

Commentary

Does Overexpression of β APP in Aging Muscle Have a Pathogenic Role and a Relevance to Alzheimer's Disease?

Clues from Inclusion Body Myositis, Cultured Human Muscle, and Transgenic Mice

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In Alzheimer's disease (AD) pathogenesis, an underlying β APP/ $A\beta$ dysmetabolism leading to neuronal toxicity is considered the essential abnormality by most investigators.¹⁻⁴ Hyperphosphorylation and filamentogenesis of tau into paired helical filaments (PHFs) is also thought to be important^{5,6} and apolipoprotein E4 genotype is considered an aggravating factor.⁷ The various hypotheses for sporadic AD (s-AD) acknowledge the milieu of aging brain cells but usually omit consideration of an initial sparking event.

For several years it was presumed that β APP/ $A\beta$ and tau abnormalities occur only in the brain (and its blood vessels in regard to β APP/ $A\beta$), until β APP, $A\beta$, and PHFs containing hyperphosphorylated tau were shown to accumulate pathologically within abnormal muscle fibers of two closely related, progressively disabling muscle diseases, sporadic inclusion-body myositis (s-IBM) and hereditary inclusion-body myopathy (h-IBM).⁸⁻¹⁰ s-IBM is a disease of aging muscle with clinical onset nearly always at age 50 or older; h-IBM begins clinically at ages 15-30 in young adult muscle.¹¹ The autosomal-recessive form of h-IBM is caused by a yet-undetermined gene on chromosome 9 p1-q1,^{12,13} not a defect of the β APP gene on chromosome 21; in two families with the autosomal-dominant form of h-IBM, no defect of the β APP-gene was demonstrable.¹⁴ s-IBM is the most common muscle disease in older patients. h-IBM is rather rare, but probably more common than hereditary AD (h-AD).

In s-IBM and h-IBM it was shown that β APP-mRNA was overexpressed in muscle fibers¹⁵ (but not exclusively; cellular prion protein and its mRNA were also overexpressed¹⁶). Several other "Alzheimer characteristic" pro-

teins, including presenilin-1 and proteins related to oxidative stress, also accumulated within abnormal muscle fibers of s-IBM and h-IBM.^{11,17,18}

Intracellular congophilic amyloid deposits are another characteristic and consistent feature of s-IBM muscle fibers.^{8,19} In h-IBM they are very rare in younger patients, but their occurrence, especially in autosomal-dominant forms, increases in older patients.¹¹ The intracellular amyloid of the IBMs is of two structural-chemical types: collections of 15- to 21-nm PHFs containing immunoreactive tau (and other components, but not $A\beta$), and collections ("microplaquettes") containing 6- to 10-nm filaments immunoreactive for $A\beta$.¹¹

Because the same proteins accumulate within s- and h-IBM muscle fibers as accumulate in the brains of patients with sporadic and hereditary forms of AD, the muscle and brain diseases might share certain pathogenic steps and knowledge of one disease might help elucidate the other. Cellular aging and evidence of oxidative stress are associated with the IBMs and the ADs. The IBMs and the ADs are both multifactorial and polygenic. The respective cascades of events leading to the specific form of AD-like IBM muscle fiber degeneration and the similar specific features in AD brain are not understood. Within both the IBM and the AD categories, the pathological phenotypes of sporadic and hereditary forms are very similar, despite the different direct causes being mainly nongenetic *versus* mainly genetic. Therefore, in each disease category it has been proposed, by our group for the IBMs^{20,21} and by others for the ADs,^{22,23} that different etiologies including different genetic defects in the hereditary forms lead to the same

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Hypothetical Model of IBM Pathogenesis

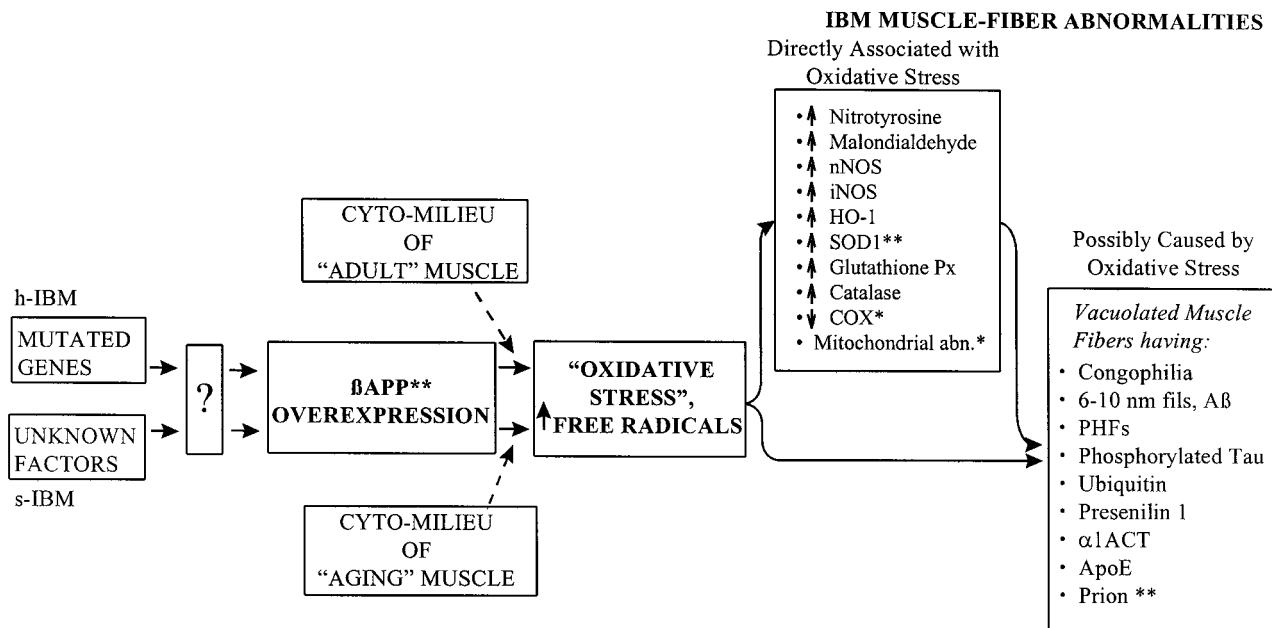


Figure 1. Genetic defects in h-IBM and various factors (including a putative virus) in s-IBM lead to presently unknown mechanisms [“?” box in diagram]. These up-regulate β APP transcription, resulting in β APP overexpression. In the milieu of aging muscle of s-IBM or of adult muscle of h-IBM, overexpression producing excessive β APP (the whole molecule or its A β fragment) leads to oxidative stress with increased free radicals, which contribute to producing the demonstrated IBM muscle fiber abnormalities. Those abnormalities are either directly associated with oxidative stress or possibly caused by oxidative stress. Perhaps some of the former contribute to producing, directly or indirectly, some of the latter. *, present in virtually all s-IBM patients and in the older h-IBM patients; **, both mRNA.

upstream step, which then promotes the final common downstream pathogenic cascade of events resulting in the specific cellular deterioration. Moreover, the intracellular pathogenic cascades of the IBMs and the ADs might have strong similarities following determination by yet-unknown factors of the initial tissue selectivity in each category, muscle *versus* brain.

In both s-IBM and h-IBM, it is of particular interest that individual muscle fibers accumulate N- and C-terminal β APP and A β , and β APP-mRNA, before other abnormalities are evident.⁸⁻¹¹ We hypothesized that overexpression of β APP might be upstream to other cellular abnormalities, including oxidative stress and mitochondrial abnormalities (Figure 1).²¹ To test this hypothesis, wild-type full-length 751 β APP was overexpressed long-term in cultured normal human muscle. It produced within muscle fibers several aspects of the IBM cellular phenotype, including vacuolization, congophilic amyloid inclusions (as microplaquettes) in a small percentage of muscle fibers, cytoplasmic 6- to 10-nm amyloid-like filaments, nuclear PHFs, mitochondrial cytochrome oxidase deficiency, and mitochondrial morphological abnormalities.^{24,25}

Transgenic Mice

This approach of overexpressing β APP in muscle has now been extended in two very interesting studies involving transgenic mice, reported in this issue of the Journal. Fukuchi et al overexpressed a wild-type 99-amino acid C-terminal sequence of β APP,²⁶ and Jin et al²⁷ overex-

pressed a C-terminal 99-amino acid sequence of β APP containing the substitution of lysine-612 to valine (K612V) resulting in a α -secretase-resistant mutation intended to increase A β production. The groups reached some important parallel conclusions.

(1) Within muscle fibers, some aspects of the IBM pathology were produced, including vacuolization and abnormal intracellular accumulation of C-terminal β APP and A β and intracellular amyloid deposits. Whereas Jin et al reported intracellular thioflavin-s-positive amyloid deposits in one-third of the muscle fibers, Fukuchi et al observed polarized-light congo-red positivity in only a few muscle fibers. It remains to be determined whether this difference is due to use of a different β APP sequence, mutant *versus* wild, to generate the transgenic mice or to different staining techniques to identify amyloid deposits. In IBM muscle fibers, because of the small size of amyloid inclusions, evaluating congo-red staining through polarizing filters is often not satisfactory. Using ultraviolet illumination and Texas-red filters greatly increases detection of congophilic amyloid,²⁸ an approach that might be helpful in further studies of the Fukuchi model.

(2) The muscle abnormalities were evident only in aging mice, not being detectable until the mice were 24 months of age or older. In the transgenic mice generated by Fukuchi et al, possibly there was strain-susceptibility.

Additional interesting findings by Jin et al included amyloid-like 6- to 8-nm fibrils within muscle fibers by electron microscopy that closely resemble those present in IBM, and lymphocytic inflammation comparable to that

seen in s-IBM muscle (but usually not in h-IBM). Although in s-IBM the lymphocytic response in muscle can be moderate to prominent, the patients respond only slightly or not at all to anti-dysimmune treatments, suggesting that the major causes of clinical weakness are noninflammatory intracellular malfunctions resulting in muscle-fiber vacuolation and atrophy.²⁹ The results reported by Jin et al now suggest that overexpression of β APP can produce, directly or indirectly, a lymphocytic response in aging mouse muscle, raising the possibility that in s-IBM the lymphocytic inflammation might not be a primary phenomenon. (The term "inflammation" in reference to s- and h-AD pathology is used quite differently and denotes a response not of circulating lymphocytes or granulocytes but of local microglia and astrocytes.)

Another abnormality described by Jin et al that is similar to that typical of IBM muscle^{11,17} is the presence of small, angular, atrophic muscle fibers; in muscle pathology these are usually considered to indicate a denervation component. This feature is especially interesting in view of the recent studies in cultured human muscle fibers demonstrating that experimental overexpression of β APP prevents formation of neuromuscular junctions, apparently by rendering those cultured muscle fibers incapable of becoming innervated.³⁰ Cultured h-IBM muscle fibers also were not able to be innervated, possibly due to their demonstrated overexpression of β APP/A β .³⁰ A synaptopathic mechanism of β APP/A β overexpression might also be responsible for denervation (and possibly more subtle synaptic abnormalities) seen in IBM patients *in vivo* and in the β APP-transgenic mouse. Likewise, in AD, β APP/A β overexpression might be a direct intracellular cause of the known synaptic abnormalities, considered by some to be the most important substratum of the dementia.^{31,32}

In summary, overexpression of β APP/A β in transgenic mice and in cultured human muscle produces changes similar to those in IBM muscle. This is the scenario we have hypothesized (Figure 1).²¹ In some transgenic mice the changes also included lymphocytic inflammation. That the IBM-like changes in the mice were not due to a nonspecific effect of an overexpressed gene is supported by a previous report indicating that in transgenic mice overexpressing the cellular prion protein gene, there was a morphologically nonspecific myopathy that lacked characteristic features of the IBM phenotype.³³

Questions

If overexpression of β APP/A β is a key pathogenic step in the IBMs, several questions become important.

(1) How does excessive β APP/A β cause other changes further downstream in mouse and human muscle? Does it directly or indirectly affect transcription, translation, or protein function of other genes, unleashing a cascade of cellular malfunction? Several of the proteins, including some associated with oxidative stress, that accumulate extrajunctionally within IBM muscle fibers are found in normal human muscle only at the postsynaptic region of the neuromuscular junctions.³⁴

These junctionally concentrated proteins include β APP and its mRNA and presenilin-1, but not tau.³⁴ We therefore proposed, as an aspect of the pathogenic cascade, an upstream junctionalization phenomenon involving initial overexpression of a putative junctionalizing master gene (jmg).^{17,34} The β APP/A β overexpression might either activate a jmg or have a jmg function itself.

(2) If aging of the patient is a key prerequisite for developing either s-IBM or s-AD, in what manner might an aging cellular milieu foster the pathogenic mechanism? This is not known but in general, possible mechanisms, such as more easily induced oxidative stress or mitochondrial dysfunction, might involve decrease of youthful protective influences and/or increase of susceptibility factors.

(3) Are there present within muscle fibers of transgenic mice mitochondrial abnormalities, such as absent cytochrome oxidase activity and ultrastructural distortions and increased markers of oxidative stress, like those found in s-IBM biopsied abnormal muscle fibers^{11,18,20} and in cultured normal human muscle overexpressing the transferred β APP gene?^{11,24}

(4) In s-IBM, what is the initial event activating the putatively β APP/A β -mediated pathogenic cascade? A virus-induced mechanism has been proposed.^{17,29} Because only a small percent of aging persons develop s-IBM, there could be a human strain-susceptibility to either viral infection or viral-induced expression of β APP/A β (eg, by a viral protein acting directly or indirectly on the enhancer/promoter of the β APP gene or on its mRNA) or to consequences thereof. This could be analogous to the possible strain-susceptibility of the transgenic mice proposed by Fukuchi et al and reported previously in relation to Alzheimer models of β APP-overexpressing transgenic mice.^{35,36} Whether a virus might be the initiating spark of s-AD remains to be determined. In contrast to Alzheimer's disease, ApoE4 is not a risk factor for the IBMs,^{37,38} but an increased frequency of the DRB1 0301/0302 allele corresponding to the DR3 HLA haplotype was reported in two studies.^{39,40}

(5) In the h-IBMs, how might a non- β APP gene defect cause β APP/A β overexpression? The same general question applies to the h-AD patients having a non- β APP mutation of presenilin-1 or presenilin-2.^{41,42} A general possibility is that the non- β APP mutant gene product might directly or indirectly affect β APP/A β expression and/or processing. For example, presenilin-1 and presenilin-2 mutations lead to overproduction of A β , as do mutations of β APP.^{1-3,41,42}

(6) Is the pathogenesis of the IBMs essentially intracellular? We have been proposing this for several years and suggested an upstream intracellular pathogenic role of overexpressed β APP/A β , through modulation of expression of other genes and induction of the demonstrated oxidative-stress and mitochondrial abnormalities.^{17,18,20} The β APP-overexpressing cultured human muscle and transgenic mice support an upstream role for intracellularly overexpressed β APP/A β . In the IBMs, extracellular amyloid plaques do not form. Even though normal muscle fibers in culture secrete A β 40 and A β 42 and those secretions are greatly increased in β APP-over-

expressing cultured muscle fibers (Xie, McFerrin, Engel, Selkoe, and Askanas, unpublished observations, 1997), medium from the latter is not myotoxic.^{24,25} *In vivo*, it is possible that clearance of extracellular A β is more effective from muscle than from brain.

IBM muscle fiber nuclei do not show apoptotic changes by the TUNEL.^{43–45} However, in all s-IBM patients, 2–3% of the muscle nuclei manifest at least a few disease-characteristic PHFs in addition to those in the cytoplasm,¹¹ and some autosomal-dominant h-IBM nuclei possess many tau-containing PHFs.⁴⁶ These indicate a disturbance of nuclear metabolism that could be accompanying malfunction of DNA and/or RNA. The cultured human muscle experimentally overexpressing the β APP gene had a few of those intranuclear PHFs.²⁵

By analogy with IBM, we raise the possibility that in AD there might be similar mechanisms of intracellularly overexpressed β APP/A β directly inducing abnormality in neurons to cause the essential damage. Although AD investigators generally have favored an initially extracellular role of A β toxicity,^{1–4,31,42} recently some have endorsed intracellular mechanisms.⁴⁷ Because extracellular A β experimentally can be neurotoxic,^{2,4} it is possible that extracellular A β in AD can exert an additional adverse effect.

In IBM muscle fibers, apolipoprotein-E (ApoE) is pathologically localized on the A β -containing 6- to 10-nm filaments and the tau-containing PHFs,⁴⁸ but it has not yet been studied in the IBM transgenic mice. In AD, astrocyte-derived intraneuronal ApoE is considered to aggravate neurotoxicity and to be isoform-dependent, ie, more prominent with ApoE4.⁷ In both IBM and AD, an ApoE aggravation of cytotoxicity might occur by enhancing intracellular damage caused by overexpressed neuronal β APP/A β . Perhaps ApoE also facilitates formation of intracellular 6- to 10-nm fibrils and PHFs.

An obvious corollary of the hypothetically intracellular mechanisms of IBMs and ADs is that a therapeutic drug must enter or at least modulate the intracellular milieu. The two new transgenic mouse models of IBM, like our β APP/A β -overexpressing human muscle cultures, may now be used to explore intracellular pathogenic mechanisms and proposed treatments of β APP/A β overexpression in muscle fibers.

(7) To support a central role for β APP/A β in the pathogenesis of s- and h-IBM, is more evidence needed in the β APP overexpression models involving cultured human muscle and the transgenic mice regarding formation of PHFs, accumulation of tau and other Alzheimer-characteristic proteins, and muscle weakness in the transgenic mice? Such evidence would certainly support the hypothesis. In the β APP-overexpressing transgenic mice, the question of hyperphosphorylated tau accumulation was not addressed, and ultrastructural PHFs in muscle were not found. In cultured human muscle overexpressing the transferred β APP gene, PHFs like those in s- and h-IBM were present in muscle nuclei but not yet found in the muscle cytoplasm.²⁵ Perhaps longer-cultured, longer-overexpressing, older muscle fibers will also manifest the cytoplasmic tau-containing PHFs typical of the IBMs. It is reported that A β up-regulates tau protein kinase I (TPK-I),

resulting in hyperphosphorylated tau, which can lead to PHF formation⁴⁹ (A β -up-regulated TPK-I can also phosphorylate, and thereby inactivate, mitochondrial pyruvate dehydrogenase, resulting in cellular depletion of acetyl CoA and consequently reduced ATP synthesis⁴⁹). From another aspect, one can ask whether in IBM and AD the accumulated hyperphosphorylated-tau PHFs contribute early and significantly to cellular malfunction, or are simply diagnostically interesting parallel markers, somewhat later-appearing and themselves relatively harmless? If not critically pathogenic, is PHF formation actually necessary to enhance validity of the models?

Conclusion

The two new reports of muscle abnormality in transgenic mice overexpressing β APP provide exciting data and intellectual stimulation for further studies of the intracellular molecular pathogenic mechanisms of β APP/A β , which may relate to both the IBMs and the ADs.

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