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Commentary

Discovery of New Lesions in Neurodegenerative Diseases with Monoclonal Antibody Techniques

Is There a Non-Amyloid Precursor to Senile Plaques?

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The manuscript by Schmidt et al reports that antibodies generated to paired helical filaments (AMY antibodies) unexpectedly labeled novel non-amyloid, plaque-like structures (AMY plaques) in aged and Alzheimer's disease brains. The full disclosure of the nature of these lesions awaits additional structural and biochemical studies, but at first glance there are interesting parallels between AMY plaques and recently described lesions composed of glia and glia-associated proteoglycans in brains of aged mice. The increasing recognition of the role of proteoglycans in paired helical filaments formation makes proteoglycans or their associated molecules attractive candidates for AMY-immunoreactive proteins. The relationship of AMY plaques to age-related glial changes that some have speculated may be precursors to senile plaques remains to be determined, as is the relationship of AMY plaques to more widely recognized amyloid-containing plaques. Future studies will determine whether AMY plaques are non-amyloid precursors to senile plaques or if they represent an independent type of structural lesion in the Alzheimer's disease brain. Ultimately, the clinical significance of AMY plaques will depend upon their characterization in brains of prospectively studied subjects. (Am J Pathol 1997, 151:7–11)

The manuscript by Schmidt et al[1] challenges the assumption that the histopathology of Alzheimer's disease (AD) is established. Despite more than a century of descriptive studies on AD,[2] with intense investigation in the last several decades, it is indeed remarkable that modern immunological methods are still able to reveal structures that have heretofore been unknown. In addition to the present study, a number of other examples might be cited in which the use of antibody technology in descriptive studies of degenerative diseases has revealed unexpected findings, which have often fostered new ways of thinking about these disorders.

Frontotemporal dementia, especially the subtype associated with motor neuron disease, has been referred to as "dementia lacking distinctive pathology"[3] because of the fact that routine histological methods show only nonspecific neuron loss, microvacuolization of the neuropil, and gliosis. On the other hand, with antibody methods, several distinctive structures are now recognized in this disorder. In particular, non-pyramidal neurons in the affected cortex and dentate fascia of the hippocampus contain cytoplasmic inclusion bodies that are not visible with silver stains or routine aniline dyes but are clearly revealed with ubiquitin immunocytochemistry (reviewed in Ref. 4). Ubiquitin, a small heat shock protein that is involved in non-lysosomal proteolytic degradation, reveals pathology in other disorders as well. Of note are the neurites that are consistently found in the hippocampus and amygdala in Lewy body disease.[5] Until immunocytochemical methods were used to study Lewy body disease, these structures were completely invisible. Ubiquitin immunocytochemistry also clearly demonstrates inclusions in

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oligodendroglial cells, so-called glial cytoplasmic inclusions, which have become the hallmark of multiple system atrophy.[6] In contrast to Lewy neurites, glial cytoplasmic inclusions can be seen with silver stains, but they were only widely recognized, and they remain most easily differentiated from other types of glial inclusions with immunocytochemistry. Another lesion that can be seen with silver stains, but which was initially recognized and best demonstrated with immunocytochemistry, is the astrocytic plaque[7] that has come to typify corticobasal degeneration.[8]

In the present study, Schmidt et al have discovered plaque-like lesions in AD brains with antibodies generated with highly purified preparations of paired helical filaments (PHFs) from AD brains. PHFs are the filaments that accumulate within vulnerable neurons and their processes in AD and, to a lesser extent, in aging and in a small number of non-AD disorders.[9]

The authors refer to the new lesions as AMY plaques, presumably because of their initial resemblance to amyloid plaques, although the name is never completely explained by the authors. AMY plaques are always found in brains with amyloid deposits and not in their absence, but they do not have amyloid properties with thioflavin-S fluorescent microscopy, which is arguably the most sensitive method for detecting amyloid in tissue sections. They often co-localize with plaques, but AMY plaques have a different distribution compared with amyloid plaques. The low power image of the hippocampus and entorhinal cortex in the manuscript clearly shows a distinctive topology of AMY plaques. In particular, they are absent from the parvocellular presubiculum and subpial regions, areas that are known to be vulnerable to early or diffuse amyloid deposits. The latter deposits are best seen with antibody methods and easily overlooked with histochemical or even histofluorescent methods.

Double staining studies demonstrated that AMY plaques sometimes formed a corona around amyloid deposits. The nature of AMY plaques remains to be additionally defined, as clearly acknowledged by the authors. There is an immediate need to determine the ultrastructure of AMY plaques. Do the lesions contain PHF or some other filamentous structures? Are they intracellular inclusions or extracellular deposits? With what cell type are they most closely associated: neurons or glia?

As previously mentioned, the antibodies that reveal AMY plaques were generated by immunizing mice with purified preparations of PHF. This approach has been used by others to discover novel proteins in AD and to decipher the molecular composition of neurofibrillary tangles. Unfortunately, most of the novel molecules have turned out to be previously defined proteins. One of the best examples of this is Alz50, which is a monoclonal antibody that was produced by immunizing mice with AD brain homogenates and screening hybridoma clones with enzyme-linked immunosorbent assay for those that had more immunoreactivity with AD than control brain samples.[10] Initially, the protein detected by Alz50 was considered to be a novel Alzheimer-specific molecule, as it had properties unlike any known protein of similar size and composition.[10] Subsequent studies have demonstrated unequivocally that the abundant Alz50 antigen in AD brains is tau protein, albeit an abnormal form of tau protein and a form of tau protein (PHF-tau) that is highly characteristic of, and enriched in, AD.[11] Nevertheless, it must be acknowledged that Alz50 also recognizes less abundant molecules that are not related to tau protein.[12] This raises an important issue that is true for the present study, which is also based upon analyses with monoclonal antibodies: namely, that monoclonal antibodies have exquisite specificity to a narrowly defined epitope that may be present on widely unrelated molecules. Such epitopes may be susceptible to factors such as postmortem delay, effects of different types and duration of tissue fixation, and differences in tissue processing, to name a few. The studies by Schmidt et al use tissue that is processed in an unconventional manner, that is, fixation in ethanol and saline. How this may compare to immunocytochemical studies in more routinely fixed (ie, formaldehyde) tissue remains to be determined.

Epitopes recognized by monoclonal antibodies may also be different in denatured proteins subjected to electrophoresis and immobilization to fixed substrates, eg, nitrocellulose, than the epitopes in tissue. There is no definitive proof that the 100-kd macromolecules recognized in brain homogenates and PHF fractions by the AMY antibodies are the same as those in tissue sections. Direct biochemical characterization of AMY plaques will be needed to prove this.

Several investigators have used an approach similar to that used in the present study, but the authors are to be commended for screening hybridoma clones with immunocytochemistry, rather than immunoblots or enzyme-linked immunosorbent assays, that are more readily scaled up for screening large numbers of supernatant samples. Most of the antibodies that have been generated to PHF have recognized structural proteins, including tau,[13,14] other microtubule associated proteins,[15] ubiquitin,[16] and

phosphorylated epitopes.[17,18] In addition there are antibodies to PHF for which no normal counterpart has yet been determined, which may possibly be antibodies to conformational epitopes.[15,19] Direct biochemical studies of PHF have consistently shown that the major structural component of PHF is tau protein;[20] other components of neurofibrillary tangles have been defined by immunocytochemical methods. Among the other components of PHF defined with antibodies methods are proteoglycans (PGs), apolipoprotein E, amyloid P component, and complement factor C1q (reviewed in 21). Could any of these minor components account for AMY plaques?

The antibodies generated by Vincent and Davies are of particular interest with respect to the present study. Vincent and Davies also generated a series of monoclonal antibodies to highly purified preparations of PHF using essentially similar methods.[22] Interestingly, they also obtained antibodies to epitopes, many of which were phosphoepitopes, that were present in proteins of a molecular mass of approximately 100 kd.[18] Some of these antibodies recognized nuclei and some also stained amyloid plaques in AD brain (P. Davies, unpublished data). In contrast to the present study, double labeling confirmed that these PHF antibodies reacted with amyloid (P. Davies, unpublished data). Immunoblots fail to show any significant contamination of PHF preparations by amyloid, but antibody recognition of a minor amyloid contaminant remains the simplest explanation for this observation. In addition, among the molecules that have been demonstrated to be recognized by these new PHF antibodies is nucleolin,[23] a 110-kd basic protein that binds extracellular matrix molecules such as laminin and to other acidic proteins and polyanions.[24]

It is recognized increasingly that PHF formation requires factors other than post-translational modification (ie, phosphorylation) of tau. In particular, association of tau with PGs has been recently emphasized.[25] When immunization of highly purified PHF generates antibodies to molecules such as nucleolin, it may suggest that polyanions are integral components of PHF and that associated molecules are very tightly bound in the macromolecular PHF complex.

What then is the nature of the non-amyloid plaques recognized by AMY antibodies? Non-amyloid plaque-like structures are uncommon, but their description brings to mind structures that have been discovered in brains of some strains of aged mice.[26] These plaque-like deposits are found in the hippocampus and entorhinal cortex within and around glial processes. Immunochemical studies suggest that these age-related, plaque-like structures contain PGs.[26] The relationship of astrocytes to plaque-like lesions is of some interest, as astrocytes are very often associated with amyloid plaques. Astrocytes are the most probable source of a number of plaque-associated molecules, including apolipoprotein E, α1-antichymotrypsin, apolipoprotein J (also known as Sp40,40), α2-macroglobulin, and even amyloid precursor protein (reviewed in Ref. 27). There is also evidence from previous studies that morphological changes in astrocytes may precede plaque formation.[28] Indeed, clusters of activated astrocytes are increasingly noted in cortical gray matter during aging independent of amyloid deposits, whereas such astrocytes are uncommon in the gray matter at younger ages.[29] In the future, double labeling studies with antibodies to astrocytes and with AMY antibodies might answer the question of whether the two lesions are related.

Based upon these and other studies, it has been suggested that deposits of PGs may be precursors to senile plaques. Furthermore, antibodies to specific PGs recognize various cellular and proteinaceous components of senile plaques.[30] Some plaque-like lesions have even been described as being positive with antibodies to PGs and negative for amyloid.[31,32] More recently, Snow et al have illustrated plaques with a central amyloid core surrounded by a corona of granular and filamentous material that is positive for synaptic vesicle-associated PG.[32] The PG-positive plaques are similar but not identical to those illustrated by Schmidt et al. That PG may be the epitope in AMY plaques would be consistent with the observation that certain types of PGs, in particular heparan sulfate PG, are present in neurofibrillary tangles.[30] Molecular masses of core proteins of PGs range from less than 100 to more than 200 kd. It will be of interest to exclude core proteins of PGs as the AMY-immunoreactive proteins. If AMY plaques contain PGs they should be relatively simple to see with standard histochemical methods, such as periodic acid-Schiff or Alcian blue stains. Arguing against this possibility is the fact that non-amyloid lesions of this type have not been noted previously in AD. On the other hand, discovery rewards the prepared mind, and staining of AD brains with routine histochemical methods might be worth revisiting.

The discovery of a new type of lesion in the AD brain opens an entirely new vista for exploration. It will be important to try to determine whether there is a precursor-product relationship between AMY plaques and amyloid plaques. This concept has heavily shaped thinking about senile plaques for

more than a century. Senile plaques are morphologically and biochemically heterogeneous. Although a precursor-product relationship has never been confirmed for plaques of various types, it is often hypothesized that pre-amyloid plaques evolve into diffuse amyloid deposits that eventually develop reticular or dense amyloid cores. Neuritic and glial components are likewise factored into this pathogenetic cascade (see Ref. 27). The problem with these hypotheses is that morphologically distinct subtypes of plaques are anatomically separate. Although diffuse plaques are unquestionably found in the cortex in AD along with dense cored plaques, in other areas such as the molecular layer of the cerebellar cortex and the presubiculum, diffuse amyloid deposits are only rarely, if ever, accompanied by dense amyloid deposits, even in advanced stages of AD. Furthermore, dense amyloid cores occur in some regions, such as the globus pallidus without ever being preceded by a diffuse precursor type lesion. It seems reasonable to conclude that anatomical and microenvironmental constraints contribute to the great diversity in plaque morphologies and to plaque pathogenesis in ways that are currently poorly understood.

Nevertheless, it is tempting to place AMY plaques into a pathogenetic cascade. Could AMY plaques (possibly composed of PGs or complexes of PGs and molecules that bind tightly to polyanions, eg, nucleolin) be a precursor to preamyloid deposits? Are AMY plaques the answer to the question of what determines where amyloid deposits are found in the neuropil? If AMY plaques are actually non-amyloid precursors to amyloid plaques, are they glia- or neuron-derived? Given the renewed interest in the role of glia in a host of degenerative diseases, in part because of the revelation of glial inclusions in many of these disorders,[8,33] it would be of more than passing interest if astrocytes were related to AMY plaques. Additional studies are needed to unravel the mystery of AMY plaques.

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