Effects of Adoptive Transfer of Beta-Amyloid Sensitive Immune Cells in a Mouse Model for Alzheimer's Disease

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Effects of Adoptive Transfer of Beta-Amyloid Sensitive Immune Cells in a Mouse Model for Alzheimer’s Disease

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
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Effects of Adoptive Transfer of Beta-Amyloid Sensitive Immune Cells in a Mouse Model for Alzheimer’s Disease

Daniel Shippy

ABSTRACT

One major therapeutic target for preventing and treating Alzheimer’s Disease (AD) is removal of excess β-amyloid (Aβ) from the brain. Both active and passive immunotherapies targeting Aβ have proven effective in reducing brain Aβ levels and improving cognitive function in mouse transgenic models of AD. However, these approaches can induce adverse neuropathologic effects and immunologic over-activation. Indeed, clinical trials of active Aβ immunotherapy in AD patients were halted due to development of meningoencephalitis, apparently resulting from wide-spread neuroinflammation. Here we show that a more restricted and specific immune re-activation through a single adoptive transfer of Aβ-specific T cells can provide long-term benefits to APPsw+PS1 transgenic mice that last at least 1½ months. Aβ-sensitive splenocytes and lymphocytes were generated in normal mice, re-stimulated with Aβ in vitro, and then adoptively transferred into cognitively-impaired APPsw+PS1 mice. Compared to control transgenic mice through 1½ month post-infusion, those mice that received Aβ-sensitive T cells exhibited a reversal of pre-infusion working memory impairment and demonstrated superior basic mnemonic processing. Step-wise forward Discriminant Function Analysis of behavioral results clearly demonstrated that T cell infused mice performed comparably to wild-type non-transgenics, further emphasizing the extent of
cognitive benefit this therapeutic technique afforded. Importantly, a global inflammatory response did not accompany these benefits. Though no overall reductions in Aβ deposition were noted for T cell recipient mice, a subset of T cell infused mice that benefited most in cognitive function had reduced hippocampal burdens, suggesting that hippocampal Aβ burdens did play a role in determining performance capabilities of these mice. Since chronically high levels of Aβ such as those found in APPsw+PS1 mice cause immune hypo-responsive/tolerance to Aβ, our results indicate that adoptive transfer of Aβ-sensitive T-cells can supercede such immune tolerance to Aβ, and may provide a safe, long-lasting therapy for AD.
Alzheimer’s Disease

Alzheimer’s Disease Background

It is estimated that by the year 2050, 13.2 million individuals in the United States will be living with a diagnosis of Alzheimer’s Disease, further reinforcing its position as the most common neurodegenerative disorder and leading cause of dementia therein (Hebert et al. 2003). Alzheimer’s is characterized by a progressive loss of cognition and retention accompanied by varied disruptions in behavior and in many cases extreme psychological disturbances. These symptoms lead to eventual loss of social integration while at the same time stripping the affected individual of the ability to care for his or her self. Death is the eventual end result of the disease, though it is often preceded by an extensive period of complete mobility loss and a comatose state. The resulting widespread social and economic impact of caring for such afflicted individuals, already devastating today for many families affected by the disease, will undoubtedly continue to grow as Alzheimer’s becomes more prevalent.

Though treatments for Alzheimer’s have been approved by the FDA, none provide long-term reversal or delay of the disease and none serve as a cure (Doody et al. 2001, Areosa et al. 2005). This, despite the fact that the disease was characterized nearly a century ago in 1907 by Dr. Alois Alzheimer, who correlated some of the behavioral impacts of the disease to the most noteworthy aspects of its pathology,
including dramatic loss of brain mass and development of both neuritic plaques and neurofibrillary tangles as well as gliosis (Alzheimer et al. 1995). Even with the surge of research activity which has occurred within this field over the past several decades resulting from both its increased prevalence and from increased exposure due to diagnosis of Alzheimer’s Disease in such noteworthy figures as President Ronald Reagan, progress in developing treatments and determining preventative measures has been slow at best. Recent innovative studies involving varied immunotherapeutic approaches have explored outside the field of typical pharmacological treatments and have shown promise, however have at the same time been a source of major disappointment in human applications. With increased understanding of the faults of such treatments, it is possible that alternate immunological approaches may lead to valid treatments for Alzheimer’s.

Behavioral Characterization of AD

Due to the progressive nature of Alzheimer’s Disease and to the incomplete understanding of its development, it has proven useful for clinicians to establish a staging sequence for Alzheimer’s behavioral impacts. Prior to development of dementia, it is thought that most individuals enter a transition state termed Mild Cognitive Impairment (MCI). It is estimated that up to 15% of individuals diagnosed with MCI proceed to Alzheimer’s development each year, though many are diagnosed with AD without a prior MCI diagnosis (Feldman et al. 2004). Using mainly the extent of cognitive impairment present, Alzheimer’s patients can then be grouped as early, moderate, or advanced phase. Only after death can a true
A diagnosis of Mild Cognitive Impairment does not always correlate to development of AD, though the term was specifically developed to describe a state of lesser impairment preceding Alzheimer’s. It is thought that some limitations of MCI application may be due to variations in symptom sets used to define it and reliance on the testimonies of patients and relatives for establishing a history of memory disruption (Davis and Rockwood 2003). Still, MCI is considered a strong risk factor for AD development and is therefore clinically relevant. MCI is characterized by reported working memory deficits, verified to be more significant than is normal for a patient’s age, with no loss of other functions. Recent studies have also found that certain patterns of neuropsychiatric symptoms are present in up to 59% of subjects diagnosed with MCI, including various mood abnormalities and psychosis. In fact, there appears to be a correlation between presence of neuropsychiatric symptoms common in AD and progression from MCI to early Alzheimer’s (Feldman et al 2004, Hwang et al 2004).

During the initial phase of AD, mild to moderate cognitive impairment with respect to short-term memory is present and is definitive for diagnosis during the life of the patient (McKhann et al. 1984). Specific behavioral deficits and abnormalities are present throughout AD and may show some correlation to staging determined by working memory deficits, though it should be noted that many of these symptoms are not consistently present on an individual basis. Other major clinical features often associated with the disease include language and visuospatial deficits, though the
former may not appear until mid or often late phase AD. Behavioral symptoms such as depression, agitation, anxiety, aggression, and insomnia, as well as psychological symptoms such as delusions and hallucinations have been found to accompany these deficits during early phase AD, though again these symptom sets are more prevalent in later AD phases (Moran et al. 2003). These symptoms are often coupled with functional behavioral impacts such as errors in judgment, untidiness, and transient confusion as the disease progresses. Not all symptoms are present in all patients, they occur in widely varied percentages of individuals in this phase of the disease. Moran et al. recently described three classes of early phase AD patients based on observed symptom sets, the first with little to no symptoms outside of memory deficits, the second with additional symptoms related mostly to anxiety and depression, and the third with additional aggression-based symptoms. Such grouping methodologies could prove useful in understanding the basis of these symptoms if correlation to neuropathology can be made.

Entry into moderate phase AD is established based on continued loss of short-term memory coupled with new, progressive loss of long-term memory. Other symptoms described for MCI and early phase AD are common to moderate phase AD as well. Language deficits, which tend to develop later than visuospatial disruptions, become especially prevalent by the time a patient enters moderate AD. Finally, it is not uncommon for both behavioral and psychological disturbances developed during MCI or early phase AD to become even more severe before being lost to immobility and stupor common to advanced phase AD (Hart et al. 2003). Advanced phase AD involves an almost complete loss of all memory forms and entry into a vegetative
state which continues until death due to illness or systemic loss of bodily functions occurs.

In a retrospective study, Hart et al. showed that depression, delusions, and anxiety are the most prevalent of the psychological symptoms present throughout AD, although hallucinations, elation, and disinhibition may also occur. Numerous studies have also shown that apathy, motor disturbance, aggression, irritability, appetite changes, and sleep disturbances are all relatively common behavioral symptoms for AD (Hart et al 2003, Moran et al 2003, Lyketsos et al 2001). Of these, apathy appears to be the most prevalent form of behavioral change which occurs.

From the time of diagnosis of AD, an individual may often live as long as twenty years. With further development of correlations between certain forms of MCI and progression to AD, the time with which an individual and his or her family may live with such a diagnosis is ever increasing, further reinforcing the importance of developing a treatment for the disease.

Pathological Characterization of AD

Although it was Dr. Alois Alzheimer who first described the general pathological characteristics of AD, an understanding of the true molecular nature of these markers could not be gained until the 1960s when electron microscopy allowed for more detailed analysis of neuritic plaques and neurofibrillary tangles (NFT’s), the two hallmark brain pathologies of the disease (Kidd 1964). In the decades since, numerous hypotheses regarding the cause of this pathology and its correlation to behavioral impacts as well as other AD pathological characteristics have been formulated. Perhaps the most widely acknowledged of these focuses on the presence
of a particular protein fragment, Beta-amyloid (Aβ), in the brain and has thus been termed the amyloid hypothesis. Other AD pathologies such as brain atrophy, neuronal dystrophy, synaptic loss, and NFT formation are thought to be tied in some way to Aβ peptide formation in the CNS under this hypothesis.

Aβ is formed from proteolytic processing of the Amyloid Precursor Protein (APP), a 695-770 amino acid protein typically found in membranes of many cell types throughout the body. APP contains three relevant cleavage sites which are discriminately cleaved by the α, β, and γ-secretase complexes. α-Secretase cleaves within the Aβ domain of the APP molecule, preventing formation of Aβ, while sequential cleavage by β and γ secretases is needed for amyloidogenesis. The activity of these latter two enzymatic complexes appears to be increased in later life and even more so in AD (Canevari et al. 2004). While Aβ is produced in and can be found in fairly low concentrations throughout the body, it is its activity within the CNS which appears to be relevant to AD, both in regards to formation of neuritic plaques as well as through its presence in a soluble form. This is not to suggest that peripheral Aβ is not relevant to the disease, however, as it has been shown that Aβ is capable of passing through the blood brain barrier (BBB) (Zlokovic 2004). Some studies in animal models have even suggested that Aβ may be able to trigger disruptions in the BBB, which is commonly disrupted in AD patients (Farkas et al. 2003).

Two isoforms of the Aβ peptide can be found within neuritic plaques. Most contain the 42 amino acid isoform (Aβ1-42), though this isoform is actually less common elsewhere in the CNS than the second Aβ1-40 peptide. The Aβ1-42 peptide is
thought to be one of the initiating factors for neuritic plaque formation, originally aggregating to form what are known as low density or diffuse plaques (Lemere et al. 1996). Inflammatory proteins such as APOE and α-ACT, along with a number of other proteins produced by various cells of the CNS, are also thought to contribute to formation of these plaques (Nilsson et al. 2004).

Though the exact nature of plaque formation progression is not understood, it appears that these diffuse plaques are precursors to the compact plaques noted by Dr. Alois Alzheimer. It is further acknowledged that as these plaques form there is an activation of microglial cells which, via secretion of various cytokines, induce activation of adjacent astrocytic cells, resulting in a co-stimulatory inflammatory response which may accelerate plaque maturation (D’Andrea et al. 2004). Other immune cells such as B and T-cells appear to be involved in regulation of this response. Cytokines such as IL-1 and TNF-α also act by inducing APP messenger RNA translation, resulting in further increase in Aβ production (Rogers et al 1999). Inflammatory proteins such as APOE and α-ACT are produced as part of the response and may be involved in further Aβ deposition by catalyzing conversion of Aβ to a β-pleated sheet form which is prone to aggregation (Nilsson 2001). Degradation of adjacent neuronal dendritic and axonal processes further increases the local inflammatory response via release of intercellular inflammatory factors as well as additional degrading lysosomal and cytoplasmic enzymes, resulting in even further damage to surrounding cells. This form of damage elicited by Aβ plaques has caused this peptide to be termed “neurotoxic”. During this process, Aβ1-42 deposition
continues coupled with Aβ1-40 incorporation, finally resulting in formation of compact (mature) neuritic plaques, each of which is composed of a dense Aβ core surrounded by reactive microglia and astrocytes and associated with inflammatory proteins such as APOE and α-ACT. (Selkoe 2001, Nilsson et al. 2004).

Diffuse versions of the neuritic plaque can be found in various regions of the brain, both inside and outside of the hippocampus and association cortices, which are the regions most affected with regard to neuronal loss and NFT formation as well as neuritic dystrophy associated with Aβ pathology. Neuronal loss and other AD pathologies, including various forms of neuroarchitectural rearrangements in these regions, are thought to correlate to the losses in working memory noted in AD. It is primarily in these regions that high levels of compact neuritic plaques can be found, further reinforcing the importance of these plaques with regards to AD pathology.

It is important to note that Cerebral Amyloid Angiopathy (CAA) is a pathological feature common in AD which is also directly caused by deposition of Aβ, this time within the vasculature. Amyloid plaques formed within blood vessels can lead to microhemmorages and in some cases instigate a stroke. Approximately 80% of individuals over the age of 65 diagnosed with AD are thought to develop CAA.

Other aspects of this pathology have been linked to Aβ presence, though not necessarily in compact, aggregated form. Aggregation is thought to begin intracellularly, with Aβ1-42 fragments twisting together to form first dimmers and then various oligomeric isoforms (Selkoe 2001). Such intracellular Aβ is thought to
influence phosphorylation of tau proteins (Cummings 2004). This phosphorylation event is known to be linked to imbalances in cellular kinases and phosphatases. Tau proteins are microtubule components that, when phosphorylated, can twist together in \(\alpha\)-helical pairs to form Paired Helical Filaments (PHFs). These accumulate to form true NFTs, resulting in disruption of neuronal function and eventually cell death (Canevari et al. 2004 and Sobow et al. 2004).

Loss of cholinergic system functions is another major hallmark of AD, and this too has been linked to A\(\beta\) activity. A\(\beta\) is thought to interact with nicotinic receptors of cholinergic neurons, specifically by binding to \(\alpha_7\) nACh Receptors (Kihara and Shimohama 2004). This interaction is thought to result in inhibition of calcium activation and acetylcholine release in these neurons, indicating a mechanism for loss of function in cholinergic pathways. A\(\beta\) is also thought to enhance glutamate excitotoxicity via NMDA receptor interactions. These specific interactions between A\(\beta\) and neurons prone to dysfunction in AD strongly reinforce the importance of the peptide in the progression of AD, as loss of memory and cognitive functions have been linked to disruption of these pathways (Kihara and Shimohama 2004).

Further A\(\beta\) involvement via activation of caspases has also been proposed, indicating a mechanism by which cellular apoptosis, a widespread occurrence within the CNS in AD, may be induced (Eckert et al. 2003). Interactions between A\(\beta\) and mitochondrial membrane transporters along with mitochondrial enzymes are thought to be involved in decreased mitochondrial activity. This has been proposed as one mechanism for high levels of oxidative stress found in AD (Canevari et al. 2004, Marlatt et al. 2004).
Aβ is also thought to disrupt Ca++ transport in neurons, astrocytes, and microglia. Alteration of Ca++ transport functionality has been linked to alterations in neuronal signal processing, increased neuronal vulnerability to excitotoxicity, and activation of both microglia and astrocytes (Cavevari et al. 2004). Aβ can also be linked to production of oxygen free radical species, which occurs during oxygen dependent formation of Aβ β-pleated sheets. Free radicals are also formed by microglia upon activation by Aβ. These reactive species are thought to play a substantiative role in triggering oxidative damage prevalent in AD. This damage tends to affect mainly lipids and DNA, and is 3 times more prevalent in AD than in normal elderly individuals (Hensley et al. 1996, Selkoe 2001). Thus, many of AD’s pathological features can be attributed to abnormal production and function of Aβ within the brain. However, it should be noted that there are also several lines of evidence which suggest that the amyloid hypothesis described above may be incorrect or at the very least incomplete.

Those malcontent with the amyloid hypothesis have emphasized an inability of the hypothesis to explain early neurophysiological developments such as synaptic dysfunction, neuronal loss, and decreased LTP, which occur in varying extents before formation of Aβ plaques in AD. It has been suggested that Aβ accumulation, like NFT formation, is truly more a consequence of a separate cellular dysfunction, not yet entirely elucidated, which occurs before overproduction and aggregation of the peptide. This is supported by findings in transgenic mice which indicate that synaptic dysfunction may occur before formation of diffuse or compact amyloid plaques (Lee et al. 2004). It has even been shown that Aβ may be involved in regulation of long-
term potentiation, indicating that the peptide is not simply a neurotoxic by-product and that its presence in AD may be an attempt at compensating for neuronal loss, not necessarily the cause of it (Koudinov and Berezov, 2004). It should be noted that other studies have shown the opposite effect of the peptide in lowering capacity for LTP (Selkoe 2002).

Other hypothesis for the actual mechanisms underlying AD focus on cell cycling and mitochondrial enzymatic disruptions which result in oxidative stress and neuronal loss and on abnormal levels of enzymes involved in acetylcholine synthesis and degradation (Raina et al. 2004, Zhu et al. 2004). While it is necessary to consider these possibilities in seeking an understanding of, and a treatment for, Alzheimer’s, it is also important to consider in the present that previous studies have shown correlation between Aβ levels within the CNS and extent of demetia in early stages of AD (Bussiere et al., 2002), though Aβ load does not correlate directly to cognitive disturbances as the disease progresses (Terry et al. 1991). Similar correlation can be made between Aβ plaque loads and cognitive impairment in transgenic mouse models of AD (Gordon et al. 2001). Synapse loss and neurodegeneration induced by neurofibrillary tangles serve as better correlates to cognitive impairment (Terry et al. 1991, Arriagada et al. 1992). However, as has been shown above, these pathological findings may be tied to and perhaps even directly caused by the presence of Aβ. Also, as elicited above, there are numerous studies which have shown mechanisms by which Aβ can be neurotoxic and/or can have other negative physiological impacts within the CNS even in soluble form. Therefore, regardless of other mechanisms involved in development of AD, there remains definite validity in searching for a
means to elicit Aβ clearance as a means of treatment for the disease or for preventing Aβ formation/aggregation as a means of prevention.

**Genetics of AD**

Further support for the amyloid hypothesis is provided by analysis of known genetic mutations linked to Familial Alzheimer’s Disease (FAD). FAD typically follows a similar time course to sporadic AD, which has no known genetic basis, and differs only with regards to age of onset. FAD diagnosis typically occurs at a much younger age, as early as 40, than sporadic AD. Each mutation linked to FAD, which by definition is congenital and runs within families, has an *in vivo* effect of increasing Aβ peptide formation, and is an autosomal dominant trait. However, only as few as one percent of reported AD cases are currently attributed to FAD. The mutations linked to FAD thus far include those of the APP gene itself, along with those of the presenilin genes (PS1 and PS2) (Zekanowski et al. 2004, Selkoe 2001).

In 1984, Glenner and Wong proposed that genetic defects in Alzheimer’s disease would be found on chromosome 21 after discovering that the amyloid protein prevalent in both Alzheimer’s disease and Down’s syndrome pathology were homologous (Glenner and Wong, 1984). Indeed, it has been found that mutations of the APP gene which have been linked to FAD are located at either end of the Aβ coding domain on chromosome 21p21. There are 16 unique mutations of the gene which promote AD pathology and 4 which do not, though only 4-6% of FAD cases are attributed to APP mutations. The result of each mutation is increased Aβ peptide formation, most likely through increased processing of the APP molecule by β or γ secretases. With regards to current research in transgenic models, two of the most
relevant of these are the Swedish and London APP mutations. The Swedish mutation
is located at positions 670 and 671 of the APP molecule and involves a double
missense mutation, while the London mutation, which is a single missense mutation,
is located at position 717. These correlate to the domains cleaved by the $\beta$ and $\gamma$
secretases. Both mutations result in dramatic increases in overall A$\beta$ production, with
varying ratios of A$\beta_{1-40}$ to A$\beta_{1-42}$ produced. Specifically, the London mutation results
in an increased ratio of A$\beta_{1-42}$ to A$\beta_{1-40}$ while raising levels of both isoform overall,
whereas the Swedish mutation results in approximately equal increases in the levels

As with the London mutation, it is production of the A$\beta_{1-42}$ peptide which is
most increased as a result of the presenilin (PS) mutations. It is thought that both the
PS1 and PS2 molecules are incorporated into the $\gamma$ secretase complex and serve as
part or all of the active site for this complex (Farmery et al. 2003). The proteins are
generally found in endoplasmic reticulum of cells and most likely act on APP during
its processing via this organelle. The result of the presenilin mutations is increased
activity of the $\gamma$ secretase complex, resulting in increased amyloidogenic processing
of APP. The PS1 gene codes for a 467aa protein and is located on chromosome
14q23.3, while the PS2 gene codes for a 448aa protein and is located on chromosome
1q31-42. The genes are highly homologous in their sequences, indicating a similar
function. PS1 mutations are the most common cause of FAD based on current data,
and more than 100 of these mutations have been identified, most of which are
missense. Only 9 PS2 mutations have been identified, all of which are missense, and
the prevalence of these mutations is significantly lower than those of PS1 (Kimberly and Wolfe 2003, Gaskell and Vance 2004).

**Diagnosis of AD**

The predominant criteria used for the diagnosis of Alzheimer’s comes from the National Institute for Neurologic and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA), which specifies diagnosis as being either definite, probable, or possible based on the extent of confirmation available. If a histological confirmation, preceded by clinical diagnosis, is obtained, then a definitive diagnosis for AD is established. Clinical diagnosis without histological confirmation is deemed as a probable AD case, while a case with clinical abnormalities but no other diagnostic approaches available is considered possible AD (Cummings 2004).

The specific clinical features looked for in diagnosing AD are the three major behavioral hallmarks of the disease; episodic and working memory impairment and both language and visuospatial deficits. Episodic memory is based on retrieval of memories of one’s specific past events, typically with events which have occurred recently being most easily forgotten (Cummings 2004, Greene et al. 1996). Working memory, or short-term memory, is typically associated only with the ability to recall occurrences from within minutes of the present. Loss of both episodic and working memory, which largely correlates to general anterograde amnesia, is perhaps the easiest of the behavioral hallmarks of AD to identify and the most important for establishing clinical diagnosis (Welsh et al. 1992, Greene et al. 1996). There are many tests which can be administered by clinicians in able to determine if such
memory loss is present and extent of impairment, including the most common, the Mini-Mental State Exam (MMSE), which involves giving a patient number of words to remember followed by a series of questions which serve as a distraction. An individual’s lack of ability to retain the words given to them at the beginning of the task (registration-recall) is an indicator of short-term memory impairment. Other tools for evaluating mental state of potential AD patients include the 7-Minute Screen and Geriatric Depression Scale (Langbart 2002).

Basic histological measurements which may be correlated to memory impairment to help strengthen a diagnosis of AD include cerebro-spinal fluid (CSF) levels of \( \text{A}\beta_{1-42} \) and both tau and phospho-tau (p-tau) proteins. Numerous studies have shown that a dramatic decrease in CSF \( \text{A}\beta_{1-42} \) is prevalent in AD (Sobow et al., 2004; Andreasen et al., 2003). This is thought to correlate to trapping and aggregation of the peptide in insoluble forms within the CNS, allowing the brain to act as an \( \text{A}\beta_{1-42} \) “sink”. At the same time, levels of both tau and p-tau in the CSF increase significantly in most cases of AD (Sobow et al., 2004; Andreasen et al., 2003). Unfortunately, CSF sample collecting is a highly invasive process, making widespread clinical application of these histological markers difficult. Current research studies in the area of \( \text{A}\beta \) and tau levels in the plasma or other more accessible fluids are ongoing (Sobow et al. 2004).

Several brain imaging techniques also serves as diagnostic tool for AD. Computed tomography (CT) and magnetic resonance imaging (MRI) have proven to be useful as multiple scans over time can show overall brain atrophy and localized atrophy of the hippocampus and entorrhinal cortex (Barnes et al., 2004; Pennanen et
al., 2004; de Leon et al., 1997). This form of atrophy is specific to AD, allowing for exclusion of other dementia types, and can also be used to monitor the progress of the disease. In some cases it is possible to detect this atrophy during MCI, allowing for anticipation of an imminent diagnosis of AD. Importantly, studies of AD patients have shown direct correlation between hippocampal atrophy and memory impairment, specifically short-term memory (Grundman et al. 2003). Similarly, overall atrophy of the cerebral cortices has been correlated to long-term memory and memory retrieval impairment in AD (Bilgler et al. 2004). As discussed above, this atrophy is likely caused by neuronal loss and restructuring common in these areas during AD progression. More recently, positron-emission tomography (PET) and single-photon-emission CT scans have been applied to diagnosis of Alzheimer’s. These scans allow for analysis of regional brain activities, and can target deficits in activity in regions affected by AD (Cummings 2004, Minoshima 2003). Recently, the possibility of a technique for imaging of amyloid plaques using a modified version of the PET scan has been explored which may prove to be a very useful diagnostic tool in the future (Klunk et al. 2003).

A diagnosis of AD, whether it be definitive, probable, or possible, in a family with a strong history of the disease with early onset is sufficient cause for genetic testing for FAD. Tests are clinically available for presenilin mutations, however their application is limited and no such tests are available for APP mutations. Since there are numerous mutations which may be present in these genes which do not result in FAD, it is important that both physician and patient be well informed before genetic testing results are reviewed. Extensive Genetic counseling is an important aspect of
this education, meaning that genetic testing for FAD must typically occur through a hospital with trained genetic counseling staff and should not be performed at a standard physician’s office. As more FAD mutations and their resulting phenotypes become better understood, it is likely that the availability of genetic testing will spread. In the present, the results of genetic testing for FAD are not definitive for diagnosis but can confirm the presence of a FAD mutation in a family, which may prove useful for such families in preparing for the disease financially and via preventative measures (Gaskell and Vance 2004, Zekanowski et al. 2004).

Risk Factors for AD

Certain risk factors for AD such as the most significant one, aging, are in no way preventable. Other examples of these include sex (females run a higher risk of developing AD than do males), having a first degree relative with AD, and carrying the ApoE4 allele of the ApoE gene. The ApoE allele is an extremely important risk factor for AD, with each ApoE4 allele carried resulting in a sizable increase in AD risk and typically earlier onset of the disease if developed (Selkoe 2001, Yao et al. 2004). Other risk factors for Alzheimer’s may be prevented or treated, making them particularly important for individuals already at a high risk for AD.

Preventable AD risk factors include head trauma (especially in young adulthood), high blood pressure in midlife, high cholesterol levels in midlife, high blood homocysteine levels, and a number of factors related directly to diet (Fleminger et al. 2003, Wellington 2004, Ellinson et al. 2004). High levels of social interaction, mental engagement, and exercise are thought to be related to lowering risk for developing AD as well via cognitive stimulation. Diet and exercise are also indirectly
important for regulation of blood pressure and cholesterol levels (Wang et al. 2004). Reduction of cholesterol by use of statins has also been strongly linked to a decreased risk for AD (Miller and Chacko 2004). Blood homocysteine levels can be regulated by intake of vitamins B6, B12, and folic acid, which are involved in metabolism of the molecule (Ellinson et al. 2004).

Other dietary considerations for preventing AD include decreasing caloric intake, increasing intake of Tocopherol (Vitamin E) and other antioxidants, and/or increasing dietary levels of Omega-3 fatty acids (Berman and Brodaty 2004, Bourre 2004). Antioxidants are important for defending the brain against oxidative damage, and some epidemiological studies looking at dietary antioxidant levels, especially Vitamin E levels, have shown a negative correlation between intake levels and risk for AD development, though clinical trials have elicited mixed results (Berman and Brodaty 2004, Sobow and Kloszewska 2003). The antioxidant properties of Vitamin E are important in AD largely because they prevent membrane lipid peroxidation, reducing oxidative damage in the brain.

It is thought that Omega-3 fatty acids such as Docosahexaenoic acid (DHA) are important for neuronal health and may directly impact APP processing. Epidemiological studies indicate that decreased dietary levels of fish, which contain high levels of DHA and other fatty acids, can be considered a risk factor for AD (Bourre 2004, Kalmijn et al. 1997). A diet high in fruits and vegetables along with fish results in increased uptake of various antioxidants and n-3 fatty acids, and patients at risk for Alzheimer’s are often encouraged by doctors to pursue such diets both because of the direct impact of these molecular components and the indirect
impact of such diets on cholesterol levels. Other dietetic factors which have been linked to decreased AD risk include regular intake of nicotine, caffeine, and alcohol, though for each of these it has yet to be determined if possible preventative capabilities of these drugs outweigh risks associated with their use or abuse (Dall'Igna et al. 2004, Teaktong et al. 2004, Letenneur et al. 2004).

Recently, the importance of environment with respect to AD risk has been a major point of emphasis in Alzheimer’s research. It is becoming apparent that education level, social integration throughout life, and engagement in mentally stimulating activities such as writing and working puzzles has some level of protection against AD development (Fratiglioni et al. 2004). Most risk factors for AD have been determined through epidemiological studies, so their exact role and mechanism of protection are difficult to define and are often tied to those of other risk factors, such as with cholesterol, diet, and exercise. Fortunately, most actions that individuals can take to help in prevention of AD are generally encouraged as part of a standard healthy lifestyle and can be undertaken without a full understanding of their effectiveness.

Current Treatments of AD

There are currently five pharmacological treatments for AD which have been approved by the FDA. Tacrine (which is no longer used clinically due to its negative impact on the liver), donepezil, rivastigmine, and galantamine are all acetylcholinesterase inhibitors, while the fifth drug, memantine, is an antagonist for NMDA-receptors (Cummings et al. 2004). Acetylcholinesterase inhibitors have been applied clinically in the United States for many years, and are directed against lost of
cholinergic function in AD. By inhibiting acetylcholinesterase, which breaks down acetylcholine, cholinergic transmissions are increased due to raised levels of the neurotransmitter in the synaptic cleft. Galantamine also acts as an allosteric ligand at nicotinic acetylcholine receptors, further improving cholinergic function. Rivastigmine inhibits both acetylcholinesterase and butyrylcholinesterase, which is another cholinesterase largely associated with glial cells (Scarpini et al. 2004). Treatment with these drugs brings about improvement or stabilization in cognitive symptoms of mild to moderate AD, however these improvements are small and do not typically last for more than 1-2 years. Studies are ongoing to determine whether long-term use is at all beneficial (Scarpini et al. 2004, Sonkusare et al. 2005).

Memantine was only recently approved for treatment of AD by the FDA. By acting as a partial NMDA-receptor agonist, this drug helps to prevent excitotoxic over activation of these receptors by glutamate, which is thought to be present in elevated levels in AD (Sonkusare et al. 2005). Normal levels of NMDA-receptor activation are desirable due to their importance in long-term potentiation, however, since these receptors allow long periods of Ca2+ influx into cells upon activation, having too many receptors open for extended periods may lead to Ca2+ mediated neuronal degeneration. Proper administration of memantine appears to be able to prevent this type of excitotoxicity while allowing for normal levels of receptor activation. This treatment leads to improved cognitive and behavioral functions in the short term over placebo. Studies combining memantine and donepezil treatments have shown great
promise in short term treatment of moderate to severe AD (Tariot et al. 2004). Still, as with the other approved treatments for AD, memantine’s effects are relatively small and short lived, emphasizing the importance of continued research in the field of alternative AD treatments (Scarpini et al. 2004).
Animal Models of Alzheimer’s Disease

Various animal models for Alzheimer’s Disease have been developed based on a persistent need for an easily reproduced and cost effective means for studying behavioral and pathological progression of the disease. These models have since become invaluable in evaluating potential therapeutics and preventatives. The most commonly used method for producing these models in mice is through random insertion of the desired gene into a fertilized mouse egg genome via microinjection or electroporation. Mice models are desirable because they age quickly for a mammalian animal, and are relatively easy and cheap to maintain as colonies. The genes inserted to create these mice are mutant-type human transgenes which are either linked to FAD, such as mutant APP and presenilin alleles, or which are known risk factors for AD, such as the ApoE4 allele. In order for these inserted mutations to exhibit profound effects in subsequent generations of transgenic mice, they are inserted with strong promoters specifically activated in the mouse brain. For FAD APP transgenes, the result is massive production of Aβ in the brains of the resulting transgenic animals which then leads to progressive deposition of this protein fragment to form neuritic plaques in the CNS. Models based on these mutations include the PD-APP, Psw (APP23), and APPsw transgenic mice. Other mutations work in conjunction with these in double transgenic models to further enhance Aβ production
and/or deposition, but are not sufficient to induce neuritic plaque formation in single transgenic models. An important example of this type of double transgenic model is the APPsw+PS1 mouse.

**PDAPP Model**

Mice transgenic for the “London mutation” human allele of the APP gene were initially described in 1995 and have since been termed PD-APP mice due to use of a platelet-derived growth factor promoter for driving expression of the gene. As a result of using this promoter, PD-APP mice express human APP (hAPP) at levels approximately 10 times greater than expression levels in human AD brains. This allows for pathology development during the relatively short lifetime of the mice. mRNA expression consists of three major variants due to alternative splicing of exons 7 and 8 of the APP gene construct. Over-expression of hAPP containing the 717 single point mutation of Valine to Leucine in the PD-APP mouse brain leads to development of pathology and behavior which correlates in many ways with that of Alzheimer’s Disease (Games et al. 1995).

*Pathological Characterization of the PDAPP Model*

AD-type pathology begins to exhibit itself in PD-APP mice between 6 and 9 months when deposits of Aβ can be detected in plaques formed solely in the hippocampus, corpus callosum, and cerebral cortex. Deposition continues past this timepoint until plaque levels parallel or exceed those found in AD. The plaques noted in PD-APP mice brains range from diffuse to compact, and compact plaques consist of an Aβ core surrounded by activated astrocytes and microglia, just as is typical for AD neuritic plaques (Games et al. 1995). While these mice do not exhibit
cerebral atrophy or widespread neuronal loss, key features of Alzheimer’s pathology in humans, they do exhibit a loss of both synapses and dendrites in the hippocampal dentate gyrus coupled with overall hippocampal atrophy (Irizarry et al. 1997). This correlates to a similar loss in this area of the human AD brain, which is thought to contribute to short-term memory loss. Further studies of synaptic transmission in the hippocampus of these mice have shown that there is a reduction of neurotransmission and LTP which precedes Aβ deposition (Giacchino et al. 2000). This reduction is also thought to play an important role in affecting behavioral impairment of these mice.

**Behavioral Characterization of the PDAPP Model**

In 1999, Dodart et al. reported that PD-APP transgenic mice show age-independent impairment in the radial arm maze task as early as 3 months of age despite no deficit in overall motor activity. By 6 months they were shown to be impaired in object recognition tasks (Dodart et al. 1999). In 2000, a correlation between object recognition and Aβ plaque loads was reported along with data correlating deficits in both spatial and working memory to changes in synaptic density and hippocampal atrophy (Dodart et al. 2000). That same year, Chen et al. (2000) reported an age related deficit in working memory using a modified version of the water maze which included use of a shifting assortment of visual cues and escape platform locations. Alterations in platform locations after several trials required that the mice tested learn each new location, forcing them to rely on working memory. For all age time points, PDAPP mice took longer to learn the location of the hidden platform than non-transgenic controls in an age-independent manner. However,
based on last day performance at each time point, impairment was not present at a 6-9 month time point but became apparent at a 13-15 month time point and became even more prominent at a final 18-21 month time point (Chen et al. 2000). In 2004, Nilsson et al. tested PDAPP mice and non-transgenic controls in a full 6 week battery of sensorimotor and cognitive based tasks starting at 2 months and 16 months for separate groups of animals. While no differences were noted between groups at the earlier time point, aged PDAPP animals showed impairment in the final block of Morris water maze and in overall RAWM performance (Nilsson et al. 2004). That same year, Leighty et al. reported a significant statistical correlation between impairment in Morris water maze, platform recognition, and RAWM performance and deposition of Aβ (diffuse and/or compact) in the hippocampus and parietal cortex in 15-16 month old PDAPP mice. A correlation was also made between circular platform escape latency and diffuse Aβ deposition in the cerebral cortex. These patterns of impairment correlated to Aβ deposition in the hippocampus and cerebral cortex of the mice brains indicate a direct mechanism for working memory loss in this model of AD.

**APPsw (APP23) Model**

The “Swedish mutation” transgene has also been used to create hAPP transgenic mice commonly used in Alzheimer’s research. This transgene carries a double mutation at positions 670 (Lys→Asn) and 671 (Met→Leu) in the APP gene, a mutation originally discovered in a Swedish family predisposed to early onset AD (Mullan et al., 1995). The most prevalent lines of mice carrying this mutation are the
APPsw/Tg2576 and the Psw/APP23 mice, which carry different promoters and have different transgene insert locations resulting in some distinct pathological features.

Pathological Characterization of the Tg2576 Model

In Tg2576 mice, the APP transgene is expressed only by neurons, predominately in the hippocampus but with a constant, lower level of expression in other varied cortical regions (Irizarry et al. 1997). By 6-7 months, insoluble Aβ is present in the brains of these mice, with levels of both Aβ_{1-42} and Aβ_{1-40} isoforms then increasing drastically through 10 months (Kawarabayashi et al. 2001). Amyloid plaque deposition occurs by 11 months in cortical and limbic regions. Past this time point and through 23 months of age, levels of both diffuse and neuritic plaques increase to levels similar to those found in AD. As a result of plaque formation, decreases in plasma and CSF Aβ levels can be noted progressively through these age time points (Kawarabayashi et al. 2001).

Amyloid plaques in Tg2576 mice have been shown to be associated with gliosis and neuritic dystrophy, though importantly no loss of hippocampal CA1 neurons have been reported (Irizarry et al. 1997). Aβ formation and aggregation has also been associated with a number of other pathological and neurophysiological features, including activation of microglia and astrocytes, induction of oxidative stress and damage including lipid peroxidation, and impairment of LTP in CA1 and dentate gyrus neurons (Frautschy et al. 1998, Benzing et al. 1999, Mehlhorn et al. 2000, Smith et al. 1998, Practico et al. 2001, Chapman et al. 1999). Activated
microglia were shown to localize with Aβ deposits and increase expression of Interlukin-1β (IL-1β) and Tumor Necrosis Factor-α (TNF-α) (Benzing et al. 1999). Immunoreactive astrocytes directly adjacent to plaques were shown to increase expression of IL-6 and Glial Fibrillary Acidic Protein (GFAP) (Mehlhorn et al. 2000). These activated immunoreactive cells serve to enhance the inflammatory response elicited by Aβ. Tg2576 mice have also been shown to have an increased risk for development of ischemic brain damage, either as a direct result of amyloid plaque formation or as a result of the inflammatory response process (Zhang et al. 1997).

Pathological Characterization of the APP23 Model

The Psw or APP23 model differs in pathology from the Tg2576 model in one major respect. These mice are known to develop Cerebral Amyloid Angiopathy (CAA), which results from amyloid plaque formation in the cerebral vasculature. This severe pathological feature is not present in Tg2576 mice but occurs at as early as 14 months in the Psw model. CAA in these mice is associated with loss of neurons in areas adjacent to CAA pathology (Calhoun et al. 1999). Synaptic abnormalities, microglial activation, and microhemorrhage are also prevalent in these regions. Damage to the vasculature can be extreme, beginning with loss of vascular smooth muscle cells, aneurismal vasodialation and microhemorrhages and eventually leading to large hemotomas and in some cases hemorrhagic stroke (Winkler et al. 2001). CAA has been noted in AD, leading some researchers to believe that the Psw mouse provides a more realistic AD model (Calhoun et al. 1999, Winkler et al. 2001).

Behavioral Characterization of the Tg2576 Model

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Behavioral deficits have been found in both Swedish mutation transgenic mouse models. Initial studies in Tg2576 mice showed behavioral impairment in Y-maze and Morris water maze tasks by 10-11 months of age, at the same time plaques developed in these animals (Hsiao et al. 1996, Irizarry et al. 1997). Impaired CA1 and dentate gyrus neuronal LTP was also found to correlate to spatial working memory impairment based on T-maze alternation impairments at 16 months compared to non-transgenic controls (Chapman et al. 1999). In two separate studies, Holcomb et al. showed that Y-maze impairment with regards to percent alternation was present at 3 and 6 but not 9 months, that no impairment was present at any of these time points for Morris water maze, and that Tg2576 mice were not impaired in a number of sensorimotor baseline tasks (Holcomb et al. 1998, Holcomb et al. 1999). In 1999, it was shown that impairment was present in 7 month old Tg2576 mice not for initial application but for reversal learning of the circular platform task (Pompl et al. 1999).

In 2002, King and Arendash behaviorally characterized the Tg2576 model at 3, 9, 14, and 19 months, providing a comprehensive overview of impairment in this model. This was done via application of a 6-week battery of sensorimotor and cognitive based tasks. Over all timepoints, it was shown that Tg2576 mice were impaired in Y-maze spontaneous alternations, by nine months in visible platform recognition, and over numerous timepoints in sensorimotor tasks such as balance beam and string agility tasks. Continued levels of performance on par with non-transgenic mice in cognitive based tasks such as Morris water maze, circular platform, and both passive and active avoidance through 19-months indicated that
profound cognitive impairment is not present in this model even into old age (King et al. 2002 (1)). Westerman et al. (2002) found that memory loss in Tg2576 mice at as early as 6 months could actually be attributed to formation of small insoluble Aβ aggregates, though at later time points a correlation between these aggregates and impairment could no longer be drawn based on Morris water maze task results alone (Westerman et al. 2002). Also in 2002, however, King et al. showed that age dependent impairment in Morris water maze and platform recognition could be correlated with synaptophysin staining levels present in APPsw mice through 19 months of age, indicating a connection between impairment and compensatory measures taken by the mouse brain to combat loss of function (King et al. 2002 (2)).

In 2004, Arendash et al. reported early impairment at 5 months in APPsw mice in balance beam but no other sensorimotor or anxiety based tasks. Between 5 and 8.5 months, these mice were significantly impaired in Y-maze percent alternations. Impairment was also noted between 5 and 7 months in Morris maze acquisition and retention, platform recognition, and RAWM, indicating that for these mice cognitive impairment is present at an earlier time point than was previously noted. This was thought to be due to actions of soluble Aβ oligomers present in the mouse brains at these timepoints, with increased affects due to continual cross-breeding of transgenics and a resulting increased influence of the APP transgene. (Arendash et al. 2004).

**Behavioral Characterization of the APP23 Model**

In the APP23 model, three recent studies have shown that cognitive impairment is present at various timepoints in these animals. In 2003, Kelly et al.
tested APP23 mice at 3, 18, and 25 months in passive avoidance as well as small and large Morris water maze and platform recognition. Passive avoidance and small pool performance impairments were shown to be age-related when comparing APP23 mice to non-transgenic controls, while large pool performance was highly impaired across timepoints. No impairment was noted for platform recognition performance, which occurred during large Morris maze testing (Kelly et al. 2003). Also in 2003, Van Dam et al. also tested APP23 mice in Morris water maze and passive avoidance at 6-8 weeks, 3, and 6 months. Impairment in Morris water maze was found to be age related in this study as well, however passive avoidance was not found to be impaired at these early timepoints. No significant differences between APP23 mice and non-transgenics were noted in a variety of behavioral and neuromotor measures at any timepoint except for increased total path length for APP23 mice in open field at 6 months. It was also noted that overnight cage activity appeared to be higher for APP23 mice than non-transgenics at various time points (Van Dam et al. 2003). In 2004, Dumont et al. tested aged (24 months) female APP23 mice in open field, elevated plus-maze, and Morris water maze. These mice showed hyperactivity compared to controls, decreased anxiety based on elevated plus-maze performance, and impairment in Morris water maze acquisition, but not retention. This impairment did not extend to probe or platform recognition trials (Dumont et al. 2004). The three studies described above give a very general picture of spatial learning impairment in APP23 mice and hint at some of the behavioral disturbances present in these animals, however comprehensive assessment of impairment including application of working
memory tasks has not been performed. Future testing may provide insight into the exact progression of impairment in this model.

**APPsw+PS1 Model**

Formation of a mouse line transgenic for a FAD PS1 mutation was thought to be the next logic step in creation of an AD mouse model based on the relatively high frequency of PS1 mutations among FAD cases. Single transgenic mice carrying human PS1 alleles, however, were found to be lacking significant Aβ pathology at any time point, despite a drastic (approximately 51%) increase in Aβ_{1-42} to Aβ_{1-40} ratios in the brains of these mice as well as in overall Aβ levels (Duff et al. 1996). It was found however, that by crossing these mice with APPsw transgenics, a double mutant model could be created which exhibited dramatically accelerated AD-type Aβ pathogenesis (Borchelt et al. 1997, Holcomb et al. 1998). These APPsw+PS1 mice have been characterized by a number of groups with respect to both pathology and behavior and have proven useful for numerous treatment and preventative studies in AD research because of their early age of onset for Aβ pathology and correlated cognitive impairment.

*Pathological Characterization of the APPsw+PS1 Model*

Early work in the APPsw+PS1 model by Borchelt et al. showed that, similar to PS1 single transgenic mice, these mice show an approximate 50% increase in soluble Aβ_{1-42} to Aβ_{1-40} ratios at an early timepoint (Borchelt et al. 1996). Double transgenics were found to develop amyloid plaques prior to APPsw single
transgenics, measured by Borchelt et al. at 12 months, and reactive astrocytes were shown to be associated with these plaques. Many of these plaques were found to stain mostly for Aβ1-40 at this timepoint, despite increased Aβ1-42 levels. It was also found that double transgenics carrying a wild-type human PS1 gene did not display accelerated Aβ deposition, indicating that the mutant allele PS1-A246E used in the study was necessary to bring this acceleration about (Borchelt et al. 1997).

In 1998, Holcomb et al. confirmed that a dramatic increase in Aβ1-42 to Aβ1-40 ratios is present in double transgenics, this time in a APPsw+PS1-M146L line at 6 weeks of age. At 13-16 weeks, it was shown that these mice also exhibit small numbers of deposits after thioflavin S staining, which stains for plaques containing fibrillar and β-pleated amyloid, with dramatically increased staining appearing at 24-32 weeks. Many of these deposits were also shown to be congophilic (Holcomb et al. 1998). A year later, the same group showed that by 6 months of age, all double transgenic exhibited amyloid deposits, though none were present at a 3 month timepoint (Holcomb et al. 1999). In contrast, Takeuchi et al. found that deposition occurs in APPsw+PS1 mice at as early as 3M in the frontal cortex and CA1 region of the hippocampus. At 12 months, they showed that 28.3% of the superior frontal cortex and 18.4% of the CA1 was covered by amyloid plaques, which is approximately 20-40 times greater than coverage levels in APPsw single transgenic mice. It was also noted that neuronal counts did not differ significantly among double or single transgenic from 3 to 12 months, despite apparent disruption of neuronal architecture in doubles by compact plaques (Takeuchi et al. 2000).
Plaque formation at 3 months was corroborated by Gordon et al. in 2002. This group also found that compact plaques continue to form through 12 months in both the hippocampus and frontal cortex, followed by formation of diffuse plaques in these as well as other regions. These diffuse plaques were shown to be composed almost entirely of Aβ_{1-42}, while compact plaques were indicated as being composed of both Aβ_{1-42} and Aβ_{1-40}, thus providing an explanation for findings by Borchlet et al. that plaques formed prior to 12 months showed higher ratios of Aβ_{1-40} relative to Aβ_{1-42}. Vascular Aβ was also detected by this group and was typically noted to be Aβ_{1-40}. Importantly, it was shown that compact plaques are associated with dystrophic neurites, GFAP expressing astrocytes, and MHC-II immunoreactive microglia even at early timepoints, indicating that these plaques elicit a similar neuroinflammatory response to those in Alzheimer’s via both neuronal degradation and direct interaction with immune cells (Gordon et al., 2002).

The effects of both soluble and insoluble Aβ in the brains of these double transgenics appear to center on loss of neuronal function, loss of LTP in memory centers and increases in inflammatory responses in areas surrounding deposits of Aβ. In 1999, Wong et al. found that cholinergic synapses in the frontal cortex of the brains of double transgenics showed a lower density than those of single transgenic and non-transgenic groups. A lowered average cholinergic synapse size in both the frontal cortex and hippocampus was also noted, though no changes were noted in basal forebrain cholinergic cell body numbers (Wong et al. 1999). In 2003, Dickey et al. reported that aged, 17-18 month-old APPsw+PS1 mice show reduced mRNA expression of genes involved in LTP and memory formation, specifically in regions
where Aβ accumulation had already occurred (cortex and hippocampus). Changes in synaptic structure, such as those noted by Takeuchi et al. (2000), were not shown to be associated with any altered mRNA levels, however inflammatory genes were upregulated. These findings were thought to provide an example of how memory capacities can be affected prior to any loss of neurons via modulation of LTP and other neuronal functions (Dickey et al. 2003). In a recent study, Trinchese et al. (2004) delved further into the study of LTP loss in APPsw+PS1 transgenics, finding that abnormal LTP is present at as early as 3 months, the same time point at which plaque formation begins and abnormal short-term working memory was first apparent, as indexed by the RAWM task. It was also shown that by 6 months, after increases in overall Aβ loads, basal synaptic transmission is impaired, coupled with a loss of long-term spatial learning/memory beginning at this time point in a plaque independent manner (Trinchese et al. 2004). These connections to cognitive impairment show correlations to many, but not all, studies focused on behavioral characterization of this mouse line.

Behavioral Characterization of the APPsw+PS1 Model

Early testing by Holcomb et al., 1998 found a similar level of performance among APPsw+PS1 double transgenics, single transgenic APPsws and PS1s, and non-transgenics in sensorimotor tasks at 3-4 months, indicating no baseline impairment in motor or sensory functions is present in these transgenics. Both the APPsw and APPsw+PS1 groups were found to exhibit decreased Y-maze spontaneous alternations compared to PS1 and non-transgenic mice at this same timepoint, indicating a decrease in cognitive performance in these groups. Only
double transgenic mice also showed an increase in overall activity in this task, which, when coupled with decreased percent alternation performance, was thought to correlate to hippocampal dysfunction (Holcomb et al. 1998). In 1999, Holcomb et al. continued testing of double transgenics in the Y-maze task, as well as in Morris Water Maze, over two additional time points. The same deficits were found in Y-maze at 6 and 9 months for these animals, though no significant differences were noted among groups for any measure in Morris Water Maze at any time point. Furthermore, it was noted that findings of decreased Y-maze performance at 3 months occurred prior to Aβ deposition in these mice, indicating loss of cognition did not necessarily require plaque formation directly (Holcomb et al. 1999). As was already shown, however, several other studies did find that some level of Aβ deposition does occur at this early time point, including the 1998 study by Holcomb et al., perhaps confounding interpretation of these results (Holcomb et al. 1998, Takeuchi et al. 2000, Gordon et al. 2002; Trinchese et al. 2004.

In 2001, Arendash et al. found impairment in aged APPsw+PS1 mice in the Radial Arm Water Maze task, and found a correlation between the level of impairment in individual mice and the level of plaque formation. Arendash et al. (2001) comprehensively tested the double transgenic mouse model in a full behavioral test battery, cross-sectionally at two time points. It was noted that transgenicity did not appear to affect cognitive performance in Y-maze, circular platform, standard water maze, or platform recognition at 5-7 or 15-17 months, and anxiety levels measured via the elevated plus maze task did not appear to differ among groups. However, double transgenics did exhibit progressive increases in
open field activity and in string agility impairment, coupled with continual impairment in the balance beam task. Most significant, though, were findings of cognitive impairment in both water maze and RAWM. Water maze acquisition was found to be impaired at the later timepoint, though importantly no significant impairment was noted during probe trials for this task. RAWM impairment was limited to the later time point for this study as well. These findings indicate that progressive impairment in spatial reference and working memory is present in these mice. (Arendash et al. 2001). In a more recent report involving the same investigators, Jensen et al. (2005) reported much earlier impairment at 4.5-6 months in APPsw+PS1 transgenic mice in Morris water maze and RAWM (Jensen et al. 2005). This early impairment is consistent with findings of early impairment in other studies such as Trinchese et al. (2004), in which it was reported that APPsw+PS1 mice are impaired in RAWM at as early as 3-4 months and in Morris water maze at as early as 6-8 months (Trinchese et al. 2004). This difference from previous studies such as Arendash et al. 2001, in which early impairment was not reported, are most likely due to the effects of crossbreeding of APP transgene bearing mice resulting in an increase of its effects. These effects were attributed to soluble Aβ constructs in the brains of these mice which resulted in their earlier impairment.

Sadowski et al. (2004) reported impairment at 8 and 22 months for APPsw+PS1 transgenics in Morris water maze as well as in Hebb-Williams maze. Impairment in spatial memory at the 22 month time point was correlated to both a loss of neurons (35.8%) in the hippocampal CA1 region and to reduced glucose utilization in the hippocampus (Sadowski et al. 2004).
Based on results from studies performed with APP transgenics, it is clear that
the resulting presence of Aβ in both soluble and insoluble form is sufficient to elicit
cognitive impairment in these mice which in many way parallels that of Alzheimer’s
Disease (Chen et al. 2000, Arendash et al. 2001). Progressive loss of short-term and
spatial memory as well as loss of long-term memory has been reported in animal
models of AD. These losses may be associated with loss of LTP and decreased basal
synaptic transmission as discussed above. This loss has been attributed to Aβ levels
(soluble and/or insoluble) as well as plaque deposition. Early decreases in LTP and
early memory impairment may result from actions of soluble Aβ oligomers including
neurotoxic protofibrils, while later deficits may be due to progressive Aβ plaque
burdens (Dickey et al. 2003, Trinchese et al. 2004). Several studies have been
conducted which have indicated that cognitive performance can be altered via
modulation of Aβ levels and/or clearance of Aβ plaques, as will be elicited in
discussion of current immunotherapeutic research in AD. These findings generate
excitement over the prospect of developing a preventative or treatment based on these
results. At the same time, however, it must be kept in mind that these models lack
complete development of all the pathological hallmarks of Alzheimer’s Disease, and
complete characterization of behavior in such models is simply not possible, making
them in many ways incomplete representations of the disease.

**Mutant APP Mice as Incomplete Models of AD**

Behaviorally, it is impossible to gauge all measures of disturbance in mouse
models to the extent that this can be accomplished in humans for obvious reasons.
Typical tasks for behavioral assessment in mice measure exploration, object
recognition, anxiety and fear, and cognitive impairment with regards to spatial short-term or long-term memory. Disparities noted during these tasks are thought to correlate to disruptions common to Alzheimer’s, from decreased mobility and motivation to visuospatial learning deficits to heightened anxiety (Janus and Westaway 2001). However, it is clear that these tasks, while useful for gaining a general picture of mouse behavior, are not specific enough to elicit findings comparable to those obtained in humans using a wide variety of interactive tests such as the MMSE, and even in humans diagnosis of AD is convoluted as a result of often insufficient and sometimes contradictory data noted in such studies.

The question also arises: are the behavioral deficits noted in animal models of AD associated with the transgene product via the same mechanism noted in humans? Mice have different cellular machinery involved in post-translational modification of proteins such as APP, and the exact process for neuronal production of hAPP in these models, as well as any subsequent modifications which occur during processing to form Aβ, has not fully been explored. Thus, the results of microinjection of such transgenes must be elicited via pathological analysis and comparison with AD pathology. Only the results of transgenicity can be compared, not the cause (Schwab et al. 2004).

In APP transgenics, a number of distinct differences can be noted immediately in comparison with human pathology. Spontaneous NFT formation has not been noted for any APP model (tau transgenicity has been necessary to induce such formation) despite the fact that FAD genes are known to directly result in AD development with full pathology in humans, including NFT formation. The cause of
this NFT formation in humans has not been entirely elucidated, but as described above it may be associated with Aβ formation. Aβ itself is thought to act differently within animal models as well. Although this peptide is known to be neurotoxic in humans, perhaps resulting in the widespread neuronal loss in hippocampal and cortical regions noted in AD, such loss of neurons simply does not occur in many APP transgenic models, and in no model does it occur at levels similar to those present in AD (Janus and Westaway 2001, Schwab 2004). Inflammatory responses to Aβ once it has deposited into plaques is significantly lessened as well in mice, with little or no complement system activation and lessened microglia activation. This can lead to misinterpretation of results in studies such as those designed to clear plaques from the nervous system without overactivation of the inflammatory system. These differences may result from variations in Aβ pathology in transgenic mice as compared to humans, such as morphological plaque density in APP models, which tend to have less compact cores. This is most apparent in the APP23 model where dense, congophilic plaques have also been noted to be still SDS soluble (Ashe 2001, Schwab 2004).

It is apparent that despite similarities in Aβ pathology between transgenic models and humans with AD, distinctions must be drawn when considering not only the biochemical nature of plaques but their effects on cognitive performance as well. Evaluation of these effects cognitively is made difficult for reasons outlined above. Thus, behavioral and pathological studies in transgenic models must be limited in application to specific and well designed treatment or prevention studies to elicit effects, and the results of such studies must be weighed against the faults intrinsic to
them. Nonetheless, the paradigm of repeated testing in various models followed by subsequent staged clinical trials is still the most efficient protocol for gaining insights into AD therapies in a safe fashion, and thus continued behavioral testing in such models, though found to be lacking, is still essential to AD research.
Inflammatory Responses in AD

Neuroinflammation is a term which has evolved to specifically describe chronic inflammation within the central nervous system involving glial cell activation and subsequent neurodegeneration, a process for which Alzheimer’s Disease provides a unique but complete representation (Streit et al. 2004). In AD, this inflammatory process appears to be induced directly by Aβ, as described above, and involves activation of glial cells, both asctocytes and microglia. Also reactive to Aβ, activated T-cells can play a formative role in the progression and severity of neuroinflammation in AD via direct action or through co-stimulation with microglia and production of cytokines. This is one main distinction between neuroinflammation in AD and other chronic forms of neuroinflammation, which do not typically involve T lymphocytes. It may be that disruption of the BBB allows these lymphocytes access to the AD brain, further influencing the inflammatory cascade which occurs progressively throughout the disease. Along with the direct affects of immunoreactive cells, cytokines and inflammatory proteins (and in some cases complement proteins expressed by these glial cells and lymphocytes) have widespread impact within regions of the CNS affected by AD.
Microglia Activation

Microglia, resident phagocytes of the CNS, are thought to be derived from the same cell lineage as macrophages and monocytes and to provide similar functions within the brain to those provided by these cells in the periphery. In neuroinflammation associated with Alzheimer’s, it appears that it is the non-phagocytic capabilities of these cells which play a major role in inflammation associated with the disease, though these may only be induced after frustration of phagocytic attempts (Streit 2004). Specifically, microglia are known to associate with neuritic plaques in AD brains as well as in hAPP transgenic mouse brains, and it has been shown that these cells can be activated by Aβ, especially the fibrillar, neurotoxic form of this peptide commonly found in neuritic plaques. Other materials associated specifically with compact neuritic plaques are also proposed to be involved in activation of these cells, as Aβ itself does not generally elicit the exaggerated response seen therein (D’Andrea et al. 2004). It is thought that these microglia are induced by aggregated Aβ and these other factors to attempt phagocytosis of Aβ aggregations; however, it is also apparent that they are largely unsuccessful at degrading the dense plaques they are typically associated with (Streit et al. 2004).

In contrast to these findings, in human brains after postmortem analysis as well as in transgenic mice brains taken after development of neuritic plaques, it has been shown that microglia are indeed capable of clearing Aβ in rodent brains and that human microglia can be induced to ingest Aβ in vitro (Frautschy et al. 1992). In rodent models, such clearance is significantly increased by brain lesioning and by injection of either Lipopolysaccharide (LPS) or Aβ antibody (Smith et al. 1998,
DiCarlo et al. 2001, Bard et al. 2000). All of these factors tend to either activate the overall immune and inflammatory systems or to interact with Aβ and microglia directly to facilitate phagocytosis. In vitro studies have also shown that complement system proteins such as the C1q protein may facilitate microglial phagocytosis of Aβ (Webster et al. 2000). Furthermore, postmortem analysis from an Aβ vaccination clinical trial indicated an association between microglia activation and amyloid clearance in humans (Nicoll et al. 2003). These and other studies have shown that microglia are quite capable of clearing Aβ in all configurations under the right circumstances, however such clearance is obviously deficient for some reason in AD. Thus it is either: 1) an overall failure of these cells to maintain clearance of plaques, due perhaps to an inherent dysfunction of these cells 2) an overwhelming effect following increased Aβ production later in life, or 3) the nature of the plaques themselves, which prevents their breakdown. If the first is true, it is possible that Alzheimer’s is due simply to a failure of microglia to maintain proper balances of Aβ within the brain, while the last hypothesis would seem to indicate that the progressive nature of plaque formation may allow this process to be too far along prior to microglia activation for these cells to be effective as phagocytes after such activation (Streit 2004). Future studies may elicit which of these is the true initial failure of microglia, thereby allowing researchers to gain insights into how to alter this process early on. In the present, however, it is the role of activated microglia in the Alzheimer’s neuroinflammatory cascade which has received the most study in AD research due to the neurotoxic potential of the cells therein.
As stated, the major role of microglia in exacerbating the inflammatory damage noted in AD does not appear to be related to the phagocytic role of these cells. Once activated, microglia often become “frustrated” due to the persistence of the compact neuritic plaques with which they are associated, in much the same way that immune cells of the periphery can, during certain autoimmune diseases or in response to mitogens, be activated excessively (D’Andrea et al. 2004). Once over-stimulated and after failed attempts at Aβ clearance, these microglia then proceed to produce a wide array of inflammatory factors including reactive oxygen species, complement proteins, cytokines (such as IL-1, IL-6, and TNFα), and a number of other factors which may be considered neurotoxic or which may stimulate further Aβ aggregation (Akiyama et al. 2000). Local neuronal degradation causes release of cytoplasmic inflammatory factors leading to further activation of glial cells. Astrocytes activated by these factors, as well as by cytokines released from microglia, are attracted to the plaque periphery where they begin to produce their own set of inflammatory proteins and cytokines, resulting in a co-stimulatory effect with neighboring microglia (D’Andrea et al. 2004). Inflammatory proteins such as ApoE (produced by subsets of both glial cell types), α-ACT (produced by astrocytes), complement proteins, and perhaps other factors all associate with neuritic plaques and may be involved not only in promoting further Aβ aggregation, but in further stimulating microglial response via receptor mediated activation of these cells (Nilsson et al. 2004, Uchihara et al. 1995, D’Andrea et al. 2004). The end result is a localized inflammatory cascade with potentially widespread repercussions due to cytokine diffusion.
T Cell Activation

In a general sense, T cells can be divided based on expression of either the CD4 or CD8 co-receptor, which primarily segregates the cells into helper and cytotoxic lines respectively. T helper (Th) cells produce a variety of cytokines when activated which are involved in mediating inflammatory and immune responses, while cytotoxic cells are largely involved in recognition and destruction of abnormal or aged self cells. Helper cells can be further divided into Th1 and Th2 subsets, each with distinct and often antagonistic roles in the immune system. The former tends to promote inflammation and cellular based immunity via activation of macrophages and cytotoxic T cells (T_C) as well as other immune cells such as neutrophils, while the latter tends to initiate a humoral based response via activation of B cells (Vallejo et al. 2004).

Each subtype of T cells requires a different combination of receptor mediated activation to initiate the effects they are known to elicit. Receptor activation also dictates if and when T cells will begin to differentiate and mature. All T cells begin as naïve cells and remain so unless they encounter a recognizable antigen properly presented by an antigen presenting cell (APC) for Th cells or by the majority of self cells for T_C cells. T cell recognition is dictated by variations in T cell receptor (TCR) subcomponants, which alters initial ability of a cell to recognize antigens presented by major histocompatibility (MHC) II complexes of APCs or to recognize peptides presented by MHC I complexes of self cells. Antigens presented by APCs are typically produced after breakdown of foreign peptides extracellularly via enzymatic degradation or intracellularly after phagocytosis. The peptides presented by self cells
are typically derived from viral or intracellular microorganisms which have infected these cells; however aged, cancerous, or otherwise altered cells may also present peptides which promote their own death (Vallejo et al. 2004, Crow 2004).

Coupling of the TCR with the antigen/MHC II complex or peptide/MHC I complex initiates T cell activation and can result in differentiation into effector T cells capable of varying functions, however further interaction of secondary cell surface proteins is also required for induction of such activities. In many cases, such proteins are not expressed unless secondary activation via cytokine receptors has occurred. Thus, activation of individual T cell subtypes depends not only on recognition of the appropriate antigen / MHC complex, but also on stimulation of regulatory cells to produce appropriate cytokines which then mediate which specific subtype of T cells will be activated. Once fully activated, memory T cells may develop which proliferate and remain present in the body, awaiting reactivation or continuing to act in the presence of persistent stimuli (Vallejo et al. 2004, Chandock et al. 2004).

The role of T C cells in Alzheimer’s neuroinflammation is thought to be primarily detrimental due to the ability of these cells to induce neuronal or glial apoptosis, and thus Th1 cells activation may also be considered disadvantageous due to subsequent activation of T C cells. At the same time, microglia can be activated by Th1 cells resulting in a positive effect if phagocytosis of Aβ can be induced and a negative effect if phagocytic frustration results, as discussed above. Th2 cell activation may prove to be beneficial in Alzheimer’s if Aβ antibody formation by B cells is then induced, enhancing clearance of Aβ, as will be discussed later, and other
distinct capabilities of unique T cell subtypes may also be either detrimental or therapeutic based on extent of activation and timing of such activation.

Microglia and T Cell Co-activation

As discussed above, both microglia and T cells are heavily reliant on activation by other cell types via cytokine receptor activation or direct receptor protein interactions with surface proteins of these other cell lines. As phagocytes, microglia are among the few cell types capable of internalizing, digesting, and presenting proteins in association with MHC II receptors in the CNS, thus acting as APCs (Hickey et al. 1988). Specific lines of T cells, largely among the Th1 subtype but also among the Th2 subtype, are capable of inducing microglia to carry out this activity, and in certain cases microglia will remain inactive without such input. Thus, though many other cell lines may be involved in the process, activation of microglia and T cells can occur concurrently and can have a tendency towards exponential propagation under certain conditions. As will be shown, however, the true interactions which have been shown to occur between microglia and T cells in the presence of Aβ are convoluted and often difficult to interpret.

In 2003, Monsonego et al. showed that mouse microglia primed with INF-γ are capable of serving as Aβ APCs for both Aβ1-40 and Aβ1-42, presenting these peptides to Aβ-specific T cells. Both Th1 and Th2 subtypes were shown to be activated by these peptides when presented by microglia. Th1 cells were shown to be activated in the short term, with increased expression of IFN-γ (a microglial activator and inhibitor of Th2 proliferation) noted. Coactivation of microglia resulted in their expression of TNF-α (an inflammatory cytokine) and IL-10. As an anti-
inflammatory cytokine, IL-10 is known to inhibit Th1 subset activation, and to inhibit monocyte/macrophage lineage production of NO and a variety of cytokines involved in inflammation. In contrast to this, it was shown that microglia were actually stimulated by Th1 cells to increase their production of NO, which is a known general T cell inhibitor. Inhibition of iNOS to reduce this production of NO resulted in restoration of Th1 cell proliferation. Anti-IL-10 and IFN-γ antibody treatments also elucidated that this inhibition could not be reversed simply by blocking these cytokines without inhibition of iNOS, indicating that the normal control of IL-10 over Th1 cell lines was not the pathway of T cell regulation in this case. Thus it appears that the interactions between microglia and Th1 subsets in mice are in many ways distinct from normal interactions between Th1 cells and cells of the monocyte lineage, and that microglia are capable of inhibiting Th1 cells as a result of Th1 cells stimulating microglia to produce NO. This high level of NO and perhaps other mediating factors is thought to then bring about apoptosis of Th1 cells (Monsonego et al. 2003).

It was also noted that in cultures of microglia with Th2 subset cells, there was an increase in production of IL-4 (promotes Th2 proliferation), IL-10, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). These T cells did not undergo apoptosis, but were instead activated and induced to proliferate. Thus it was assumed that the Th2 repertoire of cytokines is sufficient to block NO mediated apoptosis of T cells, as has been shown in previous studies, despite an up-regulation of NO which was noted in these cultures (Monsonego et al 2003).
Based on these *in vitro* results coupled with those of previous studies, some of the potential interaction which occur between T cells and microglia in Alzheimer’s may be speculated upon and are summarized in Figure 1. The two major effectors of microglia activation noted in the study conducted by Monsonego et al. (2003) were IFN-γ and GM-CSF, produced by the Th1 and Th2 subsets respectively, which promoted presentation of Aβ by microglia to these cells. Other cytokines such as IL-10 were shown to decrease production of NO by microglia *in vitro*. Depending on the type of interactions which occur during and following activation, however, microglia can then act to regulate T cell activity via production of a variety of mediating factors such as NO, as discussed above. Therefore, with development of an understanding of the interaction which occur between these cell types, it may be possible to alter activation of specific T cell types to promote clearance of Aβ by microglia while preventing inflammatory damage due to secondary activities of both T cells and microglia.
Figure 1. IFN-γ primed Microglia cultured in the presence of Aβ are capable of MHC II associated presentation of Aβ to both Th1 and Th2 cell types. This leads to short term activation of Th1 cells with potential for subsequent inhibition following release of Nitrous Oxide (NO) and IL-10 by microglia (red). Activated Th2 cells are protected from inhibition however, perhaps via autocrine action of GM-CSF and/or IL-4 cytokines (blue). In both instances, microglia remain active and produce inflammatory cytokines such as TNF-α, as well as NO (green).
Vaccination in AD Research

In the late 1990’s, *in vitro* studies reported that antibodies specific to various Aβ epitopes can disaggregate amyloid plaques and prevent Aβ fibrilogenesis. It was not long thereafter that researchers began focusing on the idea of an amyloid vaccination as a treatment or even preventative for AD. Promising results in early work *in vitro* and *in vivo* with animal models of AD culminated in early phase clinical trials in humans using Aβ immunizations. These trials were halted after death of a small number of individuals was reported due to CNS inflammation. Since that time, intense focus has been placed on finding an immunotherapeutic strategy for fighting AD which does not instigate the type of inflammatory response initiated by active Aβ immunization.

*In Vitro Aβ Vaccination Studies*

Two of the earliest studies *in vitro* which contributed to initiating the current immunotherapeutic era of Alzheimer’s research were conducted by Solomon et al. in 1996 and 1997. In the former study, it was noted that monoclonal antibodies recognizing either aa1-28 or aa8-17 residues of the Aβ peptide are capable of inhibiting normal in vitro aggregation of Aβ (Solomon et al. 1996). In the latter study it was shown that the antibodies recognizing the aa1-28 residue of Aβ were actually
capable of disaggregating insoluble Aβ fibrils into soluble Aβ (Solomon et al. 1997). In 1998, Frenkel et al. found that these same antibodies specifically bind to aa3-6 (sequence EFRH), and it was inferred from these results that this sequence must be involved in the Aβ aggregation process (Frenkel et al. 1998). A year later, this group published findings which indicated that not all Aβ antibodies recognizing the region of the peptide containing this residue were as effective at binding to it, and that low binding affinity antibodies were not capable of preventing Aβ aggregation (Frenkel et al. 1999). From the results of these four in vitro studies, it was deduced that carefully selected antibodies to Aβ had the potential to block or even reverse amyloid aggregation, paving the way for future in vivo studies in Aβ vaccination.

**Innate Immune Responses to Aβ**

On a cellular level, the response of the immune system to Aβ is three-fold. B cells can be stimulated to produce Aβ antibodies, glial cells can be stimulated to produce a variety of inflammatory molecules and in the case of microglia to attempt phagocytosis of Aβ plaques, and T cells can be stimulated to produce an array of modulatory cytokines as discussed above. Prior to Aβ vaccination, these responses are present in varying levels. Localized, high levels of glial cell activation are common near Aβ plaques (D'Andrea 2004), and aged AD patients are significantly more likely to exhibit strong T cell reactivity to Aβ (Monsonego et al. 2003). Finally, Aβ antibodies are known to be produced in AD and may be involved in triggering microglia response to plaques, thus facilitating phagocytosis (Nath et al. 2003, Wilcock et al. 2004). These reactions to Aβ are also evoked in varying degrees
following Aβ vaccination (Nicoll et al. 2003). The current goal of immunotherapeutic research in AD is to establish a means of creating a balanced response incorporating each of these responses only to the extent necessary to be beneficial.

Active Aβ Immunization in Animal Models

A wide variety of active Aβ-based immunization studies have been reported on since the first reports in 1999. Retrospectively, with the knowledge we now have of consequences of standard Aβ immunization in humans, it is perhaps most simple to divide these studies into three main categories. The first consists of those studies which provide a general understanding of the positive pathological consequences of Aβ immunization, which are based largely on amyloid plaque clearance and reduction in plaque associated pathology. The second consists of those studies which provide insight into how side-effects from such administrations in humans could be anticipated by both characterizing the neuroinflammatory response elicited by these or which provide alternative, less dangerous modes of eliciting an anti-Aβ immune response. Finally, studies providing behavioral characterization of treated animals as potentially indicative of the cognitive protection that may or may not result after administration of an Aβ vaccine.

Pathological Benefits of Active Aβ Immunization

A number of studies focused on eliciting the pathological benefits of Aβ vaccination while not reporting results showing the potential for negative impacts or the behavioral consequences that result from such vaccination. In 1999, Schenk et al.
reported results from the first attempted active Aβ immunization study in a mouse model of AD. Injections of Aβ1-42 were performed monthly for 11 months starting at 2 months in PDAPP mice. A separate group of mice was injected with SAP peptide, a separate component common to amyloid plaques. Initial injections contained complete Freund’s adjuvant, while each additional injection contained incomplete Freund’s adjuvant, a protocol used in the majority of future active Aβ vaccination studies. Titres of antibodies to the injected antigen were measured at higher than 1:10,000 for most Aβ injected mice and between 1:1,000 and 1:10,000 for SAP injected mice. The mice were sacrificed at 13 months, and it was found that Aβ immunized mice were almost completely protected against development of Aβ plaques in the CNS, while SAP immunized mice showed statistically similar levels of plaques compared to non-treated controls. This latter data would seem to indicate that an immune response elicited towards any Aβ plaque component is not necessarily capable of clearing these plaques. Almost complete protection against development of dystrophic neuritis and reduction of astrocytosis and microglia activation was also noted in Aβ immunized mice (Schenk et al. 1999).

This group also performed additional experiments to determine if reduction of plaque loads could be accomplished in aged mice. 11-month-old female PDAPP mice were again immunized with Aβ1-42. Sacrifice of the mice occurred at either 15 or 18 months. At both time points, Aβ burdens were significantly reduced compared to PBS treated transgenic controls. Reduction in both diffuse and compact plaque counts were attributed to clearance of already formed deposits. Existing plaques were
noted to be coated in IgG antibodies to Aβ\textsubscript{1-42}, indicating a direct mechanism of action for these antibodies within the CNS. Lack of plaques in regions such as the entorhinal cortex were attributed to blocked formation of plaques typically not deposited until later time points in PDAPP mice. Aβ containing cells thought to be microglia or monocytes were present in areas normally containing high plaque counts at aged timepoints, indicating an activation of these cells to phagocytize existing Aβ deposits, perhaps via antibody receptor mediated activation. Astrocytosis was noted to be reduced in these Aβ treated mice, as were other neuritic plaque pathologies. This second round of results would seem to indicate that while Aβ vaccination does show potential efficacy as a treatment due to partial clearance of plaques at an aged time point in transgenic mice and prevented formation of additional plaques, clearly the most benefit stands to be gained from earlier, prophylactic administration of Aβ (Schenk et al. 1999).

In 2000, Weiner et al. found that intranasal administration of synthetic Aβ\textsubscript{1-40} peptide, as opposed to injections of Aβ\textsubscript{1-42}, between 5 and 12 months in PDAPP mice resulted in significantly decreased neuritic plaque burden and overall Aβ\textsubscript{1-42} levels compared to controls treated with myelin based protein or standard controls. Decreased microglia and astrocyte activation, decreased neuritic dystrophy as seen in Schenk et al. (1999), and decreased reactive mononuclear cell activation was also reported. Importantly, this study demonstrated the efficacy of intranasal Aβ vaccination for the first time. (Weiner et al. 2000).
In 2001, Das et al. showed that effectiveness of A\(\beta_{1-42}\) immunization correlates inversely to amyloid deposition levels in Tg2576 mice. Mice with minimal, modest, and significant A\(\beta\) deposition (7-8, 10-11, and 18 months) were immunized with the peptide, and reductions in cerebral A\(\beta\) were noted to be highest in mice immunized at younger timepoints. It was also noted that A\(\beta_{1-42}\) was reduced at higher levels than A\(\beta_{1-40}\). This study clearly indicated the importance of early vaccination as a preventative/treatment for AD (Das et al. 2001).

Further expanding on the potential benefits of A\(\beta\) vaccination, Oddo et al. (2004) reported for the first time that A\(\beta\) immunization results in clearance of non-hyperphosphorylated (early) but not hyperphosphorylated tau aggregates in a APPsw+PS1+Tau transgenic model, along with the normal reduction in A\(\beta\) pathology expected after such immunization. It was also noted that A\(\beta\) pathology was necessary for formation of tau pathology, and clearance of the former via non-immunological methods resulted directly in clearance of the latter (Oddo et al. 2004).

Lemere et al. (2003) reported that the typical reduction in A\(\beta\) pathology associated with A\(\beta\) immunization such as that noted in the above three studies was coupled with a dramatic increase in A\(\beta\) levels in the serum of APP transgenic mice. It was suggested that the main mechanism of action of A\(\beta\) immunization is thus stimulation of A\(\beta\) antibodies largely restricted to the blood, association of these plasma antibodies with A\(\beta\), and clearance of CNS antibody/antigen complexes into the vasculature, resulting in reduced A\(\beta\) levels in the CNS (Lemere et al. 2003).
To better understand the time course for immune system reactivity to Aβ, Wilcock et al. (2001) delved further into the specific immune responses elicited by various numbers of Aβ1-42 vaccinations, reporting that anti-Aβ antibody titers remained low after only 3 inoculations in APPsw+PS1 mice but were significantly higher after nine inoculations. After 5 inoculations there was a high level of microglia activation which tapered off by nine injections. A correlation was found between microglia activation and reductions in congo red staining, suggesting that microglia may be involved in clearing compact Aβ plaques in these mice. Thus this study and that by Lemere et al. (2003) provided two distinct and probable modes of action for anti-Aβ antibodies in clearing amyloid from the CNS (Wilcock et al. 2001).

In further examining the potential for microglial phagocytosis of Aβ plaques, it was shown that Aβ1-42 immunization of Tg2576 mice crossed with FcRγ-/- (Fc receptor knock-out) mice resulted in unimpaired reduction in Aβ levels according to Das et al. (2003) compared to immunization of Tg2576, Fc receptor-sufficient mice. This would seem to indicate that microglial, FcR-mediated phagocytosis is not required for effectiveness of the Aβ vaccination and is not the main mechanism for Aβ clearance (Das et al. 2003a). Das et al. (2003) also showed that B and T cell responses to Aβ1-42 immunization vary greatly based on specific MHC class II expression patterns, indicating that some combinations of expression among these molecules result in better presentation of the Aβ peptide to these cells and once again emphasized the importance of these immune cells in determining immune response to Aβ (Das et al. 2003b).
Along with an understanding of the reactions of the immune system of APP transgenic mice to Aβ inoculation and the mechanisms of Aβ clearance, it is also necessary that the impact of expression of this human transgene on the response of these animals be understood. In comparing the immune response of APP transgenics and non-transgenics to Aβ inoculations, Monsonego et al. (2001) reported that APP transgenic mice immunized with either Aβ1-40 or Aβ1-42 displayed hyporesponsiveness to these peptides immunologically. This was thought to be associated with a lack of T cell response in these animals, perhaps due to long-term presence of Aβ peptides in transgenic mouse brains from an early time point. It was suggested that if the T cell response could be raised during the vaccination period, such immunotherapy would prove more successful (Monsonego et al. 2001).

Further emphasizing the hyporesponsiveness of the the APP transgenic mouse immune system to Aβ, Dickey et al. (2001) reported that three vaccinations with Aβ1-42 was necessary to elicit a significant immune response in APPsw+PS1 transgenic mice based on IgG levels. Subsequent administrations of the vaccine resulted in moderate immune responses through 5 months. It was also noted that the highest immune response was to the Aβ N terminus, and it was suggested that because the main humoral response was IgG1- and IgG2-based, a Th2 T cell subset activation was likely to be involved in generating this response. Importantly, it was also noted that the immunized mice displayed T-cells stimulated specifically by the Aβ peptide, again emphasizing the potential for T-cell activation to regulate the immune response to Aβ vaccination (Dickey et al. 2001). Both of these previous two studies provided
insight into the importance of proper regulation of T cell activation following immunotherapy.

Additional support for the important regulatory role of Th2 cells following Aβ immunization in transgenic mice was provided by Town et al. (2002). It was reported that a bias towards this T cell subset in both C57BL/6 non-transgenics and APP mice inoculated with Aβ₁-₄₂ was found. Importantly, this bias was not noted following Aβ₁-₄₀ immunization, indicating an altered immune response based on antigen selection (Town et al. 2002).

In 2004, Li et al. reported that while anti-Aβ levels from Aβ immunized APP transgenic mice are masked in ELISA measurements if not first treated with a low-pH solution to dissociate anti-Aβ/Aβ complexes, these levels are still significantly lower than those of non-transgenic mice of the same background following immunization even after such treatment. Only when Aβ was administered associated with papillomavirus virus-like particles (VLPs) were levels of anti-Aβ comparable in APP and non-transgenic mice, indicating that self-tolerance to Aβ in APP mice results in hypoimmune responsiveness in these mice after normal active Aβ immunization (Li et al. 2004).

Based on findings that aged Caribbean Vervets, which are primates, often develop cerebral Aβ plaques, Lemere et al. (2004) vaccinated a number of these animals for 10 months with a cocktail of Aβ₁₋₄₀ and Aβ₁₋₄₂ in a 3:1 ratio. No plaques were found in the four surviving immunized animals, while 11 of 13 controls showed plaque development. It was also noted that rises in plasma Aβ were associated with
decreases in brain Aβ levels in immunized animals, and antibodies to Aβ were noted in both the plasma and CNS. This study was the first to report Aβ clearance following Aβ vaccination in a non-human, non-transgenic mammal which normally develops Aβ plaques with aging (Lemere et al. 2004).

The above studies elucidated the potential for active Aβ immunization in AD animal models to elicit an immune response at numerous timepoints in the development of amyloid pathology, including production of various anti-Aβ antibody isoforms and stimulation of cellular-based immunity mediated by T lymphocytes. It was demonstrated that such immune system activation leads to varying levels of clearance of Aβ pathology from the CNS based on background strain interactions, antigen selection, antigen delivery, adjuvant selection, and age. This generated excitement at the possibility of developing a vaccination-based therapeutic for Alzheimer’s disease. Still left to be discovered by additional studies, however, was the potential for such immunization to also lead to devastating consequences in the AD brain, as well as the actual cognitive benefit to be gained by Aβ vaccination.

*Deleterious Effects of Active Aβ Immunization and Alternative Active Immunization Approaches.*

Two major studies in Aβ vaccination focused largely on its negative consequences. Su et al. (1999) reported that low-level infusions of soluble Aβ1-40 intravascularly in 3 month old male rats over the course of 2 weeks resulted in blood vessel damage including gross pulmonary hemorrhage, disruption of the BBB, and activation of microglia and astrocytes in the CNS. While these physiological
consequences were not coupled with impairment in water maze, they did provide evidence that even short, low-level administrations of Aβ can result in damage to both the vasculature and perhaps the CNS via initiation of widespread inflammation. This again emphasized the importance of determining a method for vaccination which does not instigate such damage (Su et al. 1999).

Furlan et al. (2003) further elucidated our understanding of the potential for neuroinflammatory damage resulting from Aβ vaccination. Young C57BL/6 mice immunized with Aβ1-42 were shown to display inflammatory foci in the brain and spinal cord associated with the vasculature of these areas. These foci were noted to contain microphages, T and B cells, and immunoglobulins. This response was attributed to Th1 subset mediation of inflammatory events coupled with high anti-Aβ levels. Importantly, these results were noted only when a co-administration of Aβ and pertussis toxin (which elicits autoimmune processes) was performed; thus the extent to which the toxin contributed to development of these foci is important in interpretation of these results (Furlan et al. 2003).

It was apparent following the Su et al. 1999 study and based on results of phase II clinical trials in humans that there are dangers associated with simple Aβ vaccinations mainly associated with increased neuroinflammation, and numerous studies which followed focused largely on searching for more unique modes of immune system activation which do not invoke such reactions. In 2000, Frenkel et al. attempted active Aβ immunization in both BALB/c mice and guinea pigs via either intranasal administration and s.c. or i.p. injection of a phage displaying the Aβ 3-6
peptide residue EFRH. Seven days following injection, serum levels of IgG reactive against both wt-phage and Aβ were measured for three injections spaced 14 days apart. Mice injected i.p. with Freund’s adjuvant developed large titres of antibodies which increased after each injection. Specific IgG and IgA antibodies were noted 7 days after only one booster injection of phages. Guinea pigs injected s.c. were noted to develop very high titres of anti-Aβ antibodies following the same injection protocol. The guinea pig EFRH sequence is homologous to that of humans, whereas in mice the sequence is EFGH, so the effectiveness of the phage administration in both species emphasized the potential of this vaccination for effectiveness in humans. It was shown that in both animal groups, sera containing antibodies produced was able to bind specifically to the Aβ peptide, and that this sera protected against Aβ neurotoxicity in vitro. Also noted was the ability of this sera to disrupt Aβ fibril formation in vitro (Frenkel et al. 2000). In 2003, Frenkel et al. again immunized mice with EFRH presenting phages, this time in the APP transgenic model. This immunotherapeutic approach was shown to again dramatically reduce Aβ pathology in these mice. Importantly, it was noted that two mice receiving the phage did not develop high titers of anti-Aβ antibodies, indicating less than perfect penetrance (Frenkel 2003).

In 2001, Sigurdsson et al. were able to show that immunization with a nontoxic, soluble Aβ homologue results in a similar reduction in amyloid pathology to those noted after standard Aβ administration in APP transgenic mice. Along with reductions in soluble and insoluble Aβ, these mice also exhibited decreased microglia
activation, thought to be associated with an overall reduction in inflammation levels (Sigurdsson et al. 2001). In 2004, Sigurdsson et al. carried out active immunization in APP mice with this same nonfibrillogenic, nontoxic derivative of Aβ, K6Aβ1-30, which was known to induce Aβ clearance in similar levels to Aβ1-42 treatments. A primarily IgM response resulted in these mice, and Aβ pathology was significantly reduced in inverse correlation to levels of these antibodies. IgM is not able to cross the BBB, so it was inferred that Aβ clearance resulted from these antibodies acting as a peripheral “sink” for these peptides.

In 2002, a study conducted by McLaurin et al. showed that antibodies recognizing the 4-10 residues of Aβ1-42 are capable of inhibiting fibrillogenesis of Aβ in TgCRND8 transgenic mice without invoking activation of the inflammatory system. This antibody was shown to target protofibrillar Aβ, which is thought to be important in formation of Aβ plaques, and provided an important mechanism for blockage of said formation. It was further reported that the immunization which led to this antibody formation caused a biased T cell response, downregulating inflammatory events associated with Th1 cell activation while upregulating some aspects of the humoral responses elicited by Th2 cells (McLaurin et al. 2002).

Koller et al. (2004) found that immunization of APP transgenic mice with Aβ1-42 coupled with DnaK, a heat shock protein 70 analogue and strong adjuvant, resulted in measurable levels of anti-Aβ antibodies after only one inoculation with maximal titers of these antibodies present after the first booster inoculation. These levels were still significantly lower than those achieved in non-transgenic controls
receiving the same vaccination. This emphasized the importance of adjuvant in
determining the immune response elicited by active immunization, and provided a
method for reducing the total number/amount of Aβ administration necessary to elicit
an appropriate immune response. Importantly, however, these immunizations were
all administered by 3 months of age, and as a result no affect was seen on Aβ levels
and plaque burdens after sacrifice at an aged time point, suggesting that prophylactic
immunization occurring too early in life, without continuation, may prove ineffectual.
(Koller et al. 2004).

Hara et al. (2004) found that oral administration of a recombinant adeno-
associated virus vector containing cDNA for Aβ resulted in expression and secretion
of both Aβ1-43 and Aβ1-21 within the intestine of hAPP transgenic mice. This resulted
in anti-Aβ antibody production through 6 months and significant decreases in CNS
Aβ levels. These decreases were not associated with any notable inflammation or T
cell response (Hara et al. 2004).

The work of Su et al. (1999), Furlan et al. (2003), and results from Aβ
vaccination clinical trials served as a warning for researchers, demanding that new
understanding of the true consequences of Aβ immunization should be gained before
any further clinical trials are conducted. Many of the studies which followed thus
searched to explore alternative Aβ vaccination methodologies to reduce
neuroinflammation and a cellular-based immune response. Even these studies,
however, are not fully sufficient to gain true understanding of the potential effects of
Aβ vaccination is AD models. Yet to be determined is the effects of Aβ vaccination
on cognitive behavior, as most of the studies mentioned above did not seek to correlate any pathological findings with cognitive impacts.

Behavioral Characterization in Active Aβ Immunotherapy

Due to the potential for neuroinflammatory damage associated with Aβ immunization, it was necessary to establish that cognitive protection could be elicited by such treatment despite these potential negative consequences. Janus et al. (2000) reported that vaccination of young TgCRND8 mice with Aβ1-42 peptide in complete Freund’s adjuvant resulted not only in plaque load reductions, but also in decreased impairment in Morris water maze acquisition, though these mice still did not perform at the same level as non-transgenic controls. 3 cohorts of mice were immunized at 6, 8, 12, 16, and 20 weeks of age with either Aβ1-42 peptide or islet-associated polypeptide (IAPP), both in β-pleated sheet formation. This control antigen was selected based on its similarity to Aβ, but also on the fact that it only causes amyloidosis outside of the CNS. Both peptides were shown to induce antibody production by 13 weeks when the first group of injected animals underwent sacrifice. A second group of animals were sacrificed at 23 weeks and showed 2-3 fold increases in antibody productions by this time point. These antibodies were shown to strongly bind to dense Aβ plaques but to only weakly interact with diffuse, non-fibrillar deposits of Aβ. Also, these antibodies did not appear to be reactive to APP, as normal neurons were not detected by the antibody. Impacts of immunization on Aβ pathology were noted to be similar to those from previous studies with regards to density and size changes of dense-cored plaques, however at both 13 and 25 weeks,
no changes in formic-acid-extractable (soluble) Aβ were noted, perhaps because of
the specificity of anti-Aβ antibodies to the β-pleated sheet formation of deposited Aβ.

Behavioral testing of mice by Janus et al. (2000) consisted of longitudinal
testing (at 11, 15, 19, and 23 weeks of age) in a reference memory version of Morris
water maze, with the submerged escape platform in a different location at each test
age. Aβ1-42 immunized transgenic mice were shown to perform significantly better
than non-immunized transgenics, however performance was not improved to the level
of non-transgenic mice. Aβ immunized mice were also shown to significantly
improve in task performance after repeated reversal testing, indicating a learning
affect in these mice. In probe trial testing following Morris water maze testing, no
significant differences were noted among groups. However, this was attributed to the
close temporal proximaty of task administration (30 minutes) to final day Morris
water maze administration. Controls studies indicated that no performance changes
were noted for non-transgenic mice following any form of PBS, Aβ, or IAPP
injection with or without adjuvant (Janus et al. 2000).

Morgan et al. (2000) and Arendash et al. 2001 found that APPsw+PS1 mice
immunized monthly with Aβ1-42 in Freund’s adjuvant (mineral oil after 3 months)
showed significant protection against impairment in the RAWM task at an aged time
point compared to controls receiving a control vaccine of keyhole limpet
haemocyanin. Behavioral assessment of double transgenic mice by Morgan et al.
(2000) and Arendash et al. (2001) consisted of pre-immunization administration of a
6-week battery of sensorimotor and cognitive based tasks between 5 and 7 months of
age followed post-immunization by Radial Arm Water Maze testing at 11.5 and 15.5
months and balance beam, string suspension, open maze, and Y-maze tasks at 16 months. No differences were noted among groups in pre-immunization performances, except in balance beam performance in which transgenic animals were impaired overall. In RAWM testing at 11.5 months, transgenic animals performed at the same level as non-transgenic controls. Results from repeated testing at 15.5 months demonstrated that Aβ immunized mice reached similar levels of performance to non-transgenics by final trials of testing, however it was demonstrated that these mice required more trials to reach this level of performance than these non-transgenics. Working memory was thus shown to be greatly improved in APPsw+PS1 mice following repeated Aβ1-42 immunizations over an 8 month period (Morgan et al. 2000).

Mice immunized with Aβ, both transgenic and wild-type, showed high anti-Aβ serum activity while non-Aβ immunized animals showed no such activity. Modest to no reduction in Aβ plaque burdens in the frontal cortex and hippocampus were noted post-immunization. Though no measures of soluble Aβ levels were noted in these studies, it was suggested that reduced levels of soluble, toxic Aβ oligomers may have contributed to the lack of cognitive impairment present in these Aβ immunized animals, despite their high levels of Aβ pathology remaining in the CNS. Based on correlation analysis of behavioral task performances and Aβ burden measures, a number of correlations were evident in the same study’s animals (Arendash et al. 2001). While there was no correlation between total Aβ immunostaining (compact and diffuse plaques) and RAWM performance, T4 error
measures did correlate with both Aβ₁₋₄₀ immunostaining and congo red staining in frontal cortex as well as with immunostaining in both the hippocampal CA1 region and dentate gyrus. However, as Aβ reduction was modest at best it is likely the impact of vaccination on soluble Aβ constructs in the CNS (neutralization and/or clearance by anti-Aβ antibodies) that played a larger role in providing cognitive benefit (Morgan et al. 2000, Arendash et al. 2001).

Jensen et al. (2005) vaccinated APP+PS1 mice with Aβ₁₋₄₂ in Freund’s adjuvant (complete for initial injection, incomplete for monthly booster injections) from 2-16.5 months of age and determined the effect of these injections at 4.5-6 months and 15-16.5 months longitudinally via administration of a 6 week behavioral test battery at each time point. The study cohort was divided into control transgenic, vaccinated transgenic, and non-transgenic mice. At the 4.5-6 month time point, both transgenic groups displayed increased open field activity, while no group differences were noted at the 15-16.5 month time point. Only young (early time point) treatment mice were impaired at the balance beam task, however this impairment was not present at the later time point and no impairment was present for string agility performance at any time point. Comparing early and late time point performances, a significant increase in percent time spent in open arms during the elevated plus maze task in control mice was coupled with a significant decrease in the same measure for vaccinated mice, perhaps indicating an anxiolytic effect in the former and an anxiety increase in the former. Consistent with open field results, vaccinated mice also showed significantly higher plus maze and Y-maze arm entries at the young time point. Only Y-maze entries remained higher at the aged time point, however. No
differences among groups were noted among percent alternations in Y-maze or circular platform acquisition, however significance was found for each additional cognitive based task administered. In Morris water maze acquisition, vaccinated mice were protected against impairment noted in controls at both time points. This same trend was noticed for young mice tested in Morris water maze retention, however both transgenic groups were impaired compared to non-transgenics at the later testing time point. No impairment at the young time point was noticed in platform recognition, whereas vaccinated mice showed protection against impairment exhibited by control mice at the later time point in this task. RAWM overall and last block performances were reported for these mice as well, and for both measures at both time points control mice showed performance impairment. In contrast, only in overall RAWM performance at the young time point were vaccinated mice impaired; in last block, young time point performance and in both last block and overall aged time point performance vaccinated mice showed were protected against this impairment. Discriminant function analysis using direct entry method revealed cognitive protection in vaccinated animals at the young time point. Stepwise-forward analysis showed similar results for all measures, as well as cognitive measures, at both time points.

At 17 months of age, mice in the study conducted by Jensen et al. (2005) were sacrificed, and Aβ histopathology was performed on mouse brain sections. No significant reduction in Aβ immunostaining (total Aβ) or Congo Red staining (compact Aβ) burdens were noticed in vaccinated mice overall, though one vaccinated mouse was noted to have significant reductions of up to 86% in Aβ levels.
Correlation analysis between the 8 Aβ deposition measures noted and nine cognitive measures taken at the aged time point in these mice revealed no correlations between Aβ histopathology and cognitive performance, in contrast to previous findings by Arendash et al. (2001) in which such correlation was made for certain measures despite a lesser duration of Aβ administration. Still, factor analysis results from Jensen et al. (2005) did suggest an existing relationship between Aβ deposition and numerous cognitive measures, despite a lack of significant correlation therein. More importantly, perhaps, is that this study demonstrated once again the potential for long-term, active Aβ vaccination to result in cognitive protection in an AD transgenic mouse model without significant overall reductions in deposited Aβ levels (Jensen et al. 2005).

Austin et al. (2003) showed that 4 biweekly administrations of Aβ1-42 to APPsw+PS1 transgenic mice at 16-18 months of age did not result in improved cognitive performance in RAWM or other tasks between 18 and 20 months compared to cognitively intact non-transgenic mice. Serologic anti-Aβ titers in these aged mice were measured at an approximately 10-fold lower level compared to the previous study by Morgan et al. (2000), indicating a possible mechanism for no improvement in cognition. Prior to immunization, RAWM and platform recognition tasks were administered, and it was noted that impairment of APPsw+PS1 mice was only present in trial 5 performance of RAWM as well as overall in platform recognition performance. Following immunizations, both immunized and non-immunized animals showed significant impairment compared to non-transgenics over a number
of RAWM measures. Furthermore, both groups showed no pre- versus post-treatment differences in RAWM performances for any trial or block combination, and in some instances these groups showed significant decline in performance measures. In contrast, non-transgenic animals showed significantly better trial 4 performances compared to testing results at a younger time point as well as stabilization of trial 5 errors at a significantly lower level compared to the high stabilization levels of transgenic groups. Impairment also continued in platform recognition performance, with significantly poorer performances displayed by both transgenic groups compared to non-transgenics. Based on these findings, it seems that short-term immunization was not effective in eliciting a strong immune response in these aged APPsw+PS1 animals resulting in no positive impact on cognitive performance. If these results can be translated to human application, it would seem that Aβ vaccination will most likely be most effective in AD if administration is begun at early time points in disease development, resulting in increased administration periods (Austin et al. 2003).

Behavioral evaluation of vaccinated mice was also performed by Sigurdsson et al. (2004) following K6Aβ1-30 immunization. Immunized mice performed significantly better than transgenic controls in the radial arm maze task, in many measures performing at a similar level to wild-type controls. It is clear based on these results that cognitive protection is possible using a non-toxic, non-T cell activating Aβ derivative such as K6Aβ1-30 as an antigen, indicating possibilities for future vaccinations with reduced negative side-effects (Sigurdsson et al. 2004).
The above behavioral based Aβ immunization studies directly tie the pathological impacts of such vaccinations with cognitive benefits in AD transgenic mice. The potential for Aβ vaccination as a therapeutic based on beneficial cognitive impacts following reduced CNS Aβ burdens is established (Janus et al. 2000), however, the potential of long-term vaccination to result in such benefit without dramatic decreases in Aβ pathology is also reported (Morgan et al. 2000, Arendash et al. 2001). Thus, the positive effects of Aβ vaccination on behavior in APP transgenic mice appear to be mediated not simply by clearance of Aβ deposits, but by some other mechanism such as clearance/neutralization of soluble Aβ constructs. Findings by Austin et al. (2003) establish the necessity for long-term vaccine administrations to achieve cognitive benefit, and it is apparent based on the overall results of the above studies that application of Aβ vaccination as a preventative as opposed to a treatment may result in increased efficacy. Finally, findings reported by Sigurdsson et al. (2004) provide support for the concept of alternative immunotherapeutic approaches to Aβ vaccination. Such results further reinforce the importance of developing AD therapeutics targeting Aβ in the CNS, and promote Aβ immunotherapy as a field of continued importance in AD research.

Active Aβ Immunization in Humans

The collective results of Phase I and Phase IIa Clinical Trials conducted by Elan Pharmaceuticals in the U.S. and U.K. represent a disappointment which could only follow the highest level of anticipation. Protocols consisted of administration of Aβ1-42 (An1792) to humans in initial and booster shots for Phase I and, with repeated
shots planned over a 12 month period for Phase II trials. In January 2002, reports of nerve inflammation in four out of 298 patients in Phase II that had begun receiving active vaccination led to further administrations being halted. Phase II was halted, in fact, after only the initial and one booster injection (after 2 shots separated by one month). 11 additional patients were reported to have developed similar symptomologies a month later, and one death was attributed to affects of the vaccine. Specifically, post-mortem analysis indicated neuroinflammation associated with T cell activation as a possible cause of death. In the end, 18 individuals, or approximately 6%, were found to develop symptoms suggesting subacute meningoencephalitis (Orgogozo et al. 2003). A number of reports of findings from these studies were still published, however. In October, 2002, Hock et al. reported on the presence of anti-Aβ antibodies in the sera of 100% of 24 patients having received one primary and one booster injection i.m. of Aβ vaccine containing QS-21 as an adjuvant. These antibodies were not found to be cross-reactive with APP or any non-Aβ derivatives. Sera taken from these patients were also found to bind to Aβ deposits in post-mortem brain tissues of AD and CAA patients. CSF samples taken from 6 patients were also analyzed for Aβ reactivity, and it was reported that 4 out of 6 samples displayed positive results. Importantly, the presence of anti-Aβ in the CSF was noted in patients with and without BBB dysfunction. It was suggested that a combination of low Aβ antibodies and intact BBB’s resulted in prevention of these antibodies from entering the CSF in the two negatively tested patients. Aβ titers in serum samples ranged from 1:50 to 1:10,000, while in the CSF the highest measurement was 1:1,000 (Hock et al. 2002).
In March 2003, the first case report from post-mortem analysis of the lone mortality of the Aβ_{1-42} vaccination trial was published by Nicoll et al. Neuropathological analysis of this woman’s brain revealed uncharacteristically low levels of plaques in various areas of the neocortex compared to seven unimmunized AD patient brains. It was noted that these areas still maintained levels of NFTs and CAA common to non-treated AD patients, but that dystrophic neurite levels and astrocytic activations typically associated with amyloid plaques were significantly reduced. Involvement of microglia in Aβ clearance was suggested by co-associated with Aβ immunoreactivity. Perhaps most important, however, were findings of T-lymphocyte meningoencephalitis, indicating the potential mechanism by which neuroinflammation occurred in patients receiving the Aβ vaccine (Nicoll et al. 2003). This provides insight into the major disparity between immune reactivity to Aβ_{1-42} in mice and humans. In mice, evidence suggests that hypopimmune responses to Aβ may be due to low levels of T cell activation; however it is clear based on the above findings that heightened T cell response is present in at least some humans receiving the vaccine.

In May of 2003, Hock et al. reported on some of the positive cognitive effects of Aβ vaccination in humans. 19 of the 28 patients administered the vaccine in Phase I trials showed decreased decline in cognitive functions compared to non-treated individuals. These patients were also noted to be the individuals who developed antibodies against Aβ. Cognitive stability was determined via administration of tests such as the MMSE and Visual Paired Associates Test of delay recall from the Wechsler Memory Scale. Declines in activities of daily living were also diminished.
in treated individuals as determined from Disability Assessment for Dimentia results. Importantly, overall results from the 300 patients tested in Phase II AN1792 clinical trials did not parallel these findings, with no significant differences in cognitive decline noted between groups. More information on the time course for antibody development was also relayed in this publication. It was reported that both IgG and IgM levels against aggregated Aβ\(_{1-42}\) increased significantly in the first month following initial Aβ inoculation, and that maximal titers were reached by a month after the booster injection (Hock et al. 2003).

The various findings resulting from vaccination studies in humans reinforce the potential for variations between mouse model systems and human systems to result in imperfect translation of results from one to the other. Important to consider, however, is the evidence shown that cognitive benefit and Aβ clearance can result from Aβ administration in humans. Thus, while the risks associated with standard Aβ immunization outweigh potential benefits, it is clear that Aβ immunotherapeutic approaches to AD warrant further investigation.

**Passive Anti-Aβ Immunization in Animal Models**

*Pathologic Effects of Passive Anti-Aβ Immunization*

Due to its neurotoxic properties, it is clear that the concept of Aβ inoculations should elicit some level of concern. Passive administration of Aβ antibodies allows for introduction of these molecules without any need for Aβ inoculation or for immune system activations associated with antibody production. The major methods proposed for vaccine efficacy are direct solublization of Aβ, peripheral sequestration...
of Aβ by circulating antibodies, and antibody facilitated phagocytosis of Aβ by microglia. Thus, the potential for Aβ clearance based solely on the administration of Aβ antibodies is viable. Bard et al. (2000) performed the first study involving passive immunization in a mouse model of AD. It was shown that peripherally administered antibodies to Aβ are capable of entering the CNS of PDAPP mice to induce clearance of amyloid plaques by as much as 93%. Two of a set of four monoclonal antibodies along with polyclonal antibodies from Aβ1-42 immunized mice were shown to induce this effect in 8-10 month-old mice. No T-cell reactivity was seen to be evoked as a result of these procedures. Analysis of microglia showed numerous Aβ containing vesicles, indicating a major phagocytic mechanism of plaque clearance. Internalized Aβ was shown to be degraded by these cells, and the internalization process was shown to be Fc receptor mediated. Additional work with 13 month old mice showed that modest reduction in existing small plaque counts could be seen as early as 3 days after treatment, while 35 days after treatment up to 60% of small and diffuse plaques had been cleared. Large, compact plaques appeared to resist clearance. Importantly, no correlation existed between binding affinity of antibodies to soluble Aβ and plaque clearing capabilities of these antibodies, indicating that affinity to deposited Aβ may play the larger role (Bard et al. 2000).

DeMattos et al. (2001) also showed that anti-Aβ antibodies are able to prevent Aβ deposition in PDAPP mice, however it was shown that the antibodies used in this study were not capable of binding to existing Aβ deposits. It was found that these antibodies sequestered large quantities of Aβ in the serum, indicating that in this case
the ability of these molecules to act as a peripheral Aβ sink was the driving force behind protection against development of Aβ pathology. CSF levels of Aβ were also found to increase, which was attributed to increasing levels of solublized Aβ (DeMattos et al. 2001).

Lambert et al. (2001) showed that antibodies generated in vivo against inoculated Aβ1-42 oligomers were capable of binding preferentially to Aβ constructs in vitro. These antibodies were also shown to block toxicity of Aβ oligomers in vitro. As mentioned earlier, a study conducted by McLaurin et al. in 2002 showed that antibodies recognizing the 4-10 residues of Aβ1-42 inhibit fibrillogenesis of Aβ in TgCRND8 mice. Again, the antibody was shown to target protofibrillar Aβ, an important Aβ plaque precursor. These studies provide a clear mechanism for prevention of plaque formation via anti-Aβ administration which may potentially occur without modulation of Aβ levels. (McLaurin et al. 2002).

In 2002, Chauhan and Siegel reported that a single ICV injection of anti-Aβ antibodies led to reduced plaque loads in the cerebrum and decreased astrocytosis in 10 month-old Tg2576 mice. In 2003, this same group reported that this same treatment was associated with reduction in activated microglia levels in areas surrounding existing Congophilic plaques- the study, however, only involved qualitative assessments of these levels. Nonetheless, it was further reported that no signs of microhemorrhage were found in these mice (Chauhan and Siegel 2003). Wilcock et al. (2004) also showed that significant reduction in amyloid was possible in Tg2576 mice, this time in 19 month-old animals. It was shown in this study that
microglia activation between 3-7 days after injection correlated to this clearance, but that other mechanisms were involved as clearance began as early as 24 hours post-infusion. Finally, a study by Lombardo et al. (2003) in aged PDAPP mice indicated that, along with clearance of Aβ as early as 4 days following a single administration of anti-Aβ antibodies, neuritic alterations attributed to neurotoxic properties of Aβ were reversed as a result of this administration. This again emphasized the importance of achieving Aβ clearance from the CNS in AD.

Pfeifer et al. (2002) produced the first results indicating the possibility for negative consequences to passive anti-Aβ administration. 21 month-old APP23 mice showed reduced amyloid pathology following such treatment, mainly due to clearance of diffuse Aβ plaques, but also displayed increased levels of microhemorrhages. It was also shown that CAA levels were not affected, only hemorrhages associated with this pathology were shown to increase in occurrence. CAA development appeared to be a requisite for this pathology to occur, as 6 month-old APP23 mice lacking CAA did not develop these hemorrhages. One suggested mechanism for triggering of these hemorrhages was that anti-Aβ antibodies are capable of binding to amyloid deposits in blood vessel walls, potentially causing an inflammatory event that served to weaken such amyloid-bearing blood vessel walls. It was suggested that because approximately 80% of AD patients over 65 develop CAA, and because only APP23 mice develop CAA at levels comparable to those found in AD, that results from other passive anti-Aβ studies may not be a perfect indicator of the response such treatment would elicit in humans (Pfeifer et al. 2002).
Behavioral Effects of Passive Anti-Aβ Immunization

Along with those studies focused solely on pathological impacts of passive anti-Aβ immunotherapy, a few studies also investigated behavioral impacts. Dodart et al. (2002) reported that 24 month-old PDAPP mice treated i.p. with m266 anti-Aβ antibodies for six weeks showed performance in object recognition tasks similar to 8 month old wild-type mice and significantly better than non-treated PDAPP mice or IgG1 isotype treated controls. No significant reduction in Aβ pathology was noted in these mice, in contrast with previous findings from this same group in younger PDAPP mice, and task improvement was therefore attributed to reduced levels of soluble Aβ in the CNS. This was supported by correlation analysis showing no correlation between Aβ burdens and behavior in m266 treated mice and by findings that 8 month old non-treated PDAPP mice were significantly worse than aged, treated PDAPP mice at recognizing novel objects despite having lower levels of Aβ deposition.

Further work by Dodart et al. (2002) studied the impact of acute, single administrations of m266 on 11 month old PDAPP mice. Again it was shown that these mice were better at recognizing novel objects than age-matched transgenic controls after receiving only one m266 administrations. To further explore the effect of acute, single-dose m266 therapy on behavior in 11 month old PDAPP mice, 4 days of the holeboard task were administered. It was reported that treated mice made significantly fewer errors in this task during the final two days of testing (data not reported for initial two days of testing), again emphasizing the potential for learning and memory benefits based on m266 therapy. Finally, statistical analysis showed a
dose dependent correlation between object recognition performance and m266 therapy dosage, and only mice receiving maximal dosage (250 µg) showed performance above chance levels in this task. The antibody used in this study was known to not decorate existing Aβ plaques and was thought to not cross the BBB, however a dose-dependent correlation was made between plasma concentrations of Aβ isoforms and m266 therapy. Based on these findings, it was suggested that the major mechanism of action for m266 therapy was for these antibodies to act as a peripheral Aβ “sink”, binding and sequestering peripheral amyloid and causing an equilibrium shift of CNS to peripheral Aβ. Thus, according to this theory, cognitive protection can be gained via modulation of CNS soluble Aβ levels without reductions in Aβ plaque burdens. (Dodart et al. 2002).

Kotilinek et al. (2002) reported similar results to those seen by Dodart et al. (2002). It was shown that administration of the monoclonal anti-Aβ antibody BAM-10 (which recognizes the N terminus of Aβ) did not lead to plaque clearance in the CNS of 9-11 month old Tg2576 mice, but that reversal of memory loss did correlate to such administration. Baseline performance was measured just prior to immunization and consisted of administration of the visible platform task followed by hidden platform coupled with three intermittent probe trials (a variation of Morris water maze application). Mice were divided into treatment groups based on mean probe trial performances. 4-5 days after baseline evaluation, treatment mice underwent 3 separate injections of BAM-10 over a period of one week. 11-12 days following baseline testing, hidden platform testing was administered using the same
protocol; however a different set of visual cues were arranged around the testing pool and a unique platform location was used. Major differences among groups based on treatment revolved around probe trial results. It was shown that percent time spent in the target quadrant for this task was significantly increased in BAM-10 treated mice; in specific probe trials, however this trend was not noted in overall percent time measures (averaged from all probe trials) and no significant difference was measured between BAM-10 treated mice and IgG treated control mice overall. No improvements were noted for hidden platform performances. The interpretation of this data by the authors was that BAM-10 treated mice showed a reverse of memory loss following treatment. As there were not significant alterations in Aβ levels in these mice, the authors contributed this reversal of impairment to neutralization of Aβ assemblies in the CNS resulting in decreased neurotoxicity of these constructs. It was shown that BAM-10 treatment eliminated the negative correlation between soluble brain Aβ and probe trial scores, indicating that this treatment did in fact reduce the impact of existing Aβ in the brains of Tg2576 mice (Kotlinik et al. 2002).

Wilcock et al. (2004) administered weekly doses of anti-Aβ antibody to Tg2576 mice for either 1, 2, or 3 months, with treatment beginning so that all mice finished the treatment regimen at 22 months of age. Behavioral assessment of these mice consisted of a single, 8 minute administration of the Y-maze task 1 day before sacrifice. Mice were treated with IgG antibody to Aβ_{28-40} with controls receiving an anti-\textit{Drosophila} amnesiac protein IgG1 antibody. Only mice treated with Anti-Aβ for 3 months were shown to have significantly higher spontaneous alternation in Y-maze performance compared to transgenic control groups. Performance for mice
treated for 3 months was at the same level as non-transgenics, with reduced performances following lesser treatment periods. However, after 1 or 2 months of treatment, immunized mice were not significantly different in performance from either non-transgenics or controls transgenics. Hyperactivity based on Y-maze arm entries was noted in non-treated APP controls but was not noted in 2 or 3 month treated animals.

Antibodies administered for all treated mice were shown to decorate plaques within the CNS, with direct proportionality between immunostaining and plaque density. Following 1 month of anti-Aβ treatment, transgenic animals were noted to have slight but not significant reductions in Aβ deposition. Following both 2 and 3 months of treatment, however, significant decreases in Aβ immunostaining were found in hippocampal and frontal cortex regions, with both compact and diffuse plaque burdens reduced. It was further shown that microglia Fcγ receptor expression was upregulated after 1 month of anti-Aβ antibody treatment, CD45 molecules after 2 months, and that expression levels of both marks then returned to normal after three months, indicating temporal activation of these cells. Based on these findings, it was suggested that Aβ clearance was thus mediated via a number of mechanisms, including solublization of deposited Aβ by antibodies, sequestering of Aβ in the plasma, as well as by microglia activation and phagocytosis of Aβ. Furthermore, it was said to be this reduction in Aβ burden in the CNS which resulted in improved Y-maze performance for treated Tg2576 mice (Wilcock et al. 2004), although no
correlation between Aβ deposition and Y-maze alternation was done for Aβ antibody treated mice.

The speed with which acute administration of anti-Aβ antibodies result in cognitive benefit in AD transgenic mice (based on findings by both Kotilinek et al. 2002 and Dodart et al. 2002) and the lack of Aβ pathology decreases in these animals would seem to support the theory that these antibodies are acting more through either alterations in CNS:sera Aβ levels or neutralization of toxic Aβ constructs. The rapid behavioral benefits reported by Dodart et al. (2002) and Kotilinek et al. (2002) could, however, result from alleviation of secondary effects of Aβ (a known vasoconstrictor) in the blood resulting in increased cerebral blood flow. In any event, findings by Wilcock et al. (2004) still support the concept of cognitive protection following reductions in Aβ pathology due to passive Aβ immunization.

Passive Anti-Aβ Immunization in Humans

While no data has been reported on passive Aβ immunization in humans, the potential exists for such studies to occur based on results noted above in assessment of such immunization in transgenic models of AD. In the wake of findings from active immunization trials in humans, however, the climate for research along immunotherapeutic lines in humans with AD has changed dramatically in the last few years. Findings such as those noted by Pfeifer et al (2002) suggest that inflammation following anti-Aβ administration can occur in transgenic animals which develop CAA, and it is not unreasonable to assume that similar side-effects would occur in humans following such treatment. As Anti-Aβ antibodies have been shown to
sequester amyloid in the blood, it is also possible that increased development of CAA or other vasculature dysfunctions may occur. Other side-effects from continual/repeated administration of antibodies in humans may result from eventual immune response to injected antibodies, resulting in accumulation of immune complexes and potentially further disruption of the vasculature (serum sickness) (Sigurdsson et al. 2002). Such activation of the peripheral immune system may also lead to development of inflammation within the circulatory system. Although many results from passive Aβ immunization in animal models of AD have been positive, it is clear that the immune environment of transgenic models is extremely variable even based solely on background differences among mice, and the major lesson to be taken from the human vaccination trials is the potential for differences between immune responses in these mice and in humans to result in devastating side-effects. Until a valid and long-lasting therapeutic for Alzheimer’s Disease is discovered, however, it is likely that the need for such treatments will eventually outweigh other concerns, and immunotherapeutic approaches such as passive Aβ immunization will undoubtedly be assessed in human subjects at some point.
Other Immunotherapeutic Strategies for Alzheimer’s Disease

Based on our current understanding of immunotherapeutic approaches to Alzheimer’s Disease, it is clear that the most probable means of reducing Aβ pathology in the CNS is limited to two mechanisms. The first is humoral based and involves clearance of Aβ from the CNS via two separate routes. Solublization of Aβ in the CNS by direct actions of anti-Aβ antibodies can result in clearance of both soluble and insoluble forms of the peptide, while sequestration of the Aβ by anti-Aβ antibodies in circulation can lead to an equilibration shift of Aβ into the serum and out of the CNS. The second is cellular based and relies on activation of key immune system cells within the CNS to clear Aβ by phagocytosis and internal degradation of the molecule or by production of extracellular peptidases capable of breaking down aggregated Aβ. Aβ immunization, both active and passive, is known to initiate reduction of Aβ levels within the CNS, both soluble and insoluble, via both pathways as described above.

Alternative immunotherapies attempt to achieve similar results without instigating inflammatory damages associated with Aβ vaccination. Currently, two potential immunotherapies in particular show promise. The first involves development of non-antibody Aβ binding agents which can be administered
peripherally and serve to sequester Aβ in a similar fashion to anti-Aβ antibodies, again resulting in increased traffic of Aβ out of the CNS. As discussed previously, these agents thus serve as peripheral Aβ “sinks” by increasing serum levels of Aβ via this mechanism. The second involves either stimulation of an appropriate T cell response via various mechanisms or direct infusion of specifically activated T cells to elicit a desired immune response.

**Alternative Peripheral Aβ Sinks**

In 2002, Matsuoka et al. reported that peripheral administration of two separate Aβ binding molecules, gelsolin and GM1, in young APPsw+PS1 mice resulted in significant Aβ clearance from the brains of these mice. These blood-limited molecules were thought to sequester Aβ in the periphery, causing a shift in Aβ equilibrium away from the CNS, as described for anti-Aβ treatments. Gelsolin in particular is unable to cross the BBB, and so its affects are attributed solely to peripheral binding of Aβ. Importantly, it was noted after treatment that plasma levels of Aβ did not increase at anywhere near the levels seen after passive Aβ immunization, making the exact mechanism of action for Aβ sequestration in the periphery difficult to interpret. Administration of these compounds in aged PDAPP mice did not result in significant reduction of amyloid plaques, apparently indicating once again that amyloid plaque burdens are more difficult to reduce in older animals (Matsuoka et al. 2002). This resiliency of established plaques in aged mice against reduction following immunotherapy has been established in numerous studies (Morgan et al. 2000, Arendash et al. 2001, Dodart et al. 2002, Kotilinek et al. 2002).
In 2003, Deane et al. showed that sRAGE, derived from the RAGE Aβ binding protein, can also act to sequester Aβ in the periphery, providing another potential for Aβ-binding protein therapy.

The exact mechanism by which Aβ is transported across the BBB to the periphery is thought to be mediated by the LRP clearance receptor for Aβ, while re-entry of the peptide into the CNS appears to be mediated by RAGE receptors. Other carrier proteins such as albumin and transhyretin are also thought to be involved in this process. Modification or inhibition of certain aspects of these pathways or treatments may provide a mechanism for altering sera:CNS Aβ ratios, providing another mode of Aβ clearance from the CNS (Zlokovic et al. 2004).

T-cell Based Therapy

As indicated above, immunotherapeutic treatments for neurodegenerative diseases such as AD result in varied levels of activation among separate T helper cell subtypes. The actions of these reactive T cells then bias the overall immune response. In the most general terms, Th1 T cells cause a cellular based response and Th2 cells a humoral response (Vallejo et al. 2004). Either response, if elicited in an appropriate fashion, may have a beneficial effect in the AD brain; the former via activation of phagocytes, the latter via activation of antibody-producing B cells and/or downregulation of neuroinflammation. The potential for such bias to result in negative consequences has also been well established, for instance it has been indicated in vaccination studies that a Th1 biased immune response results in increased inflammation, potentially leading to an aberrant auto-immunee reaction (Furlan et al. 2003). These responses are the most general aspects of T cell activities,
however, and, especially with regards to active immunization, it is unclear to what extent undesirable T cell activation and the neuroinflammatory events which result are due simply to the unique reactions elicited by the antigen and/or adjuvant used.

Modulation of the T cell response via alterations in antigen/adjuvant vaccine components, as well as delivery routes/vehicles, could potentially result in benefit without neuroinflammation (Cribbs et al. 2003), and certain immunological treatments have been shown to elicit such benefit without any T cell activation (Schenk et al. 2004). However, it is important to consider that T cell subsets as a whole are not anathema. The potential activities of these cells in response to antigen based activation are wide ranging, and it remains to be seen whether such activation in the context of a non-immunized system is beneficial or detrimental. Infusion of Aβ reactive T-cells has never been attempted in any animal model of AD, however the concept of such adoptive immunotherapy has been established for some time in other fields of study. In individuals with lowered immune system efficacies or hypo-responsiveness to specific antigens, T cell infusions act to prime these systems to counterbalance such deficits.

*Donor Lymphocyte Infusion For Non-CNS Diseases*

Infusion of activated T cells has been used in the treatment of a number of disorders and diseases, with benefit gained due to actions of both CD4+ and CD8+ T cells individually and in concert. There is great potential for such adoptive transfers to serve as anti-tumor treatments due to the activity of both cytotoxic cells recognizing tumor specific antigens and memory T helper cells capable of sustaining an immune response to malignant cells (Vonderheide and June et al. 2003). Findings
that graph-versus-leukemia anti-leukemia affects were more likely to occur following transplant of non-T-cell-depleted bone marrow transplants resulted in a number of studies being conducted to test the efficacy of T cell Donor Lymphocyte Infusions (DLI), or selective infusion of non-patient lymphocytes, in treating various leukemias (Kolb et al. 1995, Collins et al. 1997, Slavin et al. 1996) as well as myelodysplastic syndrome (Bressoud et al. 1996), multiple myeloma (Lokhorst et al 1997), and non-Hodgkin’s lymphoma (Bernard et al. 1999). Complications were most prevalent following bone marrow transplant-based treatments and consisted mainly of marrow aplasia, or failure of the bone marrow to thrive, though this was resolved in as many as 80% of patients (Collins et al. 1997). The potential for T cell infusions to serve as a treatment for numerous cancers, especially for recurrent hematalogic cancers post-bone marrow transplant, has thus been established (Szer et al. 1993).

Continuing research in immune system diseases have shown that T cell infusions serve to re-establish immune functions in patients with Epstein Barr Virus (EBV), cytomegalovirus, and Human Immunodeficiency Virus (HIV) infections, though not always without some adverse affects (Heslop and Rooney 1997, Walter et al. 1995, Levine et al. 2002). This research elicits the potential for recharging of the immune system following DLI in immune compromised individuals or in counteracting hypo-immuneresponsiveness to a toxic self peptide such as has been implicated in AD.

*Priming of the CNS Immune System With T Lymphocytes*

APP transgenic mice display a tolerance-based effect after immunization with Aβ which results in a decreased immune system response (Monsonego et al. 2001).
To elicit an immune response to Aβ in humans, it was deemed necessary to administer an adjuvant in concert with the peptide (Hock et al. 2002). Otherwise the Aβ peptide, which is formed via proteolytic processing of the endogenously expressed APP molecule found in all humans, would not likely elicit a significant immune response until late in AD development when it is associated with neuroinflammation. This self tolerance may affect the ability of Aβ vaccination to be effective as a treatment, especially as immune system activation in AD is largely restricted to local, glial based, non-systemic types (Streit 2004, D’Andrea 2004). An exploration of the ability of T cells to prime the immune systems of AD models is thus warranted to determine the potential for such cells to overcome or reverse self tolerance to Aβ, inducing either microglial phagocytosis of Aβ or B cell anti-Aβ antibody production.

As described above, APP mice display a lessened immune response following Aβ immunization compared to wild-type controls, most likely due to self tolerance. As both APP and Aβ are present under normal conditions in humans throughout aging, it is likely that a certain level of tolerance is present in humans as well following similar vaccination. DLIs have been used to combat deficient immune systems in murine models as well as humans, whether these deficiencies be congenital or due to an infectious disease of the immune system. It has been suggested a deficiency in the immune system may be present in AD which prevents the immune cells of the CNS from taking appropriate action against the insults inflicted therein during the course of disease. Both microglia and T lymphocytes are likely to be drawn to areas of inflammation associated with Aβ pathology in AD
brains (D’Andrea 2004, Farkas et al. 2003). However, these cells may not be appropriately activated early enough along the course of the disease to be affective, or may be deficient in their activity (Streit 2004, Monsonego et al. 2001). Prior to compact neuritic plaque formation, microglia could potentially be stimulated to clear deposited Aβ. At the same time, activated T cells can both regulate the immune response to Aβ associated damage and, as will be elicited later, potentially play a neuroprotective role against further damage. If activated too late, however, frustration and continual stimulation of microglia and T cells (via co-stimulation) may occur, resulting in exacerbation of the inflammatory cascade rather than protection.

Aβ-sensitive T cells are unlikely to be found in large numbers in APP transgenic mice or in humans due to selection against thymocytes reactive to self proteins which occurs during T cell development. As AD progresses and Aβ becomes more prevalent and is associated with inflammation and cellular damage, however, is likely that Aβ-sensitive T cells would be activated and proliferate. This is supported by findings by Monsonego et al. (2003) that Aβ-sensitive T cells are common in both elderly, healthy individuals and AD patients, with AD patients more likely to possess these cells. What is not clear, however, is if this immune response to an endogenous peptide is beneficial but is simply deficient in AD patients, or is a detrimental autoimmune dysfunction.

If T cells do possess the potential to combat the damage associated with amyloid pathology, it is possible that Aβ-sensitive T cells themselves could serve as a potential therapeutic in AD. These cells may then play a role in activating other
immune system cells as described above, causing an upregulation in immune system activity, or a “charging” of the AD immune system. The potential drawback of such activation, of course, is that an incorrectly balanced immune reaction can lead to a strong bias towards a cellular or humoral based response, and under certain conditions this can lead to increased neuroinflammation, as seen in human clinical trials for Aβ vaccination (Nicoll et al. 2003). Careful study of the effects of Aβ–sensitive T cell infusions in AD models will be necessary to determine the true potential of such cells.

Neuroprotective Actions of T Lymphocytes

It is thought that in AD, immune hyporesponsiveness to Aβ prevents effectiveness of some Aβ immunotherapies, as would be true for any endogenously expressed peptide due to self-tolerance. To overcome this deficit, adjuvant coupled administration of Aβ peptide was used in human clinical trials, and it was found that this led to potentially lethal inflammation in the CNS of immunized individuals. This may have occurred in part due to activation of cells sensitive to a “self” peptide. Autoimmunity of this type is typically associated with a negative connotation due to such devastating effects, which are not un-similar to those noted in autoimmune disorders. However, new research in this field has begun to reveal that autoreactive cells, specifically T cells, are actually capable of providing neuroprotective benefits in areas of CNS inflammation and neuronal damage. Understanding and harnessing of this potential may lead to potential new therapeutics for neurodegenerative diseases such as AD, in which neuroinflammation plays a key role.
Extensive research in neural injury repair has shown that T cells can play an essential role in modulating repair in neuroinflammatory-based damage. Specifically, myelin-specific Th1 cells (the same proinflammatory, encephalitogenic cells capable of instigating onset of autoimmune diseases) seem to be capable of providing protection following neuronal injury. It appears that these autoreactive T cells, capable of causing neuronal damage and inflammation when inappropriately activated, are also capable of being drawn to an area of injury and in some way acting as neuroprotectants under the correct neurophysiological paradigm (Kipnis et al 2002, Schwartz et al. 2003).

One pathway of this protection appears to be regulation of microglia activity. Microglia, known to both be activated by and activators of T cells as discussed above, can contribute to continued neuronal damage following initial insult if left unchecked. T cells do possess the ability to prevent frustrated microglia from continuing to cause neuronal damage (Avidan et al. 2004). A second route of action for T cell mediation of CNS damage may be production of neurotrophins. Infused autoimmune T cells are capable of producing such factors after reactivation due to antigen presentation. Both Th1 and Th2 subsets of autoimmune cells have been shown to produce these factors following injury to the CNS, resulting in neuroprotective effects. These effects can be blocked via inhibition of NT-receptor activity, indicating that it is the direct interactions of these factors with neurons which is providing protection, and these receptors are known to be upregulated on injured neurons (Moalem et al. 2000). Finally, the work of Michal Schwartz and associates in the past two years has displayed the ability of T cells to protect against glutamate neurotoxicity. Excessive
activation of neurons and/or glutamate release by activated cells such as microglia can result in such toxicity, and the potential of T cells to lessen glutamate toxicity based damage is another potential mechanism by which these cells are capable of providing protection following CNS damage. The mechanism of action for this protection appears to be two fold. In response to neuronal damage associated with glutamate toxicity, self-specific T cells directly decrease damage following glutamate exposure. This is attributed to the neuroprotective capabilities of autoreactive T cells as discussed above. Secondly, regulatory T cells reduce cytotoxic-type microglial activation, reducing neuroinflammatory damage associated with such activation (Schwartz et al. 2003).

These findings seem to imply an important, direct route by which T cells typically associated with inflammation in the CNS are actually important in combating damage under appropriate conditions. The damage and associated neuroinflammation present in AD may thus also be regulated and counterbalanced by the same T cell subsets which might otherwise be involved in propagating neuroinflammatory events. As has been illustrated earlier, microglia associated with neuritic plaques in the AD brain are known to instigate neuronal damage if left unchecked due to phagocytic frustration. However, microglia have been shown to be capable of acting as Aβ APCs for T cells, indicating a mechanism by which Aβ-sensitive T cells may be reactivated local to these plaques (Monsonego et al. 2003). In 2003, Farkas et al. reported that Aβ mediated break-down of the BBB followed by infusion of activated T cells resulted in migration of these cells into the brains of male Wistar rats. Based on these results, it is reasonable to assume that in murine AD
models, peripherally infused Aβ-sensitive T cells would migrate across the BBB into the brain, allowing them to associate with microglia in amyloid containing regions of the CNS.

Microglia express MHC-II in order to interact with T cells, allowing microglia to participate in T cell activation, and thus indirectly in neuroprotection. Suppressed expression of MHC-II is thought to be present in AD and may prevent early actions of microglia in clearing amyloid plaques, and it has been suggested that localized $T_{H1}$ cells may be able to stimulate such expression at an earlier time point (Schwartz et al. 2003). In the presence of inflammatory factors released by damaged neurons, and following co-stimulation with microglia, these T cells may then be stimulated by the same mechanisms outlined above to both reduce local gliosis and counteract neuronal damage associated with Aβ deposition. As a caveat, it should again be noted that $T_{H1}$ subset cells have been implicated in playing a major role in neuroinflammation in AD and other CNS disorders (Furlan et al. 2003). Interestingly, however, it appears that microglia and T cell co-activation in the CNS results in upregulated production of anti-inflammatory cytokines such as IL-10 by T cells, resulting in decreased production of neuroinflammation-inducing cytokines and other inflammatory factors by microglia and $T_{H1}$ cell lines (Chabot et al. 1999). Mallat et al. (2003) produced results emphasizing the potential benefit to be gained via this mechanism. It was shown that adoptive transfer of ovalbumin-specific Tr1 subtype T cells (which are capable of downregulating Th1 cells) reduced development of atherosclerosis in Apolipoprotein E knockout mice. Injection of these cells along with ovalbumin in complete Freund’s adjuvant resulted in activation of infused lymphocytes and a
significantly reduced overall Th1 response (based on cytokine profiles). It was suggested that this bias against a cellular based immune response resulted in decreased inflammation, which is thought to play a major role in development of atherosclerosis in these ApoE KO mice (Mallat et al. 2003). Importantly though, this study focused on overall Th1 subset activities, not on the potential for specific autoreactive Th1 subsets on the CNS side of the BBB.

Th2 subsets may also be capable of playing a neuroprotective role. Benner et al. (2004) demonstrated that Cop-1 (a Th2 phenotype specific stimulator) activated immune cells adoptively transferred into MPTP-intoxicated mice (a model for Parkinson’s disease) are capable of migrating into the inflamed nigrostriatal dopaminergic regions of these mice. Once localized to the site of inflammation, these cells were shown to be capable of suppressing microglia actions and of stimulating production of neuroprotective products such as glial cell line-derived neurotrophic factor (GDNF) by astrocytes to result in significant neuronal protection (Benner et al. 2004). Thus, without serving to reduce Aβ pathology, infused Aβ autoreactive T cells, including both Th1 and Th2 subsets, may be capable of protecting against Aβ associated damage if they too are able to produce neuroprotective agents and to combat neuroinflammation.

Perhaps the most persuasive study indicating the potential for T cell therapy in AD was conducted in 2004 by Avidan et al. This study reported that T cells specific to neuronal self antigens are capable of reducing damage associated with neurotoxic properties of Aβ. Intraocular Aβ1-40 injections in C57BL/6J mice resulted in significant death of retinal ganglion cells. In mice immunized prior to Aβ injection
with interphotoreceptor retinoid-binding protein (antigen specific cells in the eye),
loss of neurons was significantly less. To verify that this reduction was T cell
induced, interphotoreceptor retinoid-binding protein and S-antigen specific T cells
were passively transferred into C57BL/6J mice immediately following Aβ intraocular
injections, resulting once again in retinal ganglion cell protection. Further findings by
Avidan et al. (2004) would seem to downplay the potential for neuroprotection
following infusion of Aβ-sensitive T cells in APP transgenic mice. It was noted that
T cells specific to non-aggregated Aβ1-40 are capable of inducing only slight
reductions in Aβ-induced retinal ganglion cell loss. This was attributed to a lack of
microglial capacity for expressing MHC-II in response to aggregated Aβ1-40 exposure
based on unpublished results by Butovsky et al. However, the authors do suggest that
memory T cells specific to self antigens should be capable of activation and down-
regulation of cytotoxic microglia actions. This is more in agreement with findings by
Monsonego et al. (2003) which indicate that presentation of Aβ and direct activation
of Aβ specific T cell subsets by activated Microglia can occur. Furthermore,
additional findings published by Monsenego et al. demonstrate the presence of Aβ-
sensitive T cells in AD patients, and it is suggested that processing of Aβ in the CNS
by APC cells followed by migration of APCs to lymph nodes leads to eventual
peripheral activation of Aβ reactive T cells (Monsonego et al. 2003-2). The potential
for Aβ-sensitive T cells to be activated via MHC-II presentation is thus established in
both murine systems as well as in humans.
There appears to be potential for adoptive T cell transfer to serve as a therapeutic for neurodegenerative diseases. The pathways by which such protection may occur in AD, as discussed above, are outlined in Figure 2. The first pathway is based on the ability of certain T cells to stimulate the immune system early in Aβ pathology development via either a cellular (stimulation of microglial phagocytosis of Aβ) or humoral (stimulation of anti-Aβ antibody production) based response. In addition, there is the potential for T cells to provide protection against neuronal damage resulting from development of Aβ pathology later along the course of the disease via both production of neurotrophins and down-regulation of glial-based neuroinflammation. This form of protection may also extend towards prevention of glutamate based neurotoxicity. No studies to date have established the behavioral effects of T cell infusions into a neurodegenerative disease model, nor have the pathological effects of such infusions been explored in a model of Alzheimer’s Disease. Elucidation of the actions of various T cell subsets once within the CNS of AD models may provide insight into the potential for T cell therapy in AD.
Figure 2. **Mechanisms of T cell based neuroprotection.** Following re-activation in the CNS via interactions with APCs such as microglia, infused auto-reactive T cells sensitive to Aβ may act therapeutically in the AD brain via three mechanisms: 1) Early activation of the immune system to promote both humoral and cellular based immune responses to soluble and deposited Aβ (red). 2) Suppression of “frustrated” cell (microglia, astrocytes, etc.) actions in the region of Aβ mediated neuronal damage to prevent excessive neuroinflammation and associated glutamate toxicity (blue). 3) Production of neurotrophic factors which promote neuronal health and help protect against neuronal loss/dysfunction while also protecting against glutamate neurotoxicity (green).
Specific Aims

The specific aims of this thesis are:

1) To isolate, culture, and prime with human Aβ1-42 peptide, spleenocytes isolated from young, Aβ1-42 immunized wild-type mice, and to inject these activated, multi-subset, T cell enriched spleenocytes into AD transgenic mice.

2) To determine the effects of Aβ-sensitive T cell enriched spleenocyte infusion on cognitive performance in Radial Arm Water Maze, Platform Recognition, and Y-maze tasks compared to placebo-infused transgenic and wild-type mice.

3) To determine post-mortem the pathologic effects of Aβ-sensitive T cell enriched spleenocytes within the CNS of AD transgenic mice through analysis of Aβ burdens and distribution/activation levels of microglia and T cells therein.
4) To determine effects of Aβ-sensitive T cell enriched spleenocyte infusions on blood cytokines levels, specifically with regards to levels of pro and anti-inflammatory cytokines.

5) To perform correlation analysis to determine if behavioral task performance measures correlate to any specific pathologic or biochemical measures.
Materials and Methods

Animals

To obtain the APPsw+PS1 mice used in this investigation, male heterozygous APPK670N,M671L transgenic (APPsw) mice were crossed with female, homozygous PS1 mice of the 6.2 transgenic line. Female heterozygous PS1 transgenic offspring were then crossed with APPsw males of the parental generation to obtain the non-transgenic and heterozygous APPsw+PS1 mice used for this investigation. Mouse backgrounds for this generation consist of a 56.25% C57, 12.5% B6, 18.75% SJL and 12.5% Swiss Webster heterogeneous background. Mice were genotyped after weaning, and then singly housed throughout the course of the study in standard 12-hour light-dark cycle conditions, with free access to water and rodent chow. Behavioral assessment and immune cell infusions occurred during the light phase of the circadian cycle.

General Protocol

As indicated in the Timeline in Fig. 3, baseline cognitive performance for 10 non-transgenic and 15 APPsw+PS1 mice was established via a 12 day administration of the RAWM task for working memory at eight months of age. Transgenic mice were then divided into two groups balanced in RAWM performance based on overall latency and error averages so that pre- vs. post- infusion comparison could be made
between groups. Seven transgenic mice were designated as recipients for Aβ-sensitive immune cells, and 8 transgenics (as well as all 10 non-transgenics) were designated as controls, to be given PBS. To generate Aβ-sensitive immune cells, 3 additional congenic non-transgenics were repeatedly immunized with Aβ1-42 at 3-4 months of age. Spleenocytes and lymphocytes isolated from these animals were then cultured for 4 days prior to infusion into immune cell recipient mice. Infusion of immune cells into these mice along with injection of PBS into both control groups occurred two days following completion of RAWM testing (Fig. 3). After a one month delay, mice underwent additional behavioral assessment consisting of RAWM, Platform Recognition, and Y-maze task administrations (9, 4, and 1 days respectively). A neurological assessment battery was also used to test for motor and/or neurological deficits prior to sacrifice. Plasma samples and brains were taken at sacrifice. Plasma cytokine levels were evaluated via immunoassay, and half brains (bisected mid-sagittally) were coronally sectioned and used for quantification of brain Aβ deposition levels via direct Thioflavin S staining, as well as by 6E10 immunostaining. Finally, statistical analysis was conducted to determine group differences in cognitive task performances, plasma cytokine levels, and Aβ load determinations. Correlation analyses, factor analysis, and discriminant function analysis were also be used to evaluate overall group differences across these measures.
Figure 3. General protocol timeline for the adoptive transfer study.
Immune Cell Infusion Protocol

Three non-transgenic mice from the same generation as the study cohort were vaccinated with human Aβ1-42 to elicit an immune response. One day prior to injection, the site of injection on the backs of these mice was shaved. The following day, 3 separate endodermal injections of human Aβ1-42 peptide (Bachem) in complete Freund’s adjuvant (CFA) were administered to each mouse following isofluorane anesthesia. Each mouse received a total of 250 µg of non-fibrillar, soluble Aβ in 100µl of adjuvant divided equally over the three injections to induce proliferation of Aβ sensitive immune cells. Each mouse then received additional injections of 200 ng pertussis toxin 1 and 3 days post-immunization. Mice were monitored for signs of distress and paralysis for these 3 days and for 7 days thereafter. Following this 10 day period, these mice were sacrificed for harvest of spleen tissues and lymph node fluids. Tissues from all 3 mice were pooled and homogenized with a loose fitting 15 mL dounce, followed by filtration through a 70µm sieve filter, centrifugal pelleting, and resuspension in red blood cell lysis buffer. After five minutes, cells were again gently pelleted, then resuspended in full media and counted. Cells were then cultured in full media containing 8-10 µg/mL Aβ1-42 and for 4 days. After culturing, cells were once again pelleted, resuspended in phosphate buffered saline (PBS), and viable cells were counted by trypan blue exclusion. Immune cell recipient APPsw+PS1 mice were then administered approximately 2x10^7 viable immune cells in 0.5 mL PBS by tail vein injection. Control transgenic and non-transgenic mice received tail vein injections of 0.5 mL PBS. All animals were then monitored daily for signs of discomfort or paralysis.
Behavioral Assessment

Radial Arm Water Maze

The RAWM task for working (short-term) memory was administered for all mice at two time points as indicated on the study timeline. Testing occurred in a 100 cm diameter pool of water divided by an aluminum insert which establishes 6 evenly spaced radial arms surrounding a central open area. Each arm was 30.5 cm in length and 19 cm wide, while the central circular swim area was 40 cm in diameter. A transparent 9 cm diameter platform at a 1.5 cm depth was used as an escape platform for mice placed in the maze, and was placed approximately 15 cm away from the end of the randomly assigned goal arm of the maze each day of the task. Numerous visual cues surrounded the pool to provide spatial references for navigation of the maze. For each trial of the task, mice were placed in the water at the entrance of a novel start arm of the maze for that day, facing the central swim area. The start arm was never the arm containing the submerged escape platform, and both start arm sequences and goal arm location were semi-randomly changed each day. The mouse was allowed to navigate the maze freely until fully entering an arm of the maze. If the arm selected by the animal did not contain the escape platform, an error was recorded and the animal was pulled gently back to the start location for that trial. This procedure, including counting of an error, was also used if no arm selection was made within 20 seconds. If after 60 seconds, an animal did not find the escape platform, it was guided gently to it. Latency to find the platform up to 60 seconds and number of errors were recorded for each trial. The RAWM task
consisted of five daily trials, the first four of which were administered with only a 30 second delay between them during which the mouse was left on the escape platform. The fifth trial was administered 30 minutes later, and the animal was returned to its cage during this delay interval. For animals which did not make at least 3 choices during a trial and did not find the escape platform, a penalty error was recorded for that trial. The penalty error was calculated by averaging trial one errors for the first three days of testing for animals which did not find the escape platform but did make at least three arm choices during these trials.

*Platform Recognition*

The platform recognition task was administered following completion of the RAWM task to determine the ability of the mice to recognize a visible platform placed at various locations in an open, 100cm diameter pool. This required a strategy switch, as the RAWM task required that the animals search for a submerged platform hidden in a divided pool based on orientation of visual cues around the pool, whereas the platform recognition task only requires identification of the visible escape platform. Thus, this task also tested the ability of these animals to adapt to a new escape strategy. For this task, a 9 cm circular platform with a conspicuous 10 x 40 cm black and white conical visual cue attached was used, with the surface of the platform raised .8 cm above the water’s surface. Mice were placed in the same location in the pool for each of 4 daily trials. For each trial of a given day, the visible platform was moved to a central part of one of four established quadrants in the pool. The mice were allowed 60 seconds to find the platform before being guided to it.
during each trial, and a 30 second stay on the platform was allowed between each trial. Latency up to 60 seconds for each trial was recorded for 4 days of testing.

**Y-maze**

Mice were given 5 minutes to explore a black, three-armed Y-maze in order to test both general activity (based on total number of arm entries) and basic mnemonic processing (based on spontaneous percent alternation). Mice were placed facing the center of the maze at the opening of the maze arm designated arm two. During the five minute test period, the number and sequence of arm choices was recorded. Percent alternation was calculated by subtracting two from the total number of arm entries and dividing this value into the total number of spontaneous alternation events (consecutive choice of each of the 3 Y-maze arms, without re-entry into a previously chosen arm) for each animal.

**Neurological Battery**

19 sensorimotor measures were taken from a neurological battery derived from the Irwin Test (Irwin 1968). Qualitative assessment ratings of the following were obtained for each mouse: transfer arousal after placement in a new environment, ataxia, hypotonic gait, pelvic elevation (while walking), tail elevation (while walking), palpebral closure, approach to Q-tip (novel object), withdrawal from invasive Q-tip, response to touch, response to audible startle stimulus, corneal reflex, righting reflex, visual placing (reaching for paw hold while hanging), grip strength (while hanging), recognition of visual cliff, response to toe pinch, and response to tail pinch. Quantitative measures were also obtained for the following: latency to move
to the edge of an elevated platform and number of head pokes over the edge of an elevated platform in one minute.

**Brain Collection and Sectioning**

Two days following behavioral testing all study mice were anesthetized with Nembutal (1 mg/10 gm body weight). Blood (.2 ml) was collected from the heart, mixed with .5M EDTA, and centrifuged. Plasma and red blood cells were then separated, flash frozen, and stored at -80°C. Following blood collection, all mice were pericardially perfused with .9% saline. Brains were excised and halved mid-sagittally. The left half was fixed in 4% paraformaldehyde for 24 hours at 4°C, followed by graded sucrose solutions (10, 20, and 30% (w/v) sucrose in 0.1× Sorenson's phosphate buffer) prior to sectioning. Frozen 25 µm coronal sections were then collected on a sliding microtome and stored in PBS. These sections were immunostained with 6E10 antibody (for diffuse Aβ) or stained with Thioflavin S (for compact Aβ deposition).

**Plasma Cytokine Level Measurements**

Relative cytokine level determinations were obtained using a custom RayBio Mouse Cytokine Antibody Array. Briefly, provided membranes bearing ten separate anti-mouse-cytokine antibodies were treated with blocking buffer and then incubated with 1 ml of 1:10 diluted plasma samples for one hour. The membranes were then incubated with 1x secondary biotinylated antibodies diluted in blocking buffer for one hour, followed by a two hour incubation with 1,000 fold diluted labeled-strepavidin in blocking buffer to complete the conjugated secondary antibody complex. The membranes were washed following each incubation with provided 1x wash buffer
solutions. Detection signals for each membrane were detected using Fujifilm AR x-ray film following a seven second incubation with provided detection buffers. Back-lit photographs were taken of the developed film with a Kodak DC290 digital camera. Mean signal intensities minus background signal intensity for duplicate readings were determined using Kodak 1D Image Analysis Software and standardized to a zero to one scale based on minimum and maximum mean intensity readings for each cytokine. This was necessary because of the naturally occurring large variability (100x-1000x) in levels (based on signal intensities) among various cytokines. Resultant standardized signal intensities were then used for relative cytokine level comparisons among animal groups.

**Aβ Deposition Determinations**

**6E10 Immunostaining**

6E10 immunostaining was performed on mouse brain sections at the level of the dorsal hippocampus. Three 25µm coronal sections spaced approximately 600-700µm apart were used to analyze diffuse Aβ deposition in sections of the dorsal hippocampus and overlaying parietal cortex. Sections were floated in 10% ethanol and mounted on pre-treated slides. For the following steps, all incubations were carried out at room temperature on a rotating platform shaker unless otherwise noted. In order to induce epitope retrieval, slides were first placed in a 85°C 25mM citrate buffer (pH 7.3) bath for five minutes, washed at room temperature in PBS, placed in 88% formic acid for five minutes, and then washed under low pressure running de-ionized water for 10 minutes. Slides were then incubated at room temperature with pre-mixed DAKO blocking solution [49.5% DAKO (catalog #X0909; Dako,
Carpinteria, CA), 49.5% PBS, .3% H₂O₂ by volume] for 15 minutes to block endogenous peroxidase activity, with M.O.M. anti-mouse IgG antibody for 60 minutes to block non-specific binding, with a Triton X solution (.4% by volume) in two 2.5-minute PBS baths to induce permeability, and with protein concentrate solution for 5 minutes to further block non-specific binding. Slides were placed in PBS baths for 5 minutes following both DAKO and Triton X applications. Slides were then incubated with 6E10 primary antibody overnight at 4ºC. Following this incubation, slides were placed in three successive PBS baths at 60ºC for 3.5 minutes each. Slides were then incubated with secondary IgG antibody in PBS with Triton X and protein concentrate. Slides were then incubated for 30 minutes with ABC complex solution from a NovaRed (Vector) substrate kit, which was pre-mixed and allowed to incubate for approximately 30 minutes prior to application. Three 3-minute PBS baths were done following both incubation steps. Slides were then incubated with NovaRed detection reagent mixture for three minutes, followed by a five minute water bath. Slides were then washed in 45%, 60%, 80%, 95%, and 100% ethanol baths for approximately 20 seconds each or until no colors from Nova Red staining ran. A final, four second xylene bath followed by immediate application of cytoseal and coverslipping completed the staining procedure.

Thioflavin S Staining

Thioflavin S staining was used to detect compact Aβ plaques in the same brain regions used for 6E10 immunostaining. Slides were immersed for five minutes in 1% Thioflavin S in 50% ethanol. The same graded alcohol washes and xylene
wash scheme used for 6E10 protocol was applied, followed by application of cytoseal and coverslipping.

*Image Analysis*

All 6E10 and Thioflavin-s stained sections were analyzed on a Nikon Eclipse E1000 microscope at either 4x (Thioflavin S) or 10x (6E10) magnification with Plan Four objective lenses. A Retiga 1300 CCD with QImaging RGB LCD-slider was used to capture images of these sections. A Nikon BV-2B fluorescence fliter cube was used for thioflavin S staining. Image Analysis was performed using customized software written in Visual Basic 6.0 (Microsoft) that used Auto-Pro function calls to segment and quantify images according to the established protocols used by Costa et al (2004). Aβ deposition was quantified as a percent of area of interest (=Area stained_{total}/Area Measured_{total}).

*Statistical Analysis*

*Behavioral Statistical Analysis*

Inter- and Intra-group comparisons in pre/post infusion RAWM performance (errors and latency) and post infusion Platform Recognition (latency) and Y-maze (% alternation and arm entries) performances were obtained using standard one-way ANOVAs. Two-way repeated measure ANOVAs were also used to compare groups in multi-day tasks (RAWM and Platform Recognition). Daily averages over 4 days in Platform Recognition were used for this measure, and three 3-day block averages were used for RAWM. Using the Fisher LSD test, *pos-hoc* pair-by-pair differences between groups (planned comparisons) were then obtained. Swim speed in RAWM was approximated for each animal by dividing overall Trial 4 + Trial 5 average errors
into overall Trial 4 + Trial 5 average latencies. Non-performers (e.g. repeated circlers etc.) for a given task were not used for statistical analysis in that task. 16 of the 19 sensorimotor tasks comprising the Neurologic Screen were analyzed non-parametrically using the Kruskal–Wallis test and Mann–Whitney U-test. The remaining 3 measures (visual cliff, head poke latency, and number of head pokes) were analyzed using ANOVA.

*Pathological/Histochemical Statistical Analysis*

Standard one-way ANOVAs were used to determine group differences for Aβ load determinations (6E10 and Thioflavin S) and relative cytokine signal intensities. Using the Fisher LSD test, *pos-hoc* pair-by-pair differences between groups (planned comparisons) were then obtained for each measure.

*Correlation Analysis*

To determine if a direct relationship existed between behavioral and pathological measures, correlation analysis was performed using Systat software. A correlation matrix was established between 18 RAWM behavioral measures and 4 Aβ histochemical measures, as well as between these same behavioral measures and 10 plasma cytokine measures. Finally, Y-maze % alternation and arm entry measures were correlated to Aβ histochemical measures.

*Factor Analysis*

Factor Analysis (FA) was performed using Systat software to group behavioral measures into common, distinct factors. Each factor corresponds to a discrete component of behavior or cognition such as sensory motor function or working memory. This allows for the relationship between behavioral measures to be
determined, and also indicates how individual task performances may correlate with other task performance levels. FA was performed for 13 behavioral measures obtained.

**Discriminant Function Analysis**

Discriminant function analysis (DFA) was performed using 11 similarly loading measures from FA analysis as well as with all 13 behavioral measures used for FA analysis to determine if experimental animal groups (Tg+/T cell, Tg+/PBS, and NT) could be distinguished from one another behaviorally. All DFA analyses were performed using Systat software using both direct entry and stepwise forward DFAs. The direct entry method attempts to discriminate between groups using all behavior measures available, while the stepwise-forward method sequentially selects measures based on their variance contribution to best discriminate between the three groups.
Results

Behavior

RAWM

Pre-Infusion Testing. In pre-infusion RAWM testing (Fig. 4), transgenic (Tg+) mice were significantly impaired in working memory, as evidenced by an overall group effect for average combined Trial 4 and Trial 5 errors over 4 blocks of testing [F(1,23)=6.6823, p<.02]. After completion of pre-infusion testing, animals in the Tg+ group were divided into two groups for infusion of Aβ-sensitive immune cells (Tg+/T cell group) or PBS (Tg+/PBS group). Pre-infusion RAWM performance of these two sub-groups were identical prior to infusion (data not shown).

Post-Infusion Testing. Looking at overall post-infusion RAWM performance, Tg+/PBS mice made significantly more Trial 4+5 errors than Tg- mice (p<.05), while Tg+/T cell errors were not significantly different from either Tg- or Tg+/PBS mice (p=n.s.). Thus, overall Tg+/T cell animals performed at a level between that of Tg+ and Tg- control groups in RAWM working memory. Looking at individual blocks, Tg+/PBS mice maintained high Trial 4+5 errors during block 1 of post-infusion testing (Fig. 5), whereas Tg+/T cell mice made significantly fewer Trial 4+5 errors (p<.05) and performed identically to Tg- mice (p=n.s.). No group
Figure 4. Pre-infusion overall working memory performances. Tg+ mice averaged significantly more errors in overall Trial 4+5 testing than Tg- mice prior to their division into treatment groups, indicating a clear transgenic impairment in working memory. * = significant difference between Tg- and Tg+ groups (p<.02).
Figure 5. Post-infusion Block 1 RAWM errors. In contrast to Tg+/T cell mice, which performed similarly to Tg- mice, Tg+/PBS mice averaged significantly more errors in Block 1 Trial 4+5 post-infusion testing. * = significant difference between Tg+/PBS and both Tg+/T cell (p<.05) and Tg- mice (p<.02).
differences were evident on the semi-random Trial 1 (Fig. 5). Additionally, no group differences were noted for Trial 1 or Trial 4+5 errors in either block 2 or 3 of testing (data not shown). For Block 3 post-infusion testing (Fig 6) however, both Tg+/T cell mice (p<.05) and Tg- mice (p<.05) were able to significantly reduce their combined Trial 4+5 errors from Trial 1 “naive” performance levels, whereas Tg+/PBS mice were not (p=n.s.). Neither Tg+/T cell nor Tg+/PBS mice were able to improve Trial 1 vs. Trial 4+5 performance in Block 1 and Block 2, whereas Tg- mice showed improved Trial 1 vs. Trial 4+5 performance for all 3 blocks of testing (data not shown).

To ensure that post-infusion performance differences were not attributable to differences in time taken by animals to navigate the RAWM apparatus, an average number of seconds per arm choice in overall Trial 4+5 was calculated for each group by dividing overall Trial 4+5 latency by overall Trial 4+5 errors. No group differences in number of seconds per choice were noted for this measure (Tg+/T cell: 10.9 ± 1.2, Tg+/PBS: 9.8 ± 1.1, Tg-: 11.2 ± 1.0 sec.).

Pre-Infusion vs. Post-Infusion Testing. To further determine the effects of immune cell infusion on working memory performances in Tg+ mice, a block by block comparison of pre-infusion vs. post-infusion performances in the RAWM task was performed for all groups (Fig. 7). It was determined that Tg+/PBS mice did not significantly lower their number of errors for any individual block of testing (p=n.s.). In contrast, Tg+/T cell mice did significantly lowered their number of errors from Block 1 pre-infusion testing to Block 1 post-infusion testing (p<.01) as
Figure 6. RAWM during post-infusion Block 3 Trial 1 vs. Trial 4+5. Both Tg+/T cell and Tg- mice were able to significantly reduce their number of Trial 4+5 errors compared to Trial 1 “naive” performances. In contrast, Tg+/PBS mice failed to obtain a significantly lower number of errors for Trial 4+5 compared to Trial 1. * = significantly lower Trial 4+5 errors compared to Trial 1 errors (p<.05).
Figure 7. RAWM pre-infusion vs. post-infusion comparisons for Trial 4+5 combined errors by group. Tg+/PBS mice did not significantly lower their errors for any block of post-infusion testing. In contrast, Tg+/T cell mice were able to significantly lower their errors for both Block 1 and Block 2 of post-infusion testing compared with Trial 4+5 error averages for these blocks from pre-infusion testing. Tg- mice maintained relatively low errors throughout and thus also lacked significant improvement post-infusion. * = significantly lower Trial 4+5 error in post-infusion testing vs. pre-infusion testing (p<.05 or higher level of significance).
well as from Block 2 pre-infusion testing to Block 2 post-infusion testing (p<.05) but
did not show this error reduction for Block 3 performance (p=n.s.). Tg- mice did not
show significantly decreased errors for any block comparison, most likely due to
relatively good pre-infusion performance levels. Comparisons of Trial 4+5 errors
during the final block of pre-infusion testing vs. the first block of post-infusion testing
showed that Tg+/PBS mice became significantly worse (p<0.05) during initial post-
infusion testing (Fig. 8). By contrast, Tg+ mice that received T cell infusions
maintained their final pre-infusion performance level, as did Tg- controls, on the
initial post-infusion block (Fig.8).

Y-Maze

Post-infusion performance in the Y-maze task revealed a significant overall
effect indicating a direct treatment effect on basic pneumonic processes
[F(2,18)=6.8962; p<.01] (Fig. 9). Tg+/PBS mice demonstrated significantly lower
percent spontaneous alternations than Tg+/T cell mice (p<.01) or Tg- mice (p<.005).
In sharp contrast, Tg+/T cell mice performed identically to Tg- mice. No group
differences were noted in number of Y-maze arm entries (data not shown).

Platform Recognition

Analysis of average latencies for individual days and over all 4 days of
Platform Recognition testing found no group differences in performance for this task
(Fig. 10). There was a highly significant day effect [F(3,66)=7.02; p<.0005),
indicating that all animals collectively improved their performances across the 4 days
of testing.
Figure 8. RAWM Trial 4+5 performance for pre-infusion (final block) vs. post-infusion (first block). Tg+/PBS mice significantly worsened their working memory performance from pre- to post-infusion testing, whereas Tg+/T cell and Tg-animals maintained lower levels of errors in post-infusion testing. * = significantly higher post-infusion RAWM errors compared to pre-infusion performances (p<.05, paired t-test).
Figure 9. Post-infusion Y-maze spontaneous percent alternation performances.

Tg+/PBS mice displayed significantly lower percent alternations compared to Tg- animals, whereas Tg+/T cell mice performed identically to Tg- controls. * = significantly lower percent alternation compared to Tg- and Tg+/T cell mice (p<.01 or higher level of significance).
Figure 10. Post-infusion Platform Recognition latencies by day. No group differences were noted for any day or overall in Platform Recognition performance.
Neurologic Battery

While no group differences were noted between Tg+/T cell and Tg+/PBS groups for the 19 measures of our Neurologic Battery (data not shown), direct comparison between these groups and Tg- animals did elicit group differences. Tg+/PBS animals showed significantly decreased transfer arousal upon placement into a novel environment compared to Tg- mice \([p<.05]\). Tg+/T cell mice showed decreased transfer arousal as well \([p<.05]\), and also scored significantly lower in evaluation of pelvis \([p<.01]\) and tail elevation \([p<.05]\) compared to Tg- animals. Finally, Tg+/T cell mice showed an increased score for hypotonic gait qualifications compared to the Tg- group \([p<.01]\). The lack of differences between Tg+/T cell and Tg+/PBS groups may indicated that infusion of T cells did not result in any deleterious neurologic effects in Tg+ mice.

Pathology

Brain Aβ Deposition Quantification

6E10 Immunohistochemistry/Thioflavin S Histochemistry. Tg+/T cell and Tg+/PBS mice showed no group differences in % area Aβ plaque deposition, staining either for diffuse deposits (6E10) or compact deposits (Thioflavin S) (Fig. 11). Comparisons were made between groups for both hippocampal and parietal cortex Aβ burdens; no significant differences were noted for either brain region \((p=n.s)\).

Plasma Cytokine Analysis

For the ten cytokine measures taken (Fig. 12), there were no group differences between Tg+/T cell and Tg+/PBS mice or between Tg+/PBS and Tg- mice \((p=n.s)\).
Figure 11. Quantification of 6E10 and Thioflavin S Aβ burdens. No group differences between Tg+ groups were noted for either Aβ burden measure in either hippocampus or parietal cortex.
Figure 12. Standardized mean signal intensities for 10 plasma cytokines. Tg+/T cell mice showed significantly lower levels of plasma IL-10, TNF-α, and GM-CSF compared to Tg- mice. * = significantly lower mean signal intensity compared to Tg- animals (p≤.05).
However, the Tg+/Tcell group did display significantly lower plasma cytokine levels than Tg- mice for GM-CSF (p≤.05), IL-10 (p <.05), and TNF-α (p≤.05). Thus, T cell infusion into Tg+ mice did not result in a sustained, global increase in plasma pro-inflammatory cytokines.

**Multi-metric Statistical Analysis**

*Correlation Analysis*

**RAWM vs. Aβ Burdens.** A correlation matrix comparing Aβ deposition quantifications in hippocampus and parietal cortex vs. RAWM performances uncovered numerous correlations between these measures. All correlations were positive unless otherwise noted (e.g. higher RAWM errors/latency associated with higher Aβ deposition). For all 15 Tg+ animals combined (Table 1), these correlations were found exclusively between 6E10 hippocampal measures and the following 8 RAWM working memory measures: overall Trial 4 errors (p≤.05), Block 1 Trial 4 errors (p<.01), Block 1 Trial 4+5 errors (p<.01), overall Trial 4 latency (p<.05), overall Trial 4+5 latency (p≤.05), Block 1 Trial 4 latency (p≤.001), Block 1 Trial 5 Latency (p<.05), and Block 1 Trial 4+5 Latency (p≤.005). No correlations were found between Thioflavin S burdens in either brain area and working memory. Thus, lower hippocampal levels of diffuse Aβ deposits correlated with improved working memory.

A strong correlation was noted between RAWM Trial 4+5 errors for Block 1 of post-infusion testing and hippocampal 6E10 burdens when considering all Tg+ mice (p<.01). This correlation is of particular interest because Block 1 performance for Tg+/T cell mice was identical to that of Tg- mice, while Tg+/PBS mice made
Table 1. For all Tg+ animals, a correlation matrix of RAWM error and latency measures vs. 6E10/Thioflavin S measures of Aβ plaque burdens in the parietal cortex and hippocampus.

**RAWM Errors**

<table>
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<tr>
<th></th>
<th>Overall Trial 4</th>
<th>Overall Trial 5</th>
<th>Overall Trial 4+5</th>
<th>Block 3 Trial 4</th>
<th>Block 3 Trial 5</th>
<th>Block 3 Trial 4+5</th>
<th>Block 1 Trial 4</th>
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<th>Block 1 Trial 4+5</th>
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</table>

**RAWM Latency**

|                | Overall Trial 4 | Overall Trial 5 | Overall Trial 4+5 | Block 3 Trial 4 | Block 3 Trial 5 | Block 3 Trial 4+5 | Block 1 Trial 4 | Block 1 Trial 5 | Block 1 Trial 4+5 |
|----------------|-----------------|-----------------|-------------------|                |                |                   |                 |                 |                   |
| **6E10: Cortex** |                 |                 |                   |                |                |                   |                 |                 |                   |
| r              | 0.19            | 0.094           | 0.15              | 0.048          | 0.24           | 0.162             | 0.309           | 0.232           | 0.287             |
| p              | 0.497           | 0.738           | 0.594             | 0.864          | 0.389          | 0.565             | 0.263           | 0.405           | 0.299             |
| **6E10: Hippocampus** |             |                 |                   |                |                |                   |                 |                 |                   |
| r              | **0.589**       | 0.384           | **0.51**          | 0.379          | 0.337          | 0.394             | **0.772**       | **0.515**       | **0.687**         |
| p              | **0.021**       | 0.158           | **0.052**         | 0.163          | 0.219          | 0.146             | **0.001**       | **0.049**       | **0.005**         |
| **Thioflavin S: Cortex** |         |                 |                   |                |                |                   |                 |                 |                   |
| r              | -0.027          | -0.156          | -0.093            | -0.024         | 0.025          | 0.001             | 0.028           | -0.093          | -0.029            |
| p              | 0.924           | 0.578           | 0.743             | 0.932          | 0.929          | 0.996             | 0.741           | 0.919           | 0.919             |
| **Thioflavin S: Hippocampus** |        |                 |                   |                |                |                   |                 |                 |                   |
| r              | 0.287           | 0.182           | 0.246             | 0.151          | 0.396          | 0.305             | 0.169           | 0.238           | 0.211             |
| p              | 0.299           | 0.517           | 0.376             | 0.591          | 0.144          | 0.269             | 0.546           | 0.394           | 0.451             |

Correlations (bold font) were noted between 6E10 diffuse plaque burdens in the hippocampus and several overall and Block 1 RAWM measures of working memory. Most of these correlations were also noted when looking at only Tg+/T cell animals, however none were found when considering Tg+/PBS animals (data not shown), indicating the Tg+/T cell animals as the driving force behind correlations between working memory and diffuse Aβ deposition. r = Pearson product-moment correlation coefficient. p = probability.
significantly more errors than either group. Looking at the graph for this comparison (Fig. 13), it is apparent that Tg+/T cell mice are driving the correlation between working memory performance and hippocampal diffuse Aβ burdens. Interestingly, it appears that this correlation occurs as a result of segregation of the Tg+/T cell group into two subgroups. Of the seven total Tg+/T cell mice, a group of four mice maintained low errors in Block 1 and displayed low diffuse Aβ burdens in the hippocampus, whereas the remaining three Tg+/T cell mice made a high number of errors in testing and displayed high hippocampal burdens. In contrast, seven of eight Tg+/PBS mice displayed high errors and diffuse Aβ burdens. This stark difference between Tg+/T cell and Tg+/PBS animals clearly illustrates not only how Tg+/T cell animals are driving correlations between RAWM and 6E10 measures, but would also seem to indicate that a treatment effect is apparent in the majority of Tg+/T cell animals. It should also be noted that even the remaining three Tg+/T cell animals with higher Block 1 RAWM errors were able to reduce their errors by Block 3 of testing, indicating that good working memory performance was not limited to only a portion of the Tg+/T cell animals.

When considering only T cell recipient mice (n=7), correlations were also exclusive to hippocampal 6E10 burden when compared to the following measures: overall Trial 4 errors (p<.05), Block 1 Trial 4 errors (p<.05), Block 1 Trial 5 errors (p<.05), Block 1 Trial 4+5 errors (p<.05), Block 1 Trial 4 latency (p<.05), and Block 1 Trial 4+5 latency (p<.05). The lack of correlations between Tg+/PBS mouse RAWM measures and hippocampal 6E10 measures once again indicates that it is the Tg+/T cell mice which are driving these correlations for all Tg+ mice combined.
Figure 13. For all Tg+ mice, a correlation between RAWM Block 1 Trial 4+5 Errors and Hippocampal 6E10 burden. A strong correlation between diffuse hippocampal Aβ burden and working memory impairment in Tg+ mice is apparent. This correlation is clearly driven by the Tg+/T cell group, which show a distinct segregation into two subgroups, one with good working memory performance and low Aβ burdens (left side of the graph), and one with poor working memory performance and high Aβ burdens (right side of the graph).
This is especially true for 6E10 vs. overall and Block 1 measures where correlations were found when considering all Tg+ mice as well as Tg+/T cell mice alone, but not when considering Tg+/PBS mice alone. When considering PBS recipient transgenic mice only (n=8), correlations noted were between hippocampal Thioflavin S burdens and the following behavioral measures: Block 3 Trial 5 errors (p<.05), Block 3 Trial 5 latency (p<.01), and Block 3 Trial 4+5 latency (p<.05).

_Y-maze and Platform Recognition vs. Aβ Burdens._ No correlations were observed between Aβ burdens in the hippocampus and parietal cortex vs. Y-maze percent alternations for Tg+/T cell mice or for all Tg+ mice combined (p=n.s.). Similarly, no correlations were noted between Aβ burdens and Day 4 or overall Platform Recognition Latencies for these groups of animals.

_RAWM vs. Plasma Cytokines._ A correlation matrix between plasma cytokine levels and RAWM performance measures was established for all mice, for Tg+ mice combined, and for both PBS recipient Tg+ mice and T cell recipient mice alone. All correlations noted were negative (e.g. higher plasma cytokines associated with less RAWM errors). For all mice combined, 9 negative correlations were found, including one between Block 3 Trial 5 errors and plasma GM-CSF levels (p≤.05). Negative correlations were also found between IL-1β levels and the following measures: overall Trial 4 errors (p<.05), overall Trial 4+5 errors (p<.05), Block 1 Trial 4 errors (p<.05), and Block 1 Trial 4+5 errors. As for IL-1β correlations, the same negative correlations were also noted for plasma TNF-α levels: overall Trial 4 errors (p<.01), overall Trial 4+5 errors (p<.05), Block 1 Trial 4 errors (p<.005), and Block 1 Trial 4+5 errors (p<.05).
For all Tg+ mice combined, only 2 negative correlations were noted: between IL-4 levels and overall Trial 5 errors (p<.05) and between IL-1β and Block 1 Trial 5 errors (p<.05). When considering only PBS recipient Tg+ mice, the only (negative) correlation noted was between IFN-γ and Block 1 Trial 5 errors (p<.05). In sharp contrast, numerous negative correlations (13) were noted between levels of plasma cytokines in T cell recipient Tg+ mice and RAWM measures (Table 2). IL-1α was found to correlate negatively with Block 3 Trial 5 errors (p<.05). IL-1β was found to correlate negatively with overall Trial 4 errors (p ≤ .05) and Block 3 Trial 5 errors (p<.05). IL-2 was found to correlate negatively with Block 3 Trial 4 errors (p ≤ .001). IL-4 was found to correlate negatively with overall Trial 4 errors (p<.05), as well as with Block 1 Trial 5 (p<.05) and Block 1 Trial 4+5 (p<.05) errors. IL-12 was found to correlate negatively with both overall Trial 4 (p<.05) and Block 1 Trial 5 errors (p<.05). Finally, TNF-α was found to correlate negatively with overall Trial 5 errors (p<.005), overall Trial 4+5 errors (p<.05), and Block 1 Trial 4+5 errors (p<.05).

The strong negative correlations noted between RAWM measures and both TNF-α and IL-1β when considering all animals as well as T cell animals alone were not noted when looking at all Tg+ animals alone. This would seem to indicate that both T cell and Tg- mice are driving these negative correlations, implicating plasma IL1-β and TNF-α levels as being particularly important in terms of RAWM performance. Other cytokines such as IL-4, IL-1α, and IL-12, which were only negatively correlated to RAWM performance when considering Tg+/T cell animals, may also have an impact. However, the lack of such correlations for all animals combined
Table 2. Correlation matrix for RAWM errors vs. plasma Cytokine measures for Tg+/T cell animals.

<table>
<thead>
<tr>
<th></th>
<th>Overall Trial 4</th>
<th>Overall Trial 5</th>
<th>Overall Trial 4+5</th>
<th>Block 1 Trial 4</th>
<th>Block 1 Trial 5</th>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.296</td>
<td>-0.454</td>
<td>0.316</td>
<td>-0.433</td>
<td>-0.411</td>
<td>0.236</td>
<td>-0.572</td>
<td>-0.972</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-4</td>
<td><em>r</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.131</td>
<td>-0.854</td>
<td>0.030</td>
<td>-0.790</td>
<td>-0.278</td>
<td>-0.844</td>
<td>-0.815</td>
<td>-0.566</td>
<td>-0.761</td>
</tr>
<tr>
<td>IL-6</td>
<td><em>r</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.353</td>
<td>-0.439</td>
<td>0.384</td>
<td>-0.464</td>
<td>0.499</td>
<td>0.035</td>
<td>-0.815</td>
<td>-0.646</td>
<td>0.014</td>
</tr>
<tr>
<td>TNF-α</td>
<td><em>r</em></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><em>p</em></td>
<td>0.060</td>
<td>-0.950</td>
<td>0.004</td>
<td>-0.892</td>
<td>-0.369</td>
<td>-0.790</td>
<td>-0.827</td>
<td>-0.437</td>
<td>-0.643</td>
</tr>
</tbody>
</table>

Negative correlations (bold font) were noted for numerous measures when considering Tg+/T cell animals alone; correlations which did not exist when considering Tg+/PBS mice alone, or both groups of Tg+ mice together. (data not shown).
would seem to indicate that they are not universally associated with behavioral performance in this task.

**Factor Analysis**

Factor Analysis of 13 post-infusion behavioral measures from RAWM, Y-maze, and Platform Recognition was performed to determine the underlying relationships between these measures/tasks (Table 3). FA involving all 13 behavioral measures resulted in 11 of those measures loading on a common first factor, which accounted for 58.2% of the total variance (a measure was considered “significant” for loading on a factor if its component loading value exceeded 0.65 for that factor). Factor 1 was thus considered the primary cognition-based factor, as nearly all cognitive measures contributed to it. The two Platform Recognition measures used in this analysis co-loaded in factors 1 and 2. Only one RAWM measure (Block 1 Trial 5 errors) and Y-maze percent alternations did not load in the cognition-based factor 1. Block 1 Trial 5 errors did not load in any factor, whereas Y-maze percent alternations loaded alone in factor 4.

**Discriminant Function Analysis**

DFA was utilized to determine if behavioral performances of the three groups (Tg-, Tg+/PBS, and Tg+/T cell) could distinguish the groups from one another during post-immune cell infusion testing. Both “direct entry” and “stepwise-forward” DFA methodologies were employed. The direct entry method included all behavioral measures evaluated, while the “stepwise-forward” selects behavioral measures from the total number evaluated based on their contribution to the variance. For each methodology, all 13 measures used in Factor Analysis were included. Additionally, both
Table 3. Factor loadings of behavioral measures.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variance (%)</th>
<th>Post-Infusion Behavioral Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(58.2)</td>
<td>RAWM:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall Trial 4+5 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall Trial 4 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall Trial 5 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 1 Trial 4+5 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 1 Trial 4 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 3 Trial 4+5 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 3 Trial 4 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 3 Trial 5 Errors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y-maze:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arm Entries</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Platform Recognition:</td>
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<tr>
<td></td>
<td></td>
<td>Overall Latency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 4 Latency</td>
</tr>
<tr>
<td>2</td>
<td>(12.3)</td>
<td>Platform Recognition:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall Latency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 4 Latency</td>
</tr>
<tr>
<td>3</td>
<td>(9.6)</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>(8.7)</td>
<td>Y-maze:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent Alternations</td>
</tr>
<tr>
<td>Did not load:</td>
<td>-</td>
<td>RAWM:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block 1 Trial 5 Errors</td>
</tr>
</tbody>
</table>

Eight of nine RAWM measures loaded with Y-maze entries and two Platform Recognition measures in factor 1- the primary cognitive factor. Both Platform Recognition measures co-loaded in factors 1 and 2. Only Y-maze alternations loaded in a separate factor (factor 4), whereas Block 1 Trial 5 RAWM errors did not load in any factor. Percent of total variance explained by a given factor is indicated in bold type within parentheses.
forms of DFAs were run using only the 11 measures that loaded on factor 1 of Factor Analysis. Results of these DFA’s are summarized in Table 4. Direct entry DFAs, both for all 13 measures and factor 1 measures only, could not discriminate between the three groups based on their behavioral performance (p=.19; p=.48, respectively). In sharp contrast, the stepwise-forward DFA method (for both behavioral measures sets) was very effective in discriminating between Tg+/PBS mice and the other 2 groups. When all 13 behavioral measures were analyzed, RAWM Block 1 Trial 4+5 errors and Platform Recognition Day 4 latencies were retained in step-wise forward DFA. 86% of Tg+/PBS animals were correctly classified by this analysis, whereas only 50% of Tg+/T cell mice and 60% of Tg- mice were correctly classified. A similar pattern was noted for step-wise forward analysis of factor 1 behavioral measures, for which RAWM Block 1 Trial 4+5 errors was retained. 88% of Tg+/PBS animals were correctly classified by this analysis. In contrast, only 48% of Tg+/T cell and 50% of Tg- mice were correctly classified by this procedure. Thus, while step-wise forward DFAs were capable of discriminating between Tg+/PBS groups and the other 2 groups, this analysis could not effectively discriminate between Tg- and Tg+/T cell groups, indicating that behavioral performances in these latter two groups were too similar for distinction between them to be made.
Table 4. Summary of discriminant function analyses.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Direct Entry</th>
<th>Stepwise Forward</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Significance:</td>
<td>Significance:</td>
</tr>
<tr>
<td>All 13</td>
<td>N.S.</td>
<td>*p &lt; .01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor 1</td>
<td>N.S.</td>
<td>*p &lt; .05</td>
</tr>
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</table>

p-values are from Wilks's $\lambda$. * = significant for Tg+/PBS vs. both Tg+/-T cell and Tg- groups.
Discussion

General Summary

In the present study, we evaluated the behavioral and pathological effects of adoptive transfer of Aβ-sensitive immune cells into AD transgenic mice. These cells consisted of spleenocytes and lymphocytes taken from Aβ-immunized, congenic wild-type mice. The cells were cultured to enrich for T cells prior to infusion into recipient animals. We determined that such an infusion of T cells led to reversal of working memory impairment and also improved basic mnemonic processing at a 1-1½ month post-infusion time point. While overall no reductions in brain Aβ deposition were found, strong correlations between diffuse hippocampal deposition and RAWM working memory performances in T cell infused mice were noted, indicating that cognitive benefit was likely Aβ dependent.

It is also important to note that cognitive benefit in immune cell infused animals was not accompanied by increases in plasma pro-inflammatory cytokines. This would seem to indicate that a systemic inflammatory response was not evoked by adoptive transfer of immune cells into AD transgenic mice. Results of our Neurologic Battery would seem to confirm this, with no differences noted between transgenic controls and T cell recipient mice. Considering the lack of differences between transgenics noted in the Neuorologic Battery, as well as the presence of cognitive benefit extending over several
domains following only one immunotherapeutic infusion of T cells, such adoptive immunotherapy shows great promise for future human application in treating AD.

Behavioral Results

Immunotherapeutic techniques directed against Aβ have been tested in AD transgenic mice since 1999, however only a handful of these studies incorporated comprehensive behavioral testing of treated animals. In 2000, Morgan et al. found that repeated immunizations with Aβ1-42 in Freund’s adjuvant over an 8 month period resulted in protection against impairment in the RAWM task for working memory, though the 15 month old APPsw+PS1 mice tested did take more trials to learn the task than non-transgenic controls. In 2003, Austin et al. found that short term (4 biweekly injections) administration of this same vaccine to aged APPsw+PS1 mice did not result in reversal of impairment in the RAWM task. In a longitudinal study, Jensen et al. 2005 reported that APPsw+PS1 mice immunized with Aβ1-42 from 2-16.5 months of age were protected against impairment in RAWM testing performed at both 5 and 16 months of age. Thus it has been established that protection against impairment in RAWM can be incurred via active Aβ immunization in APPsw+PS1 mice. However, it is also clear that repeated administrations over an extended time period are necessary to establish this behavioral benefit. In the current study, we show that a single immunotherapeutic administration of Aβ-sensitive T cell results in improved RAWM performance at a 1 month post-infusion time point.

In pre-infusion RAWM testing, Tg+ mice were clearly impaired in working memory performance compared to Tg- mice, making significantly higher Trial 4+5 errors overall. In post-infusion RAWM, Tg+/PBS mice maintained a poor level of working
memory performance, with significantly higher Trial 4+5 errors overall compared to Tg-
mice. In contrast, Tg+/Tcell mice were not significantly different from either Tg- or
Tg+/PBS mice for this measure, indicating an overall improvement in working memory
performance to a level between that of Tg- and Tg+ controls. Indeed, for Block 1 of
testing, Tg+/T cell mice performed identically to Tg- animals in Trial 4+5 errors, while
Tg+/PBS mice made significantly more errors than either group. Further demonstrating
that improvement in working memory performance was present in Tg+/T cell animals, it
was noted that these mice showed a significant learning effect from Trial 1 to Trial 4+5
during the final block of RAWM testing. This effect was not noted for Tg+/PBS mice for
any block, whereas Tg- mice showed a significant learning effect for each block of post-
infusion testing.

To further demonstrate the level to which Tg+/T cell animals were able to
improve their working memory performances from pre-infusion levels, a block by block
comparison of pre- vs. post-infusion RAWM errors was performed. This analysis
revealed an inability of Tg+/PBS animals to improve their performance for any block. In
contrast, Tg+/T cell animals did show significant improvements in this measure for both
Block 1 and Block 2 of post-infusion testing. One final analysis of the RAWM task
comparing final block pre- vs. first block post-infusion also demonstrated this effect.
Tg+/PBS animals significantly worsened their working memory performance following
PBS infusion and a 1½ month hiatus from testing, whereas Tg+/T cell animals as well as
Tg- animals maintained significantly low levels in post-infusion testing. Post-infusion
and pre-vs. post-infusion analysis revealed that overall and for each block of post-
infusion RAWM testing, Tg+/T cell animals displayed either an improvement in working
memory performance from pre-infusion performances, and/or an ability to perform at a level greater than that of Tg+/PBS controls. In many cases, this performance level was identical to Tg- animals. This benefit was most prominent during the initial phase of post-infusion testing, but was evident throughout the entire post-infusion treatment period. Thus, post-infusion analysis confirms the potential for a single Aβ-sensitive immune cell infusion to reverse cognitive impairment in transgenic animals. Trinchese et al. (2004) found impairment in APPsw/PS1 mice in RAWM at as early as 3-4 months of age, while our laboratory has found impairment at as early as 5.5 months (Jensen et al. 2005) but has found no impairment at a 3 month time point (unpublished observation). These findings combined with pre- vs. post-infusion analysis from the current study confirm that the treatment effect noted above was a reversal of cognitive impairment, specifically in the domain of working memory.

In the Y-maze task of spontaneous alternations, Tg+/PBS animals displayed significantly lower alternations than both Tg+/T cell and Tg- animals. Therefore, it can be said that T cell infused animals were clearly protected against basic mnemonic processing impairment found in un-infused transgenic controls. Thus Y-maze, which is a relatively insensitive task, was still capable of eliciting a treatment effect in 10 month old mice. The results demonstrate how both working memory (RAWM) and basic mnemonic processing (Y-maze) benefited from T-cell infusion in Tg+ mice. In early Y-maze studies, Holcomb et al. (1998; 1999) found decreased Y-Maze alternations in 3-4 month, 6 month, and 9 month old APPsw/PS1 mice, whereas other studies did not find impairment at multiple time points through 16 months (Arendash et al. 2001, Jensen et al. 2005). Thus, the direct treatment effect observed confirms that T cell infusion promotes
improved performance in the Y-maze task, but does not provide insight into whether this is due to prevention or reversal of impairment. In 2004, Wilcock et al. showed that 3 monthly infusions of anti-Aβ antibodies were required to see behavioral improvement in the Y-maze task in 22 month old Tg2576 mice. The improved performance noted for this task in the current study occurred one month post-infusion of a single dose of Aβ-sensitive T cells. This again underscores the potential of this immunotherapeutic technique to provide cognitive benefit without numerous administrations.

No differences were noted between treatment groups in Platform Recognition performance. The relatively poor performance of all 3 groups compared to prior studies (Arendash et al. 2001, Jensen et al. 2005) may have resulted from Platform Recognition testing immediately following post-infusion RAWM. All groups may have had difficulty adjusting to the new, working memory-independent strategy of this task. However, it should be noted that, for all groups collectively, there was an overall learning effect, with significantly improved latencies from first to last day of testing.

Importantly, no differences were found when comparing the Neurologic Battery results of Tg+/PBS and Tg+/T cell animals. This would seem to indicate a lack of EAE (Experimental Allergic Encephalomyelitis) in T cell animals, which can occur following adoptive T-cell transfer (Kipnis et al. 2002). However, differences noted between Tg+/T cell animals and Tg- animals may indicate potential for some hind-leg motor control difficulties in T cell animals, beyond those found in control Tg+ mice. Future studies must look further into the effects of adoptive immunotherapy on neurological function to determine if these effects are consistent and, if so, to identify their cause.
Pathology

No group differences were noted for 6E10 or Thioflavin S burdens in the hippocampus or parietal cortex, indicating no overall reduction in diffuse or compact (insoluble) Aβ deposition following T cell infusion into APPsw/PS1 mice. Potential does exist for reductions in soluble Aβ levels, which would go a long way in explaining the behavioral improvement noted in Tg+/T cell animals. Previous immunotherapeutic studies in AD models have found improved behavior associated with reduced insoluble Aβ levels (Janus et al. 2000, Wilcock et al. 2004). Additionally, it has been noted in several studies that behavioral improvement can be independent of overall reductions in Aβ deposition (Morgan et al. 2000, Arendash et al. 2001, Jensen et al. 2005) following immunotherapy, as noted in the current study. This may be due to reductions in soluble Aβ levels, as other immunotherapeutic studies have reported reductions in soluble Aβ levels in the CNS following immunization (Sigurdsson et al. 2001, Sigurdsson et al. 2004). Future studies must be conducted to analyze soluble forms of this peptide, most importantly the Aβ1-40 and Aβ1-42 isoforms, to determine if reduced soluble amyloid levels contribute to the cognitive improvement noted in Tg+/T cell animals.

The lack of significant difference between Plasma cytokine levels in Tg+/T cell and Tg+/PBS groups suggests that T cell infusion into Tg+/T cell mice did not result in a sustained, global immune response. Thus, cognitive benefit associated with such an infusion was found to occur independently of an enhanced systemic immune response, although strong correlations were evident between plasma cytokines and cognitive
performance (see below). However, it should be noted that these cytokine measurements are for plasma only, and may not be indicative of regional brain cytokine profiles. Future studies must be conducted to analyze brain specific cytokine levels to determine if this trend for reduced cytokine levels is carried over into the CNS following infusion of Aβ-sensitive T cells. Compared to Tg- mice, there was a trend for Tg+/T cell mice to display reduced plasma cytokine levels, with significance noted for IL-10, TNF-α, and GM-CSF vs. Tg- controls. This reduction in cytokine levels may indicate an overall immune system inhibition by immune suppressive regulatory T cells (T regs), or down regulation of specific immune cell types by T helper cell subsets. The lack of significance between cytokine levels in Tg+/PBS and Tg- animals suggests no overt immunological response to Aβ in Tg+ animals. This may be indicative of an established immune tolerance to this human peptide, as would be expected for any peptide produced endogenously at high levels throughout the life of these mice. In 2002, Town et al. found that APPsw mice immunized with Aβ1-42 displayed decreased IFN-γ and increased IL-10 plasma cytokine levels. Levels of IL-2 and IL-4 were deemed immeasurable in this same study. Based on these findings, the authors proposed that Aβ1-42 immunization elicited a Th2 biased response in APPsw mice. Microglia and astrocytes are known to display increased expression of IL-1β, TNF-α, and IL-6 in the brains of Tg2576 animals, however this is not necessarily indicative of plasma levels of these cytokines (Benzing et al. 1999, Melhorn et al. 2000).

Correlations

Correlations to RAWM measures were limited specifically to hippocampal 6E10 diffuse brain Aβ burdens when considering all Tg+ mice. The majority of these
correlations were shown to be driven by the Tg+/T cell group however, as strong correlations also exist when considering only these animals. Analysis with Tg+/PBS animals only showed correlations with hippocampal Thioflavin S compact brain Aβ burdens exclusively. Thus it is clear that hippocampal Aβ burdens do have an impact on cognition, and diffuse hippocampal burdens seems to play the most important role following infusion of Aβ-sensitive T cells into AD transgenic mice. Without such infusion, compact plaques seem to take on a more important role in determining level of impairment.

Perhaps the best example of the overall correlation effect involved correlation data between Tg+ animals in RAWM Block 1 Trial 4+5 errors vs. hippocampal 6E10 Aβ burdens. Clearly, Tg+/T cell animals drove this correlation, with 4 animals displaying low errors and low burdens, and 3 animals display higher errors and higher burdens. In contrast, all but 1 Tg+/PBS animal displayed high errors with high Aβ burdens. This correlation may also demonstrate one mode of action for T cell adoptive immunotherapy. Clearly the four Tg+/T cell animals with lower RAWM errors displayed decreased Aβ burdens, and it may be possible that these infused animals benefited behaviorally due to clearance of hippocampal diffuse Aβ, thus explaining why this measure was of particular importance.

We have previously demonstrated that cognitive performance in a number of tasks can correlate with brain Aβ burdens in AD transgenic mice (Leighty et al. 2004, Gordon et al. 2001, Arendash et al. 2001b), though these correlations are not always present (Jensen et al. 2005). When present, correlations often involve the RAWM task,
although they can also involve Morris Water Maze and Platform Recognition (Leighty et al. 2004). RAWM is a task for working memory, thus performance in this task is heavily hippocampus dependent. This directly explains the cognitive benefit noted during Block 1 of post-infusion testing in 4 out of 7 Tg+/T cell animals. What these correlations do confirm is that both diffuse and compact Aβ burdens in the hippocampus can contribute to cognitive impairment in Tg+ animals, and also that modest reductions in diffuse hippocampal Aβ burden are associated with improved performance in the RAWM task.

Several correlations were noted between RAWM measures and plasma cytokine levels when considering all animals. Higher plasma cytokine levels correlated with better cognitive performance. All but one of these correlations were between either IL-1β or TNF-α and RAWM measures. These and several other correlations were also found when looking at Tg+/T cell animals alone, but not when looking at Tg+ animals combined or Tg+/PBS animals alone. Clearly then, these negative correlations are being driven by both Tg- and Tg+/T cell animals. These correlations were all negative (e.g. higher plasma cytokines with lower RAWM errors). Therefore, it would seem that despite a trend for decreased plasma cytokine levels in Tg+/T cell animals, higher levels of individual cytokines (especially IL-1β and TNF-α) may play a role in improving cognitive performance capabilities. It is interesting that the two key cytokines driving these correlations are pro-inflammatory cytokines which have been found to be over-expressed by microglia local to Aβ plaques in transgenic models of AD (Benzing et al. 1999, Akiyama et al. 2000). In humans with AD, CSF levels of both cytokines are increased (Cacabelos et al. 1991, Tarkowski et al. 1999). However, several lines of study suggest that while these cytokines are involved in the neuroinflammatory cascade of AD,
under situations of appropriate immune activation they may actually act beneficially in the CNS to reduce inflammatory damage (Correale and Villa, 2004). This may indicate that the actions of certain cytokine producing cells may be important in determining extent of behavioral improvement. Once again, this emphasizes the importance of analyzing brain cytokine levels, and perhaps also quantifying levels of activated immune cells such as microglia, astrocytes and T cells that would contribute to production of these cytokines in the brain.

**Factor Analysis/Discriminant Function Analysis**

All but one RAWM measure loaded in Factor 1 of FA, as did both PR measures, indicating this factor as cognitively-based. Stepwise forward DFA results for all behavioral measures as well as Factor 1 measures alone indicate that Tg+/T cell animal performances could not be separated from Tg- performances, whereas Tg+/PBS animal performance levels were poor enough that they were clearly distinguished from the other two, better performing groups. Our laboratory has shown in the past that “stepwise forward” DFA analysis is capable of distinguishing not only between transgenic treatment groups and wild-type mice, but also between different strains of transgenic mice (Jensen et al. 2005, Leighty et al. 2004). Thus, the lack of discrimination between Tg+/T cell animals and Tg- animals when applying this methodology across multiple cognitive measures strongly emphasizes the similar performance levels of these two groups. This reinforces the hypothesis that infusion of Aβ-sensitive immune cells leads to normalization of Tg+ animal performances in RAWM to that of Tg- levels.
Proposed Mechanisms of Aβ-Sensitive T Cell Mediated Cognitive Improvement in APPsw/PS1 Mice

Although the current literature is limited with regards to application of adoptive immunotherapy in neurodegenerative diseases, it is possible to speculate as to the mechanisms by which infused T cells could act therapeutically in a mouse model for AD. It has been shown that immuno-tolerance to Aβ is present in APP transgenic mice (Monsonego et al. 2001), and it is therefore necessary to administer Aβ vaccinations in adjuvant designed to activate the immune systems to elicit an effect. One mechanism of action for Aβ-sensitive T cells infused into such an immuno-tolerant environment could be to break this tolerance and initiate an immune system-based clearance of Aβ from the CNS. Neuroinflammation is thought to be a major contributor to the progression of AD, and is present to a large extent in AD mouse models. A second mechanism for T cell action could be for regulatory T cells and/or Th2 cells to down-regulate this type of cellular-based neuroinflammatory response. Finally, it is possible for T cells and the cells they activate (astrocytes, microglia etc.), provide neuroprotection via a number of mechanisms.

*Stimulation of the immune system against an endogenous ‘self’ peptide, Aβ*

T helper cells play a central role in determining the type of immune response elicited against a given immunogen. Depending on the major sub-type of T cell involved in stimulating such an immune response against Aβ, it is likely that one of two reactions, either cellularly or humorally based, would be elicited against this peptide. If Th1 T helper cells were the major contributor towards developing this reaction, a phagocytic immune cell activation would be anticipated. This would potentially result in clearance

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of deposited amyloid via phagocytic mechanisms, a mechanism of clearance found to be present in several active immunization studies in AD models (Das et al. 2003a, Das et al. 2003b). In the CNS, the primary phagocytic cells are the microglia, and thus such a reaction would most likely be associated with increased microglial activation in the areas of A\(\beta\) plaques (Wilcock et al. 2001). While correlation data from the current study suggests that decreased diffuse hippocampal A\(\beta\) may mediate RAWM improvement in some T cell infused mice, no overall reductions in A\(\beta\) pathology were noted, indicating that this effect was not widespread in Tg\(^+\)/T cell mice. In futures studies, it will be important to determine to what extent microglia and other components of the cellular immune system are activated in the CNS of T cell infused mice.

If following T cell infusion a Th2 biased response was elicited, in contrast to the cellular based response to A\(\beta\) promoted by Th1 cells, a humoral response would be anticipated instead. This would be mediated by the ability of Th2 subset cells to stimulate B cells to produce antibodies, in this case antibodies specific to A\(\beta\). Based on the results of passive immunizations studies in models of AD (DeMattos et al. 2001, Wilcock et al. 2004), it is likely that such a humoral based immune response would also trigger the clearance of insoluble as well as soluble A\(\beta\) from the CNS via standard clearance mechanisms for antigen-antibody immune complexes. A down-regulation of cellular immunity present in Th1-biased immune reactions would also be expected in a Th2-biased response, perhaps leading to decreased neuroinflammation as discussed below. To determine if these effects were present in T cell animals, it may be beneficial to look at antibody titers and regional brain cytokine levels in future studies.
Overall down-regulation of the immune system, or of specific immune cells which may contribute to neuroinflammation.

As previously mentioned, microglia and other cellular components of the CNS immune system may be down-regulated by the Th2 subset of T helper cells. This would be especially beneficial in the Tg+ animal brain paradigm, in which immune cells such as microglia are perpetually activated by Aβ and Aβ associated neuronal damage. Without the influence of regulatory cells, this form of activation may result in a state of “frustrated phagocytosis”, which results in production of neuroinflammatory mediators involved both in inappropriately activating other cells (such as astrocytes) and in directly causing damage/dysfunction in adjacent neurons (Streit et al. 2004, D’Andrea et al. 2004). Th2 subset cells could down-regulate such activation, blocking perpetuation of the AD neuroinflammatory cascade. Regulatory T cells may also be involved in this process, down-regulating numerous immune cell types indirectly via de-activation of T-cells or directly via interactions with microglia and other components of the cellular immune system (Avidan et al. 2004). Again, in future studies, looking at regional cytokine profiles would be beneficial to determine if such an effect was present. Analysis of activated immune cell levels in the CNS would also be extremely beneficial in determining the exact actions of adoptively transferred T cells.

Neuronal protection

The potential for T cells to enhance neuronal health and protect against neuronal damage has been established in the current literature (Moalem et al. 2000, Benner et al. 2004, Avidan et al. 2004). This could involve a down-regulation of immune cells which instigate neuroinflammation (and resulting neuronal damage) as described above, or a
direct production and/or induced production of neuroprotective agents by astrocytes. Whether cells in the Aβ-sensitive T cell populations infused into Tg+ animals possess this potential is unknown, and analysis of either neurotrophic factor levels in the CNS would indicate if this is a viable method by which T cells provide cognitive benefit in these animals.

Clinical Implication of T cell Study Findings and Proposed Future Investigations

As with all immunologically based protocols, special care must be taken before application of such protocols in humans. Further investigation into the potential for Aβ-sensitive immune cells to promote EAE or other deleterious auto-immune type reactions is of the utmost importance, especially in lieu of the adverse effects resulting from clinical application of active Aβ immunotherapy (Orgogozo et al. 2003). Additionally, to help elucidate the exact mechanism of cognitive improvement/protection provided by immune cell infusion, it may be beneficial for futures studies to look at the effects of infusion of specific T cell subsets into transgenic animals. Analysis of the effects of these subsets on behavior and pathology, as well as added analysis of brain cytokines, activated immune cell (such as microglia) stains, and neurotrophic factor levels may provide insight into the mechanisms by which Aβ-sensitive immune cells improve cognitive-based performances in these AD transgenic mice. In addition, a comparison of neuronal health and activity, especially in the hippocampus, in treated vs. untreated Tg+ animals may elucidate the exact mechanism by which cognitive performances in tasks such as the RAWM are being improved. These and additional studies would be desirable before application of this procedure in humans is initiated.
From a methodological standpoint, Aβ-sensitive T-cells could be isolated from an AD patient’s blood, or even spleen, expanded in vitro and returned to the patient on a periodic basis. While invasive, this process is not unprecedented and has been used in donor lymphocyte infusion protocols for many years (Kolb et al. 1995). Only one infusion of T cells into AD transgenic mice was necessary to observe cognitive improvement 1-1½ months later in this study. Future studies would be necessary to determine if these effects would continue without additional infusions. If such follow-ups were unnecessary or could be spaced far apart, this immunotherapeutic approach may prove to be less invasive than other immunotherapeutic approaches to AD (specifically active and passive immunization therapies against Aβ), which require regular injections of either Aβ in adjuvant or anti-Aβ antibodies. Both of these forms of treatment have potential for side effects as elucidated by studies both in animal models of AD and, for active immunotherapy, in humans. Both active and passive immunotherapeutic approaches have been shown to induce meningoencephalitis (Lee et al. 2005) due to excessive immune system activation. This form of inflammation may not be present following a single infusion of Aβ-sensitive T cells independent of adjuvant. Findings of reduced levels of cytokines in the plasma of T cell infused mice would seem to suggest that inflammation such as that noted in human active Aβ immunization trials is not present, though further work will confirm or refute this hypothesis. Thus, adoptive immunotherapy may provide a means of providing long term cognitive benefits in AD, with decreased potential for negative side-effects.
References


King DL, Arendash GW. Behavioral characterization of the Tg2576 transgenic model of Alzheimer's disease through 19 months. Physiol Behav. 2002 Apr 15;75(5):627-42.


Su GC, Arendash GW, Kalaria RN, Bjugstad KB, Mullan M. Intravascular infusions of soluble beta-amyloid compromise the blood-brain barrier, activate CNS glial cells and induce peripheral hemorrhage. Brain Res. 1999 Feb 6;818(1):105-17.


