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Commentary

Inheritance of the *E4* allele of the apolipoprotein E gene (*APOE4*) substantially increases the risk of developing late-onset Alzheimer disease (AD). A large body of evidence has firmly established a role for apoE in modulating the risk of developing the amyloid plaque pathology that is pathognomonic for AD. In this issue of the *JCI*, Liao and colleagues discovered that antibodies against a nonlipidated form of apoE4 are highly effective in delaying the deposition of amyloid β (Aβ) peptides in mouse models of AD pathology. Using a combination of passive immunization and viral-mediated expression of recombinant antibodies, the authors show that Fc receptor–mediated clearance of the nonlipidated apoE4 was critical in delaying Aβ deposition. Collectively, this study identifies a new therapeutic target that could be exploited to prevent, or possibly reverse, the Aβ pathology of AD.

Targeting the accomplice to thwart the culprit: a new target for the prevention of amyloid deposition

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Inheritance of the *E4* allele of the apolipoprotein E gene (*APOE4*) substantially increases the risk of developing late-onset Alzheimer disease (AD). A large body of evidence has firmly established a role for apoE in modulating the risk of developing the amyloid plaque pathology that is pathognomonic for AD. In this issue of the *JCI*, Liao and colleagues discovered that antibodies against a nonlipidated form of apoE4 are highly effective in delaying the deposition of amyloid β (Aβ) peptides in mouse models of AD pathology. Using a combination of passive immunization and viral-mediated expression of recombinant antibodies, the authors show that Fc receptor–mediated clearance of the nonlipidated apoE4 was critical in delaying Aβ deposition. Collectively, this study identifies a new therapeutic target that could be exploited to prevent, or possibly reverse, the Aβ pathology of AD.

Introduction

One of the most replicated genetic association studies in Alzheimer disease (AD) is the discovery that the apolipoprotein E (*APOE*) genotype modulates the risk of developing AD (1, 2). Notably, apoE immunoreactivity was first described in association with both amyloid β (Aβ) plaques and neurofibrillary tangles in humans well before genetic studies linked *APOE* to AD (3). The major alleles of human *APOE* are *E2*, *E3*, and *E4*, all producing 299 aa-secreted products that differ at aa 112 and 158 as follows: *E2*, Cys/Cys; *E3*, Cys/Arg; *E4*, Arg/Arg (reviewed in ref. 4). *E2* is the least common and *E3* is the most common allele. Forty percent of all patients with sporadic AD have 1 allele of *APOE4*, and the risk increases 5- to 10-fold in subjects with 2 alleles (5). *APOE* has been implicated in a plethora of pathways that could potentially modulate the risk for AD, including modulating the toxicity of tau pathology (6), modulating neuroinflammation, impairing mitochondrial function, and altering lipid metabolism (reviewed in ref. 7). However, studies in humans and preclinical mouse models have firmly established that the presence of *APOE4* leads to earlier onset of amyloid pathology (recently reviewed in ref. 8), suggesting that the primary mechanism by which apoE modulates the risk of AD is by modulating the deposition of Aβ.

Antibodies against nonlipidated apoE emerge as new biotherapies for AD prevention

The study by Liao and colleagues evaluated a series of antibodies against human apoE (9). Through careful analysis they determined that antibodies against a nonlipidated, possibly aggregated, form of apoE4 are highly effective in delaying the deposition of Aβ peptides in mice that express human mutant amyloid precursor protein (APP), human mutant presenilin (PS1), and human apoE4. Beginning with passive immunization to screen antibodies, the antibody that emerged as most efficacious was poorly reactive to lipidated forms of apoE3 or apoE4. Using adeno-associated virus–mediated CNS expression of recombinant antibodies, the authors show that Fc receptor–mediated clearance of the nonlipidated apoE4 was critical in delaying Aβ deposition. Passive immunization with antibodies that recognized the lipidated forms of apoE was ineffective, due partially to binding to lipidated apoE in plasma and rapid clearance of the immune complex. The antibodies to nonlipidated apoE4 were highly reactive to cored-neuritic plaques in the APP/PS1 mice, suggesting that the nonlipidated protein closely associates with Aβ aggregates. Thus, the presumptive mechanism by which these antibodies delay the deposition of Aβ is through microglial-mediated phagocytosis of APOE4/Aβ complexes that form early in the formation of Aβ deposits (Figure 1). Whether these antibodies could be effective in promoting the clearance of preexisting Aβ deposits requires further investigation.

Whether nonlipidated apoE is an active or passive accomplice in the deposition of Aβ is unclear. Studies in transgenic mice have generally shown that apoE plays a pivotal role in Aβ deposition. Targeted inactivation of the endogenous *apoE* allele in mice that overexpress mutant APP profoundly inhibits Aβ deposition (10). Targeted replacement of endogenous *apoE* alleles with human *APOE* alleles has demonstrated that the *APOE4* allele is significantly more amyloidogenic than the *E2* or *E3* alleles (11, 12). More recently, a pair of studies demonstrated that apoE is critical in the early stages of Aβ oligomerization and assembly, showing much less influence once deposition has taken hold (13, 14). Most effort in the field has focused on the lipidated forms of apoE. Studies have shown that lipidated apoE4 preferentially stabilizes Aβ oligomers (15), selectively promotes Aβ fibrillization (16, 17), and has a greater affinity for Aβ peptides (2, 18). Early studies with recombinant apoE isolated from *E. coli* reported that nonlipi-

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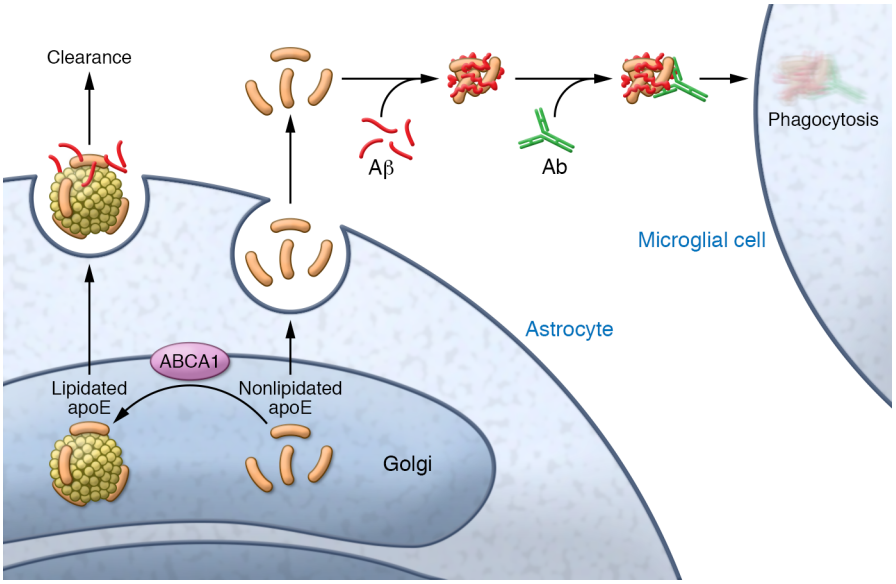

Figure 1. Antibodies stimulate phagocytosis of apoE/Aβ conglomerates to slow the formation of pathological amyloid deposits. Through the action of the ATP-binding cassette transporter A1 (ABCA1) most apoE acquires lipid in the Golgi and is secreted as proteolipid particles that bind monomeric Aβ and facilitate clearance through multiple pathways. A small fraction of apoE, particularly apoE4, fails to assemble into proteolipid particles and is prone to bind assemblies of Aβ that ultimately seed the formation of pathological Aβ deposits. Antibodies that selectively bind the nonlipidated apoE opsonize the apoE/Aβ conglomerates, leading to phagocytosis and degradation by resident microglia.

dated apoE may interfere with Aβ fibrillogenesis in vitro (19). However, studies in which ATP-binding cassette transporter A1 (ABCA1), a key cholesterol transporter in apoE lipidation, has been inactivated in mutant APP mice suggested that poorly lipidated murine apoE was associated with more severe Aβ deposition (20). Moreover, the effect of ABCA1 deletion in APP/PS1/APOE4 mice on Aβ deposition was more severe than the effect of ABCA1 deletion in APP/PS1/APOE3 mice (21). If nonlipidated apoE4 is an active accomplice in promoting Aβ deposition, and if this isoform of apoE is indeed more prone to escape lipidation, then one might predict that transgenic overexpression of human *APOE4* in mutant APP or APP/PS1 mice should dramatically worsen Aβ pathology. However, 2 separate studies using different transgenic approaches did not show that overexpression of human *APOE4* exacerbated Aβ deposition (22, 23). Thus, although the evidence is clear that poor lipidation of apoE4 is associated with more severe Aβ deposition, it is still not entirely clear whether this effect is due to an active role of nonlipidated protein in promoting amyloidogenesis, or due to diminished abilities of poorly lipidated protein to participate in the clearance of Aβ in the CNS (mechanisms reviewed in ref. 8).

Summary and future directions

Whether nonlipidated apoE4 is an active or passive accomplice does not diminish the potential utility of targeting this particular species of apoE by immune therapy if removing it also removes the initial assemblies of Aβ that are the culprit in initiating Aβ deposition. One advantage of targeting nonlipidated CNS apoE is that it appears to be a relatively minor fraction of the total systemic apoE burden. In this setting, passively administered antibody should survive longer in the plasma and potentially provide efficacy at a lower or less frequent dose. Indeed, data from the Liao study demonstrated that antibodies specific for nonlipidated apoE4 had longer plasma half-lives (9). These apoE antibodies also have the potential advantage of avoiding on-target side effects of Aβ-targeting antibodies that produce amyloid-related imaging abnormalities. Moving this avenue of therapy forward will require a better understanding of the level of nonlipidated apoE4 in humans. It is noteworthy that a recent study identified the *ABCA7* gene, which encodes a cholesterol transporter expressed in CNS, as the strongest risk factor after APOE for early Aβ pathology (24). The Liao study did not address whether treatments with apoE antibodies could clear preexisting Aβ deposits — a key unknown in translating this therapy to patients who already have significant Aβ pathology. Whether antibodies against nonlipidated apoE could displace Aβ-directed immune therapies as treatments to remove preexisiting Aβ deposits is uncertain. The Aβ-directed immune therapies are poised for phase 3 clinical trials, and if they are the first to be approved in human therapy, then any new therapy would have to be evaluated in comparison to or in combination with the existing therapy. However, for the reasons outlined above, apoE antibodies targeting nonlipidated protein could ultimately emerge as the better choice for a preventive biotherapy in high-risk *APOE4* carriers.

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