What evidence would prove the amyloid hypothesis? Towards rational drug treatments for Alzheimer’s disease

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As one of us (DM) listened intently to the debates presented at the meeting on Challenging Views of Alzheimer’s Disease, I was struck by the strong desire by some in attendance to find fault with the amyloid hypothesis of Alzheimer’s disease (AD). Arguments were presented in multiple discussions leading to the conclusion that the relationship between amyloid and AD was so tenuous that we should not even waste time developing drugs directed at reducing amyloid.

What was most surprising was the level of proof to which the amyloid hypothesis was being held by those attempting to refute it. I was left with the impression that if every symptom in every patient regardless of patient age, gender, state of disease progression or concurrent disorders, did not correlate linearly with the amyloid burden, then the assumption was the amyloid hypothesis must be false. For example, some patients die with brains loaded with enough amyloid to reach the criterion for AD, yet they are cognitively fit as fiddles. Alternatively, patients die with full blown dementia, diagnosed as AD, yet they have little or no amyloid pathology.

The animal models are argued to be failures because not all aspects of the AD phenotype are manifest. They have brains filled with amyloid, yet there is little neuron loss or tangle formation. Thus, the amyloid hypothesis must be wrong or the mice should recapitulate the disease. Even the memory loss found in these mice is suspect because the relationship with Aβ deposits is not linear under all circumstances.

The arguments against the amyloid hypothesis never address the strengths of the hypothesis, which is largely the genetics of the disease (AβPP, presenilin and Down’s syndrome), but dismiss these data as affecting too few individuals to be meaningful.

It took me some time to understand why I was so uncomfortable with these arguments. It was ultimately because the level of proof being required by the anti-amyloid group was beyond the reach of any similar disease and its causal entities. I began to consider the analogy of current Alzheimer’s disease research in light of cardiovascular disease research two to three decades ago. Both of these disorders have plaques in their etiology. The plaque in AD is neuritic and the one in cardiovascular disorders is atherosclerotic. The plaque in AD has amyloid as a major component; a major component of atherosclerotic plaques is cholesterol. At the present time, most regard the connection between elevated serum cholesterol levels (particularly serum LDL-cholesterol) and cardiovascular disease as well established. Indeed, we doubt anyone would name their pet “Cholesterol”, so extensive is the public perception of this substance as the great life-shortener. What level of evidence has been required to reach this consensus?

Does everyone with atherosclerotic plaques develop symptoms of cardiovascular disease? The answer certainly is no. But that wasn’t enough to kill the hypothesis. Does everyone with symptoms of cardiovascular disease...
disease have atherosclerotic plaques? In this case, since most have some, the answer is probably yes. However, do the symptoms of cardiovascular disease covary one for one with the extent of plaque formation? Certainly not. On a statistical basis, and the relationship is nonlinear owing to threshold pathology required for symptomatic expression of disease. Occult coronary artery stenosis increases dramatically with age [7].

What evidence supports a role of cholesterol in this disease? There is a modest association of elevated cholesterol with increased risk for cardiovascular disease. However, it is not linear and requires large sample pools to gain statistical significance. Some individuals have massively elevated total cholesterol, yet no plaque formation. This mismatch led some to argue that the evidence associating cholesterol with atherosclerotic cardiovascular disease was so weak that the theory should be discarded (e.g., see [8]). Yet these objections did not squelch the hypothesis relating cholesterol levels to cardiovascular disease. Instead it led to intensive evaluation of different types of total cholesterol, leading to the recognition that one cholesterol fraction was closely linked with pathology while another was, in fact, beneficial. I find it intriguing that we are now recognizing multiple Aβ fractions (soluble, oligomeric, fibrillar, intracellular, etc) with various data suggesting that some forms may be more closely linked to pathology than others. To me this cries out for further evaluation, not dismissal of the hypothesis.

Genetics. Certainly one of the strongest arguments in favor of the cholesterol hypothesis was the identification of families in which atherosclerotic cardiovascular disease occurred at early ages, with pathologies that closely resembled the more general disease found in older patients. Finding that these families exhibited hypercholesterolemia due to a defect in the LDL receptor certainly strengthened the association between cholesterol (particularly LDL-cholesterol) and cardiovascular disease [2]. Subsequent studies [9] using transgenic mice carrying defects in ApoE (which binds to the LDL receptor) offered strong confirmation of the association between elevated serum cholesterol and atherosclerosis.

Animal models. One would certainly predict that if high cholesterol caused atherosclerotic plaques and cardiovascular disease, then feeding mice or rats large amounts of cholesterol and elevating their plasma cholesterol should lead to plaque formation. However, it does not [5]. Did these studies relegate the cholesterol hypothesis to myth? Fortunately, they did not. Rodents differ significantly from humans in that they are protected from cholesterol-induced atherosclerosis, due primarily to the efficient hepatic uptake of cholesterol-remnant lipoproteins in these species [1]. Other animal models were required to demonstrate the effects of cholesterol on plaques. Had transgenecs been available then, and researchers studied only mice as models, as we largely do in AD research today, we might have abandoned the cholesterol hypothesis. Mice differ from men in more than size and lifespan.

However, the most convincing evidence supporting the cholesterol hypothesis comes from the effects of the statin drugs. The Scandinavian Simvastatin Survival Study [6] demonstrated that randomization to simvastatin reduced the risk of major coronary artery events by over one third. Additional studies continue to confirm that these agents, developed specifically because of the cholesterol hypothesis, reduced the risk of a second heart attack, and recently reduced risk of a first heart attack in those with elevated cholesterol. Anecdotally, the Japanese were the first to discover the statins, but they discarded them for human use because the drugs performed poorly in rodents. Merck subsequently showed that the drugs work well in dogs, and went on to human studies — eventually realizing a billion dollar a year market in Mevacor.

Not surprisingly, the first attempts to reduce cholesterol were not very successful in minimizing disease risk [1]. Dietary manipulations, niacin, bile acid sequestrants, and other agents had only modest effects on cholesterol levels, and on disease risk. Importantly, the fact that drugs directed at targets other than cholesterol also mitigate disease progression (e.g. antihypertensive drugs) does not detract from the role of cholesterol in cardiovascular disorders.

Hopefully, the relevance of this comparison to the present debate regarding the amyloid hypothesis is apparent. The pathology found in Alzheimer’s disease brains first suggested that plaques were important. The chemistry of plaques led to identification of Aβ-containing amyloid. The genhetics of familial disease led us to view the long variant of the Aβ peptide as more likely to be toxic. Certainly the amyloid hypothesis is sufficiently flexible that it can accommodate the possibility that not all forms of Aβ are equally pathogenic, as turned out to be the case for cholesterol.

However, the real challenge to the amyloid theorists is now to develop effective medications. The ultimate proof of the hypothesis, as for cholesterol, will require specific therapies designed to effectively diminish Aβ levels which ultimately prevent, slow, arrest or reverse the disease. These might be amyloid vaccines, gamma
secertase inhibitors. BACE inhibitors, plaque busters or other not yet described drug targets that unfold as we understand more about \( A_\beta \) production and clearance. If the first attempts fail, that should not lead to premature abandonment of the hypothesis. It will likely take the next decade of drug trials to fully test the amyloid/A_\beta plaque hypothesis of Alzheimer’s disease. Still, the identification and characterization of other potentially druggable sites should be encouraged. Antioxidants, and excitotoxin inhibitors (NMDA antagonists) modifying downstream effects on A_\beta also merit controlled clinical evaluations. Similarly, drugs modifying tangle formation should be tested as such agents develop. But, these other initiatives should not preclude the continued development of anti-amyloid therapies.

It is most important in this context to separate the concepts of causality and risk. Clearly, no one can claim that elevated serum cholesterol necessarily causes death from coronary artery disease. The same is true for amyloid and AD. They are risk factors. Another analogy is blood alcohol levels and automobile accidents. Elevated serum ethanol is a risk factor. So too are poor brakes, disregard of traffic lights, and talking on a mobile phone. But there are many impaired drivers who do not have accidents. And not all accidents involve drunk drivers. But driving very drunk (analogous to an APP transgenic mouse with unavoidably elevated levels of cholesterol or amyloid) almost ensures an accident. And driving drunk through red lights while talking on the phone almost guarantees a collision, sooner or later. Similarly, elevated cholesterol along with smoking, hypertension and a genetic predisposition, almost guarantees an early death from coronary artery disease. And elevated amyloid, along with age, apo E status, and other (unknown) factors greatly increases the risk of AD. To carry the analogy further, eliminating serum ethanol from drivers will not eliminate accidents – it will reduce them because it eliminates a risk factor. Reduction of serum cholesterol with statins did not eliminate cardiovascular disease deaths, since it similarly is a risk factor. (In contrast, elimination of tuberculobaccillus by antibiotics will eliminate tuberculosis, because it is a causative agent). By similar reasoning, is it not logical to develop drugs that lower the levels of one of the major risk factors for AD?

A final comment on this dialectic regarding the amyloid hypothesis derives from the history of science. While hardly a scholar in this regard, DM has been continuously struck by the descriptive accuracy of Kuhn’s book on the Structure of Scientific Revolution [4]. Kuhn argues that old scientific theories are never disproven, but that rival theories emerge which ultimately fit the data better and supercede the older ideas. This is what has happened to the cholinergic hypothesis of Alzheimer’s disease. It was never disproven, but has been displaced by the amyloid treatise. Part of the reason is the rather modest efficacy of medications based upon the hypothesis in treating the disease. Another fascinating point from Kuhn is that the diehards never really relent; they go to their graves chastising the young turks for not having been there when. The real challenge to those deriding the amyloid hypothesis is to develop evidence regarding the alternative hypotheses and to convince students that these ideas fit the data better. The ultimate proof will require development of effective treatments based on the hypothesis. The challenge to the amyloid theorists (or theists as Joseph et al. [3] might have it) is to examine the critiques of the hypothesis, and explain why the relationships are not as tight as they might be if biology were physics. Yet, the ultimate proof will require development of medications designed to mitigate the purportedly deleterious effects of amyloid. Supported by AG 18478, AG 15490 and AG 20227.

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I wish to thank the organizers of this meeting for arranging the debates which were lively and informative. I welcome the development of potentially new, improved approaches to curing this disorder, but until they come along, make mine anti-amyloid.

References

