomegaly, or as a neurological disease, characterized by cerebellar ataxia, bulbar dysfunction, and variable degrees of cognitive decline.[16,17] Both systemic and neurological features ultimately coexist although visceral symptoms predominate in perinatal and infantile forms of NPC, while motor deficits and cognitive and psychiatric dysfunction predominate in the more common late infantile/juvenile forms and the rare adult cases. Death in the juvenile form, which is attributed

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largely to the progressive neurodegeneration, often occurs in the teens or twenties.

NPC neuropathology at the anatomical level differs considerably from that of AD. The most vulnerable neurons are Purkinje cells, accounting for the prominent ataxia seen clinically. Neurodegeneration, however, is progressive and widespread within cortical and subcortical neuronal populations. A "dying back" or retrograde pattern of degeneration is suggested by the striking degeneration of large fiber tracts in the human disease and in animal models of NPC.[18–20] In the juvenile form of NPC, NFTs develop in significant numbers in the third decade[1] and contain PHF-tau, which is structurally and immunologically similar to that in AD tangles.[21–23] Mouse, dog, and cat models of NPC exist, all arising from spontaneous mutations, and these models reproduce the main neurological and pathological features of the human disease.[16,24,25]

The failure of cholesterol trafficking in NPC can be understood in part from its genetic basis, which involves mutations of either of two functionally related genes, NPC1 and NPC2, accounting respectively for 95% and 5% of the cases.[26,27] The NPC1 protein, normally located primarily in lysosomes[28] contains a putative sterol-sensing domain common to other proteins involved in cholesterol homeostasis.[29] NPC1 influences the trafficking of NPC2, a protein that resides in the *trans*-Golgi network and late endosomes and is regulated by the cation-independent mannose-6-phosphate receptor (MPR215). Cells lacking MPR215, like cells in NPC, accumulate cholesterol in late endosomes[30] suggesting that MPR215 binding to NPC2 is important for endocytic cholesterol transport.

Cells with dysfunctional NPC1 or NPC2 accumulate unesterified cholesterol in late endosomes, which reflects a failure of cholesterol to efficiently exit this compartment and travel to the plasma membrane and ER. NPC cells can internalize LDL cholesterol, transport it to endosomes, and hydrolyze the LDL moiety, but once it is delivered to NPC1-containing late endosomes, a "lipid traffic jam" develops involving cholesterol, some other lipids and gangliosides, and MPR215.[18,28,31,32] The traffic jam also traps endogenously synthesized cholesterol,[33] and impedes its delivery into distal axons, where it is required for membrane maintenance. Cholesterol in the brain, unlike other tissues, originates mainly from endogenous synthesis.[34] This transport failure in NPC, therefore, likely accounts for the special vulnerability of axons in the disease and their lowered potential for regeneration, as well as the impairment of ApoE-mediated cholesterol scavenging ability after axon injury.[35]

Jin and colleagues used both primary cortical neurons and NPC brain tissue to assess the consequences of this lipid traffic jam on the metabolism of APP, which is known to be actively processed in the endocytic pathway.[36–40] Exploiting an observation that U18666A, a class-II amphiphile, directly inhibits NPC1 function and induces an NPC-like phenotype in cells,[41] they tracked cholesterol accumulation and β- and γ-cleaved products of APP within different endocytic compartments identified with antibody markers. As expected, cholesterol accumulated in late endosome/lysosome compartments of U18666A-treated cells, as seen in NPC. Also consistent with earlier reports,[9,10] Aβ42 and the β-cleaved C-terminal fragment of APP, C99, accumulated in the cells, including a significant proportion of formic acid-insoluble and -soluble aggregated forms of these peptides. The absence of a U18666A effect on C99 and Aβ levels in a cell line overexpressing C99 suggested that the effects seen in the U18666A treated neurons were due at least in part to the increased β-cleavage of APP. A surprising finding revealed by immunocytochemistry, however, was that the antibodies recognizing Aβ and C99/APP-CTF did not decorate mainly the late endosomes/lysosomes that accumulated cholesterol but, instead, labeled rab 5-positive early endosomal compartments. The result was even more clear-cut in the Purkinje cells of a small set of human NPC cases where increased APP fragment immunoreactivities, corresponding presumably to the C99 elevations by Western blot analyses, co-localized with rab 5-positive early endosomes rather than the late endosomes.

Early endosomes were also abnormal in other ways. They were substantially enlarged and contained high levels of the lysosomal hydrolase cathepsin D suggesting that cathepsins were being partially rerouted to early endosomes in NPC Purkinje cells. Hippocampus and cerebral cortex, which are affected late in the course of NPC, did not display these abnormalities although intraneuronal Aβ42 was detected in late endosomes of neurons in the CA1 area and entorhinal cortex of older adult subjects. These results on endosomal alterations in NPC are supported by findings in the mouse model of NPC[11] where brain levels of βCTF, Aβ42, and Aβ40 are also increased and presenilin is redistributed from the ER to early endosomes. Early endosomes in NPC1-disrupted CHO mutant cells and NPC1-deficient mouse brain also acquire more βCTF, Aβ40, and Aβ42, although Aβ accumulates in cholesterol-containing late endosomes in U18666A-treated CHO cells, suggesting that differences in cell type or the nature of the experimental cholesterol perturbation, including the source of cholesterol, may influence the details of the trafficking dysfunction.[42,43]

The convergence on endosomes of many factors that promote amyloidogenesis is another remarkable example, in addition to neurofibrillary neurodegeneration, of the overlap between cellular mechanisms operating in NPC and AD. In Alzheimer's disease, alterations of rab 5-positive endosomes are the earliest appearing disease-specific cellular pathology. Rab 5-positive endosomes are enlarged in some neurons even before birth in Down syndrome, and this abnormality is evident in many neurons decades before the onset of dementia in these individuals.[14] In sporadic Alzheimer's disease, early endosomes are already abnormal in pyramidal neurons of the neocortex at a stage of disease when Alzheimer-like (plaque and tangle) pathology is limited only to the hippocampus and entorhinal cortex. The endosomal changes in the neocortex at this very early stage of the disease coincide with the appearance of Aβ40 and Aβ42 in early endosomes and the initial rise in soluble Aβ levels.[15] Rab 5-positive compartments are abnormally

enlarged, proteins facilitating rab 5 function such as EEA1 and rabaptin 5 are mobilized to early endosomes, and the expression of rab 4, an index of endosome recycling, is increased. Together these findings suggest that endocytosis is up-regulated and possibly dysfunctional. A similar endosomal phenotype is produced in cell lines overexpressing rab 5. As in NPC cells, βCTF is overproduced and appears in early endosomes of the rab-5-transfected cells, which also generate substantially higher amounts of Aβ40 and Aβ42 than control cells.[38] The enlarged early endosomes in Alzheimer's disease, as in NPC, also contain abnormally high levels of lysosomal hydrolases.[13] This is at least partly due to a rise in MPR46 expression in the brain at the incipient stages of AD.[44] Overexpression of MPR46, or modified MPR constructs that deliver lysosomal hydrolases to endosomes substantially increases Aβ40 and Aβ42 production in cell lines.[44] Thus, two abnormal features of endosomes seen in AD and NPC, endosome enlargement and elevated hydrolase content, are tied mechanistically in AD brain to other known biochemical abnormalities that promote amyloidogenic cleavage of APP within the endocytic pathway. It will be interesting in the future to examine how MPRs promote mistrafficking of cathepsins and other proteins in NPC, especially in view of the mannose content of NPC2 and the importance of MPR215 in NPC2 function and cholesterol trafficking.[30]

Endosomal pathology also develops in a genetic model of Down syndrome, the segmental trisomy 16 mouse (Ts65Dn), within the basal forebrain, hippocampus, and neocortex, which later exhibit aging-related atrophy and degenerative changes.[45] Triplication of *App*, one of the genes in the trisomic segment of chromosome 16, is required for endocytic abnormalities to develop,[15] highlighting the importance of this AD-related gene in the development of this endosomal phenotype. The degenerative changes in these mice develop in the absence of β-amyloid deposition or neurofibrillary tangles, although soluble Aβ peptide is overproduced in amounts disproportionately higher than the 50% increase expected based on A*pp* gene dosage. Recent immunocytochemical studies place this Aβ mainly within rab 5-positive endosomes (Anne Cataldo, personal communication).

β-CTF and soluble, intracellular forms of Aβ are increasingly being investigated as *App*-related moieties that may be the most critical to Alzheimer's disease pathogenesis.[46–53] Soluble Aβ levels correlate better than insoluble Aβ with synaptic changes and neurodegeneration in AD,[47] and lowering soluble Aβ levels improves behavioral deficits without reducing amyloid burden in some transgenic models.[50,54] The recent findings in NPC and DS, therefore, raise interesting questions about the cause and effect relationship between endosomal dysfunction and intracellular βCTF and Aβ and how each contributes to disease progression. In NPC, primary genetic defects of endosomal proteins clearly precede and cause βCTF and Aβ overproduction, but whether these APP mutations play a role in the further neurofibrillary tangle formation and neurodegeneration is an interesting question. These same endosomal protein defects in NPC, however, also have more global effects on endosomal/lysosomal function and trafficking including impaired transport of cholesterol and other constituents to distal axons.[43] These effects could easily account for neurodegeneration, based on growing evidence linking primary endosomal-lysosomal dysfunction to selective neurodegeneration.[55–60]

In the Ts65Dn mouse model of Down's syndrome, endosomal abnormalities and intracellular Aβ accumulation develop in some neuronal populations that are affected in AD and that, in these mice, later develop neurodegenerative changes. Intracellular or extracellular Aβ accumulation, by itself, does not seem to cause this endosomal pathology,[14,15] indicating that, like in NPC, endocytic pathway dysfunction likely precedes and leads to Aβ overproduction.[37,38,44] Evidence also supports the possibility that, as in NPC, endocytic dysfunction in AD and DS could contribute to neurodegeneration through mechanisms that are Aβ-independent.[61–64] For example, key signaling functions of early endosomes are defective in Ts65Dn mice, leading to impaired neurotrophic stimulation of neurons.[59] Growth factor signaling in normal neurons involves receptor-mediated endocytosis and a subsequent cascade of signaling events involving proteins associated with the early endosome, collectively comprising the "signaling endosome."[65,66] Successful trophic action likely requires the signaling endosome to be transported back from nerve terminals to the cell body.[66] In the Ts65Dn mouse, retrograde NGF signaling is defective[59,66] in basal forebrain cholinergic neurons, which seems to be responsible for the age-related neurodegenerative changes in these neurons. Whether or not this signaling failure involves impaired transport of the signaling endosome in these neurons is unknown. But if it turns out to be the case, it is tempting to speculate that such a defect might be linked to *App*, which is critical to development of the abnormal endosomal phenotype in Ts65Dn mice. Indeed, APP undergoes anterograde and retrograde axonal transport[67,68] and is believed to act as a receptor for the axonal transport of kinesin-related vesicular cargo.[69] It can be imagined, therefore, that modifying the kinesin-interacting cytoplasmic tail of APP by phosphorylation or proteolysis, including elimination of the tail by γ cleavage, could greatly influence trafficking. It would be interesting to know, in this context, how cholesterol may influence APP's role in endosomal transport. Whether or not all of these events turn out to be related, investigations of NPC will undoubtedly reveal additional insights into the close relationship between dysfunction of the endosomal-lysosomal system and neurodegenerative disease.

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