As neuroscientists, we are taught that the brain is immune privileged and thus unlikely to be affected by the peripheral immune system. Accordingly, initial results demonstrating the effectiveness of β-amyloid (Aβ) immunotherapy in mouse models of Alzheimer’s disease (AD) were viewed with considerable surprise and some skepticism. Many groups have since demonstrated efficacy with Aβ immunotherapy in models of AD, using Aβ-based immunogens and anti-Aβ antibodies. Clinical trials involving Aβ immunotherapy for AD are in progress and are providing a wealth of information around the amyloid hypothesis of AD. Aβ immunotherapy is also raising new opportunities and questions about the general role of the immune system in neurodegenerative diseases.

Introduction

Although Alzheimer’s disease (AD) is characterized histologically by β-amyloid (Aβ)-containing plaque lesions and tau-containing neurofibrillary tangles in the brain [1], the primary cause of the disease is currently not well understood. Genetic evidence has implicated Aβ as a possible causative element, and evidence supporting this ‘amyloid hypothesis’ includes the observation that a chronic reduction in Aβ, most notably by Aβ immunotherapy (Figure 1), leads to a reduction in AD pathology and improvements in cognitive performance in animal models of the disease and, potentially, in AD patients [2–12,13]. These encouraging observations must be balanced with the finding that AN1792, the 42 amino acid form of Aβ, resulted in an adverse event involving meningoencephalitis in a subset of patients that were treated during clinical trials [14].

The general target of Aβ immunotherapy is clearly some form or forms of the Aβ peptide; however, the precise mechanisms involved are still under investigation and several theories currently exist. Accordingly, thorough consideration of the immune system, its components, and the positive or negative role they play in Aβ immunotherapy is warranted. We are continuing to gain additional information from preclinical animal models and ongoing clinical trials to better understand the potential of Aβ immunotherapy for the treatment of AD.

Beta-amyloid immunotherapy in animal models

Early work in amyloid precursor protein (APP) transgenic mice that exhibit widespread amyloid plaque pathology has demonstrated that immunization with Aβ can halt and perhaps even reverse the development of cerebral amyloid plaques. In very young PDAPP mice, immunization of Aβ1-42 plus adjuvant over the course of one year resulted in an almost complete absence of Aβ plaque pathology [2]. In a study in older PDAPP mice that had pre-existing plaques, the same immunization protocol resulted in levels of plaque pathology lower than normal baseline levels, and significantly lower than in age-matched, untreated controls [2]. In these treated older mice, clear evidence of active clearance of amyloid by microglial cells was noted. This first report of an immunotherapeutic approach that could strongly reduce plaque pathology in APP transgenic mice resulted in a myriad of questions to better understand the fundamental principles underlying the phenomenon.

The key question of whether a humoral or cellular response mediates the effectiveness of Aβ immunization was clarified by Bard et al. [3], who demonstrated that peripheral administration of antibodies to Aβ is sufficient to reduce Aβ plaque pathology in PDAPP mice (Table 1). These authors also demonstrated that anti-Aβ antibodies bind to plaques (Figure 2) and trigger microglial clearance of amyloid plaques through an Fc receptor-mediated mechanism in ex vivo brain sections from PDAPP mice and AD patients. Other investigations into the mechanism of antibody-induced Aβ plaque clearance have yielded slightly different results. DeMattos et al. [10] showed that a highly specific monoclonal antibody to Aβ, first described by Seubert et al. [15], could apparently reduce Aβ pathology in transgenic mice in the absence of antibody binding to plaques. It was hypothesized that the antibody reduced brain plaque density by serving as a peripheral sink to sequester Aβ away from the brain and prevent deposition of new plaques.

**Abbreviations**

Aβ beta-amyloid
AD Alzheimer’s disease
APP amyloid precursor protein

**Addresses**

1 Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, California 94080, USA
2 Wyeth Research, 401 N. Middletown Road, Pearl River NY 10965, USA
*e-mail: dale.schenk@elan.com

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Beta-amyloid (Aβ) immunotherapy strategies. Immunotherapy can be achieved through either active immunization with full length Aβ or an Aβ immunoconjugate, or passive administration of monoclonal anti-Aβ antibodies. After active immunization with Aβ1-42, the peptide is processed by antigen-presenting cells and Aβ fragments are presented to T cells. Subsequently, various B-cells that can recognize epitopes on Aβ1-42 are engaged, proliferate and produce polyclonal anti-Aβ antibodies. The second type of active immunization approach involves the administration of small fragments of Aβ conjugated to an unrelated carrier protein. The immunological response after immunization of an Aβ peptide-carrier protein conjugate is similar to the first strategy, with the exception that the T cells are stimulated by the carrier protein rather than the Aβ fragment (which lacks T-cell epitopes). The third strategy involves direct administration of monoclonal antibodies directed against Aβ and, as such, does not require any type of immunological response from the host.

### Table 1

**Overview of preclinical studies investigating the mechanism of immunotherapy-induced β-amyloid plaque clearance.**

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Immunization</th>
<th>Mouse model</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacskaia et al. (2002) [16]</td>
<td>3D6 (anti-Aβ1-5)</td>
<td>PDAPP, Tg2576</td>
<td>Clearance of diffuse Aβ deposits after central administration. Clearance of diffuse Aβ deposits after central administration of Fab2 fragments of anti-Aβ antibodies.</td>
</tr>
<tr>
<td>Wilcock et al. (2003) [18*]</td>
<td>Tg2576</td>
<td></td>
<td>Reduction in Aβ load 4–24 hours after central administration, maintained at three and seven days. Microglial activation three days after central administration.</td>
</tr>
</tbody>
</table>

**Abbreviations:** Aβ, β-amyloid; CNS, central nervous system.
Bacskai et al. [16,17] further elucidated the mechanisms of Aβ immunotherapy-induced plaque clearance through the use of anti-Aβ antibodies applied directly to the brains of APP transgenic mice, and found that within two days, the number of plaques was reduced. This study provided direct evidence that plaques could be physically cleared by the presence of anti-Aβ antibodies. Surprisingly, the same authors also showed that Fab fragments were sufficient to reverse plaque pathology, raising questions as to whether Fc receptor-mediated phagocytosis was required for plaque clearance, as originally reported [3]. This apparent contradiction in findings has recently been resolved in part by observations from Wilcock et al. [18], who have demonstrated a two-phase mechanism of anti-Aβ antibody-mediated plaque clearance. The first phase is fairly rapid and involves clearance of diffuse Aβ without microglial activation; the second is slower, involving clearance of compact Aβ through activation of microglial Fc receptors and phagocytosis of pre-existing plaques. The studies by Wilcock et al. and Bacskai et al. do have the caveat that antibodies were delivered directly to the brain, raising the possibility that the effects seen were, in part, a result of the relatively high concentrations of antibodies present.

**Effects on cognitive performance**

The ability of Aβ immunotherapy to modify central Aβ deposits is encouraging; however, the obvious next question is whether immunotherapy is able to improve cognitive performance in animal models of AD and, even more importantly, in patients. To approach this question, several laboratories have investigated the effect of Aβ immunotherapy on the ability of APP mice to accomplish several cognitive tasks. Active immunization with Aβ1–42 was effective in increasing the performance of APP mice to levels similar to nontransgenic littermates in a traditional Morris water maze and in a radial-arm water maze [4,5]. These beneficial observations after interference with anti-Aβ antibodies in mice with AD-like pathology appear to support the amyloid hypothesis of AD.

It is also of interest that certain deficits in animal models of AD occur in young (preplaque-bearing) animals and
appear to be related to the overproduction of neuroactive Aβ species. Whether these acute behavioral phenomena are relevant in AD or are peculiar to the APP transgenic animal models is an active area of investigation. However, it should be noted that the antibody m266, which only binds to soluble forms of Aβ, is also effective acutely in behavioral models of AD [19], suggesting that immunotherapy can act through plaque removal and through neutralization of neuroactive Aβ species. Ongoing and future clinical studies should reveal whether both plaque removal and capture of soluble species are required for optimal efficacy in AD patients.

**Immunological considerations**

Although Aβ immunotherapy has important implications for AD, it also raises several questions regarding the interaction of the immune system with the brain, and opens a new avenue of research to explore this interrelationship.

After immunization with Aβ, the peptide is processed by antigen-presenting cells in the periphery and then presented to T and B cells. Epitope mapping of these events following Aβ immunization in AD patients indicates that the predominance of T-cell epitopes lies in the central to carboxy-terminal region of the Aβ peptide [20]. This agrees with the dominant ****Aβ T-cell epitope region identified in nontreated AD and control elderly individuals [21]. By contrast, most of the B-cell or antibody-producing epitopes detected from immunized patients reside in the amino-terminal region of the peptide [22]. The latter point is reinforced by the observation that most anti-Aβ mouse monoclonal antibodies that have been produced are directed to the first 16 amino acids of the peptide. We know from the passive immunotherapy studies described above that an antibody response is sufficient to demonstrate a reduction in amyloid pathology in animal models of AD. Either as a coincidence or a consequence of plaque reduction, these same antibodies also improve other aspects of neuropathology in APP transgenic mice, including neuritic dystrophy and synaptic density [2,23,24]. These findings are difficult to reconcile with the observation that antibodies (such as m266), which only bind to soluble forms of Aβ, are also effective acutely in behavioral models of AD [19]. The simplest explanation for these concurrent results is that both soluble and plaque-related forms of Aβ are deleterious in the brain. This suggests that an antibody that binds both plaques and soluble forms of Aβ would be optimal for therapeutic administration in AD.

Although it is widely agreed that antibodies are eliciting positive effects in AD models of the disease, two negative immunological consequences of the response against Aβ have been demonstrated. In a model of hemorrhagic stroke involving very high overexpression of Aβ and severe congophilic angiopathy, passive administration of an antibody to Aβ resulted in an approximately twofold increase in the frequency and severity of strokes [25]. However, clinical experience with AN1792 (Aβ1–42) in AD patients did not result in an increased stroke incidence, nor were strokes a clinical feature of those patients (described below) that developed meningoencephalitis [14**]. A further report has shown that immunization with Aβ1–42 combined with multiple injections of pertussis toxin can result in cellular infiltrates in immunologically sensitive C57/BL6 mice but this rarely occurs when either agent is injected alone [26]. Again, the relevance of these findings for patients receiving AN1792 is not clear, and additional studies are underway to better understand the immunological implications underlying the observation.

Several reports have investigated the question of whether a pre-existing immune response to Aβ occurs in patients or elderly individuals in the absence of any immunization [27–29]. Overall, these studies have supported the view that low levels of antibodies to Aβ do sometimes exist in elderly individuals without AD but do not result in any measurable toxicity, and antibody levels tend to be reduced in patients with the disease. Although these findings make it tempting to speculate that naturally occurring antibodies to Aβ are protective against AD, the levels of antibody are low and inconsistent, making the conclusion difficult to substantiate. Nevertheless, it is possible that a very low anti-Aβ titer present for many years might be sufficient to protect against AD, although only lengthy studies, in many individuals, will be able to confirm or eliminate this possibility.

**Clinical observations with AN1792**

Improvements in pathology and cognition in mouse models of AD after treatment with AN1792 (Aβ1–42), together with the observed safety characteristics in a broad range of animal species, led to the initiation of Phase 1 clinical studies in AD patients. Extensive animal work, plus previous experience, suggested that AN1792 combined with the saponin QS-21 as an adjuvant would be optimal for clinical studies. In two Phase 1 studies involving administration of single or multiple doses of AN1792 (QS-21) to approximately 80 patients, the treatment appeared to be well tolerated with no clear drug-related safety concerns [30]. Based on these findings, a larger Phase 2 study involving 372 patients was initiated. However, this study was halted because of the development of symptoms consistent with meningoencephalitis in some patients
who received AN1792(QS-21) [14**]. The study remained blinded to patients and physicians for one year to allow for investigation of potential efficacy as a result of, on average, two injections of AN1792(QS-21). Analysis of the Phase 1 multiple dose study shows an advantage on measures of disability in patients treated with AN1792 compared with placebo [30*]; analysis of the Phase 2 study is in progress. Hock et al. analyzed the data from a single center (30 patients) participating in the Phase 2 study and reported positive results for some efficacy measures in AN1792 antibody responders compared with nonresponders [13**]. Confirmation of these results will await analysis and publication of the full, unblinded study. The wealth of information being accumulated from AN1792 studies is highly informative at two levels: firstly, it is relevant to the potential utility of Aβ immunotherapy in AD; and secondly, to the testing of the amyloid hypothesis.

Encephalitis associated with AN1792

Although Aβ1–42 immunization was thoroughly tested in various animal species, under numerous conditions, and no safety concerns arose during Phase 1 clinical trials to preclude initiation of larger studies, 18 patients (6%) receiving AN1792(QS-21) developed meningoencephalitis in the Phase 2 study [14**]. A subsequent postmortem diagnosis of encephalitis was made in one patient treated with AN1792(QS-21) in a Phase 1 trial who died approximately one year after her last injection from a cause not directly attributable to study treatment [31**]. The symptoms and signs of encephalitis included headache, confusion, and changes on magnetic resonance imaging scans; of the 18 patients in the Phase 2 study, 12 have returned to their baseline status and six have experienced some type of prolonged neurological deficit [14***]. Extensive immunological analysis of humoral and cellular responses was performed in these patients. The majority of patients had IgG responses to Aβ, and all patients mounted at least a small IgM response. There was no correlation of the severity of encephalitis with either the level or epitope specificity of the antibody response, suggesting that the encephalitis was caused by something other than anti-Aβ antibodies. Moreover, the vast majority of individuals who mounted an antibody response to Aβ did not develop encephalitis.

Effects on pathology

In mouse models of AD, both passive administration of anti-Aβ antibodies and active immunization with Aβ resulted in clearance of Aβ-containing plaques [3,10, 15,16,18*] and lessening of cognitive impairment [4,5, 9,32,33]. The fundamental question of whether similar findings might be seen in AD patients receiving Aβ immunotherapy is beginning to be addressed through postmortem reports (consent was given by family members prior to post-mortem examinations) of patients who received AN1792(QS-21) in clinical studies (Table 2). In the three cases (two with encephalitis) reported to date, the brain tissue clearly had neuropathological findings consistent with AD, but there were also brain regions that were uncharacteristically devoid of plaques [31**,34**] (E Masliah, personal communication), concurrent with a high level of microglial staining for Aβ. It was concluded that immunization with AN1792(QS-21), rather than a chance occurrence, resulted in the plaque clearance observed in these cases [31**,34**].

In contrast to the case without encephalitis, marked T-cell infiltrates were apparent in the two encephalitis cases (Table 2). This suggests that the encephalitis observed in some individuals treated with AN1792(QS-21) might result from a T-cell response against Aβ1–42. Preliminary immunological analyses of patients who received AN1792(QS-21) have shown that the predominant T-cell

<table>
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<th>Table 2</th>
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<tr>
<td>Overview of neuropathological findings in Alzheimer disease patients who received AN1792(QS-21).</td>
</tr>
<tr>
<td>Authors (year)</td>
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<tr>
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</tr>
<tr>
<td>Nicoll et al. (2003) [31**]</td>
</tr>
<tr>
<td>Ferrer et al. (2004) [34**]</td>
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<tr>
<td>E Masliah, personal communication</td>
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</table>
epitopes are in the carboxyl terminus of Aβ [20]. Of particular interest, patients from the Phase 1 multidose trial generated primarily a Th-2 immunological response, whereas those in the Phase 2 study generally exhibited a Th-1 response [20]. These two studies differed only by the introduction of a detergent (polysorbate-80) to aid the manufacturing and stability of the peptide. Further investigation will be required to determine whether this formulation change affected the immunological profile to Aβ1-42. These findings of the neuropathological and the cellular response to AN1792(QS-21) are important in designing improved approaches for both passive and actively administered Aβ immunotherapy.

Future directions with β-amyloid immunotherapy

Although the success of Aβ immunotherapy as a treatment for AD remains speculative, the early clinical data are encouraging. The most near-term approaches involve three different strategies (Figure 1), with the greatest amount of information available for the forerunner, AN1792(QS-21). Development of this compound has been terminated at Phase 2 for safety reasons, although efficacy signals, including evidence of plaque reduction in treated patients and improvement in some preliminary efficacy endpoints, have been observed [13**,30**,31**,34**]. The observation that Aβ immunotherapy can reduce Aβ plaque burden in AD patients means that, at the very least, immunotherapy will provide a fundamental clinical test of the amyloid hypothesis.

Overall, the findings with AN1792(QS-21) strongly support further development of Aβ immunotherapy towards the obvious goals of eliminating or reducing safety issues and in optimizing the efficacy signals. For example, in an attempt to avoid safety concerns, approaches that avoid a T-cell response to Aβ, such as passive immunotherapy with a humanized monoclonal antibody or active immunization with immunoconjugates, are now in development.

Imunoconjugates of β-amyloid peptides

Aβ immunoconjugates are being pursued by several academic institutions and pharmaceutical companies as potential treatments for AD. These immunoconjugates are typically composed of a fragment of the Aβ peptide, usually derived from either the amino-terminal or the central region, linked to a carrier protein that provides T-cell help (Figure 1). By design, the immunogen-induced T-cell response from such an immunogen cannot be directed to Aβ and hence a classical T-cell-mediated autoimmune response is not possible. This approach also offers the possibility of generating a more consistent and heightened antibody response to Aβ compared with that generated by injection of the entire Aβ peptide. Studies in APP transgenic mice and other species have confirmed these assumptions [6,24,35,36]. At least one such immunoconjugate is in late-stage preclinical development for the treatment of AD (data on file, Elan Pharmaceuticals/Wyeth Pharmaceuticals).

Monoclonal antibodies

Passive immunotherapy using a humanized monoclonal anti-Aβ antibody will entirely eliminate a cellular response to Aβ. Moreover, should any adverse event occur after administration, dosing can be stopped and thus the therapeutic entity is eliminated from the body, limiting prolonged exposure. This approach will also ensure that essentially every individual treated will receive the same dose of antibody — a factor difficult to control with a traditional immunization. Thus, passive immunotherapy has advantages over active immunization from both efficacy and safety perspectives. At the time of writing, one such monoclonal antibody, AAB-001, is in Phase 1 clinical trials in the USA, although it will be some time before we understand its potential for efficacy in AD or obtain meaningful information on its safety profile. Although an argument can be made that a passive antibody approach might adequately cover the overall field of Aβ immunotherapy, depending upon the required dose and duration of treatment, immunization might also be a preferred approach. This is particularly true if prophylactic immunization against AD emerges as a viable strategy for prevention.

Implications for other neurodegenerative disorders

The idea of using antibodies to reduce or eliminate neuropathology in vivo is very exciting and has been actively discussed by several individuals since the initial publication in 1999, reporting attenuation of AD-like pathology by Aβ immunotherapy [2]. Antibodies have subsequently been shown to be highly effective in mouse models of prion disease [37,38]. Recent studies have shown, for example, that conversion of the nonpathogenic form of prion protein to the toxic scrapie form can be blocked by the presence of some, but not all, antibodies to the prion protein [39,40]. It is anticipated that, with time, additional efforts in other neurodegenerative disorders such as Huntington’s disease and Parkinson’s disease will be attempted, and it will be interesting to discover whether similar efficacy occurs.

Conclusions and future directions

Considering that the first publication describing the efficacy of Aβ immunotherapy in an AD animal model occurred only 5 years ago, it is astonishing to consider that Phase 2 data are already becoming available. Unsurprisingly, it is still too early to know its ultimate potential in AD specifically, and neurodegeneration generally. Perhaps the most intriguing aspect of the approach is the finding that it can reduce pathology in both animal models and in patients, supporting the utility of these models to guide future treatments. Clinical results will hopefully soon also confirm the relationship between
cognitive improvements seen in the animal models to those in the human disease state. Several crucial outstanding questions remain regarding Aβ immunotherapy, but it is anticipated that at least some investigational findings will have a profound effect on our understanding of the interplay between the immune and neurological systems and on the potential for using immunotherapy in the clinical treatment of AD and other neurodegenerative disorders.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

● of special interest

●● of outstanding interest


