Commentary

Aβ immunization: Moving Aβ peptide from brain to blood

Virginia M.-Y. Lee*

Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

After many years of intense research on the etiology and pathogenesis of Alzheimer's disease (AD), the amyloid β (Aβ) peptide, the major component of senile plaques, has become a realistic target for developing effective therapies for AD. A recent study showing that simple immunization with the more amyloidogenic 42-aa-long Aβ peptide (Aβ42) can reduce Aβ levels, inhibit the deposition of amyloid onto existing plaques, and clear established senile plaques that are present in brain of a mouse model of AD amyloidosis has raised hopes for a potentially important new therapeutic approach to treat AD (1). This observation surprised the AD community, and the significance of this finding has already resulted in a request for applications of grants initiated by former President Clinton from the National Institute on Aging targeted specifically at providing an understanding of how and why this approach may work as potential therapy for AD. Thus, although the underlying mechanism(s) of Aβ immunotherapy remain unclear, it has already opened up a whole new area of research to gain insight into why such an approach can lead to the elimination of amyloid deposits in the brains of transgenic mice that develop AD amyloidosis. In a recent PNAS issue, DeMattos *et al*. (2) provide mechanistic insights on this remarkable effect. These authors peripherally administered an anti-Aβ monoclonal antibody m266 by i.v. injection into transgenic mice (PDAPP) that overexpressed a mutant amyloid precursor protein (APP) in which valine, the normal amino acid residue at position 717, is mutated to phenylalanine, and they showed a dramatic 1,000-fold increase in plasma Aβ level. Because the plasma levels of Aβ in the untreated animals were very low and because Aβ is produced only in the brains of these mice, the authors proposed that m266 in the plasma acts as a "peripheral Aβ sink" to facilitate the efflux of Aβ from brain to plasma in the PDAPP mice. They then went on to show that long-term peripheral administration of m266 to PDAPP mice markedly reduces Aβ burden without the antibody actually crossing the blood brain barrier and binding to Aβ deposits in the brain. Because recent studies have shown that exogenous 40-aa-long Aβ peptides (Aβ40) can be transported rapidly from cerebral spinal fluid (CSF) to plasma (3–5), the authors conclude that the likely mechanism to explain why peripherally administered m266 can remove Aβ deposits from brain is by altering the dynamic equilibrium of Aβ between brain, CSF, and plasma such that a reduction of plasma Aβ can lead to an efflux of brain Aβ to the CSF and into the circulation.

However, this conclusion differs significantly from several recently published studies proposing other possible mechanisms to explain the basis of Aβ immunotherapy. For example, Bard *et al.*, also used peripherally administered anti-Aβ antibodies in the PDAPP mice, but they showed that the antibodies cross the blood-brain barrier (BBB), enter the central nervous system (CNS), bind to amyloid plaques, activate microglial cells, and induce the clearance of preexisting amyloid (6). Indeed, these authors went on to demonstrate that, in an *ex vivo* assay using brain sections from PDAPP mice or AD cases, exogenously added anti-Aβ antibodies triggered exogenously added microglial cells to clear plaques through Fc receptor-mediated phagocytosis and subsequent peptide degradation. Significantly, another study also showed that the direct application of anti-Aβ antibodies on the surface of the cortex of living PDAPP mice also resulted in a decrease in Aβ deposits in the immediate vicinity of the application (7). Because microglial activation was also observed, these authors concluded that the direct binding of anti-Aβ antibodies to senile plaques is an essential first step leading to their clearance. Based on the foregoing, there is little doubt that, once anti-Aβ antibodies gain entry into the brain and bind to amyloid, microglia would clear them. However, the big question here is whether or not sufficient anti-Aβ antibodies cross the blood-brain barrier and enter the CNS. Although the endogenous immunoglobulins in brain parenchyma of mice represent about 0.1% of the antibody concentration in serum, DeMattos *et al*. were not able to detect any anti-Aβ antibodies bound to senile plaques after peripheral administration, whereas Bard *et al.* did detect plaque-bound antibodies. The only difference between these two studies is the route of this peripheral administration. Whereas DeMattos *et al.* injected the antibodies i.v., Bard *et al.* administered the antibodies via i.p. injection. It is conceivable that the different routes of administration account for the ability of anti-Aβ antibodies to cross the BBB in one study but not in the other. However, based on published reports and the data presented in DeMattos *et al.*, it is likely that circulating anti-Aβ antibodies did not cross the BBB, but instead acted as a "peripheral Aβ sink" to remove Aβ from the brain. The reasons for making such conclusions are as follows.

First, as stated before, soluble Aβ can be transported from the brain to plasma because direct injection of radiolabeled Aβ into brain results in recovery of labeled Aβ in plasma (5). This transport appears to be bidirectional because Aβ can be transported from the plasma to CNS and vice versa (3–5). The mechanism whereby Aβ moves in and out of the brain and across the BBB is through a receptor-mediated transport mechanism. Thus, these results support the existence of a dynamic equilibrium between soluble Aβ in the CNS and various peripheral compartments. Accordingly, alteration of Aβ levels in one compartment perturbs this equilibrium, resulting in the transport of Aβ from one compartment to the other until a new equilibrium is reached. Indeed, the results of DeMattos *et al.* are consistent with the ability of Aβ to move from one compartment to another because they demonstrated that the accumulation and sequestration of Aβ by m266 in the plasma resulted in the massive efflux of brain Aβ

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*E-mail: vmylee@mail.med.upenn.edu.

into the circulation. Second, Aβ immune therapy appears to be much more efficacious in younger transgenic mice without amyloid deposition than older mice that contain extensive brain amyloid plaques (1, 6, 8, 9). This observation is more consistent with the peripheral Aβ sink hypothesis because, in the absence of Aβ deposits, the sequestration of soluble Aβ by anti-Aβ antibodies in the plasma of young PDAPP mice effectively reduces soluble brain Aβ levels such that there would be insufficient Aβ left in the brain of these mice to aggregate into insoluble deposits. On the other hand, the reduced effectiveness of Aβ immunotherapy in older mice could be explained by the inability of aggregated insoluble Aβ to convert into freely diffusible soluble Aβ. In this scenario, although circulating anti-Aβ antibodies can still sequester newly synthesized soluble Aβ and limit further amyloid deposition, the highly insoluble amyloid plaques could only be eliminated slowly by a normal turnover process. Other examples of a process for plaque turnover have been shown previously in a transgenic mouse model of amyloidosis (10). By contrast, if Aβ immunotherapy is working by the antibodies crossing the BBB, gaining entry into brain, binding to existing amyloid plaques resulting in Aβ being eliminated by microglial cells, then the reversal of plaque formation should be as efficient in older mice with plaques as in younger mice without plaques, but this phenomenon was not observed in several published studies (6, 8, 9). Thus, additional work is required still to resolve how Aβ immunotherapy occur.

However, irrespective of the exact mechanism of Aβ vaccination therapy for AD, the most important question is whether or not it will work in patients. Preventing and reducing plaques in transgenic mice and reversing the course of AD in humans are two very different problems. In transgenic mouse models of amyloidosis, very high levels of Aβ are already present in the plasma and CSF before amyloid deposition, compared with nontransgenic animals and their levels remain very high even after plaque formation (11). By contrast, only very low levels of Aβ are found in the plasma and CSF of control and AD patients with the exception of those with familial AD bearing the Swedish mutation (12). The high circulating levels of Aβ in transgenic mice might explain the tremendous immune response when these mice were injected with aggregated Aβ42 peptide, but it is unclear whether or not similar immunization in AD patients will elicit strong immune responses. Furthermore, if the peripheral Aβ sink hypothesis is indeed correct in explaining plaque clearance in mice, removal of extensive plaque burdens from AD patients' brains may not be feasible or it might be a very slow process. Therefore, for Aβ vaccine to work in humans, it may be necessary to select AD patients with low plaque burdens. On the other hand, if Aβ vaccination in AD patients works by crossing the BBB and binding to amyloid plaques and facilitating clearance by microglial cells, AD patients with abundant amyloid plaques should be responsive to this treatment. Finally, Hyman *et al.* demonstrated in a recent study the presence of autoantibodies to Aβ in individuals with or without AD, but these authors were unable to correlate the presence or the levels of these Aβ autoantibodies with the likelihood of developing dementia or with plasma levels of Aβ peptide. This observation suggests that low levels of Aβ autoantibodies, although frequent in the elderly population, do not confer protection against developing dementia (13). Thus, whether or not Aβ immune therapy will work in AD patients awaits the results of the ongoing vaccination trials.

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Significantly, Aβ vaccine is but one of several emerging therapeutic approaches targeting the production, clearance, and aggregation of the Aβ peptide. For example, the identification of the two crucial enzymes responsible for Aβ production (i.e., BACE as the β-secretase and the presenilins as possible γ-secretase) provide therapeutic targets for their inhibition (14, 15). Furthermore, recent studies have shown that commonly used cholesterol-lowering drugs can reduce Aβ levels in cell culture and animal models (16) thereby providing yet another therapeutic approach to reduce Aβ levels and amyloid deposition. Other approaches, including the use of soluble Aβ derivatives and small compounds that bind to and inhibit Aβ fibrillization, are also becoming available (17, 18). Thus, these and other therapeutic strategies should allow us to address the fundamental question regarding the importance of amyloid deposition in AD patients. More important, one or another of these avenues could be the beginning of the long hoped for road to a world without AD.

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