CD4 T cells in immunity and immunotherapy of Alzheimer’s disease

Alon Monsonego, Anna Nemirovsky and Idan Harpaz
The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel

do:10.1111/imm.12103
Received 17 January 2013; revised 13 March 2013; accepted 18 March 2013.
Correspondence: Alon Monsonego, The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, and The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel. Email: alonmon@bgu.ac.il
Senior author: Prof. Alon Monsonego

Summary
Alzheimer’s disease (AD) is the most common form of dementia, with prevalence progressively increasing with aging. Pathological hallmarks of the disease include accumulation of amyloid β-protein (Aβ) peptides and neurofibrillary tangles in the brain associated with glial activation and synaptotoxicity. In addition, AD involves peripheral and brain endogenous inflammatory processes that appear to enhance disease progression. More than a decade ago a new therapeutic paradigm emerged for AD, namely the activation of the adaptive immune system directly against the self-peptide Aβ, aimed at lowering its accumulation in the brain. This was the first time that a brain peptide was used to vaccinate human subjects in a manner similar to classic viral or bacterial vaccines. The vaccination approach has taken several forms, from initially active to passive and then back to modified active vaccines. As the first two approaches to date failed to show sufficient efficacy, the last is presently being evaluated in ongoing clinical trials. The present review summarizes the immunogenic characteristics of Aβ in humans and mice and discusses past, present and future Aβ-based immunotherapeutic approaches for AD. We emphasize potential pathogenic and beneficial roles of CD4 T cells in light of the pathogenesis and the general decline in T-cell responsiveness evident in the disease.

Keywords: Alzheimer’s disease; amyloid β-protein (Aβ); CD4 T cells; Aβ antibodies; immunotherapy.

Introduction
Alzheimer’s disease (AD) is the most common form of dementia in the elderly, characterized by progressive memory loss and cognitive decline. One of the primary pathological features of the disease, in addition to neurofibrillary tangles, dystrophic neurites and significant neuronal loss in affected brain regions, is the extracellular aggregation of the amyloid β-protein (Aβ) peptide in the brain.1-4 Amyloid-β is produced from the amyloid precursor protein (APP) following proteolytic cleavage by β- and γ-secretases. Mutations in the presenelin-1 gene (PS1), which encodes for a transmembrane protein that functions as part of the γ-secretase complex, are associated with increased production of Aβ42 over the less aggregative form Aβ40, and are considered among the primary causes of early-onset familial AD.5,6

A growing body of evidence demonstrates that Aβ plaques induce an inflammatory reaction in the brain,7-9 whereas oligomeric forms of Aβ exert synaptotoxicity.3,4,10 In addition, in recent years information has accumulated demonstrating the marked pathological effects of Aβ on brain vasculature, a phenomenon termed cerebral amyloid angiopathy, that causes vascular inflammation, brain haemorrhages, compromised perivascular drainage and altered blood flow.11-13 Inflammatory processes such as microgliosis, astrocytosis, dystrophic microglia, complement activation, cytokine elevation and acute-phase protein changes are thought to represent, at least in part, a response to the accumulation of Aβ in the vasculature and parenchyma of the brain. A compromised immune system associated with aging may substantially impact on these processes and lead to compromised brain function and neuronal repair processes, which enhance the progression of AD. The current review summarizes the existing knowledge regarding the characteristics of Aβ-reactive CD4 T cells in animal models and in humans, and discusses Aβ-based immunotherapeutic approaches for AD in the context of disease pathogenesis and immunosenescence.
Main body

Aβ autoimmunity in humans and mice

More than a decade ago a new concept emerged in the study of AD, namely eliciting adaptive immune responses to attenuate the accumulation of Aβ in the brain. This was the first time that a self peptide was introduced to the body as a vaccine, similar to classic vaccination approaches used against various pathogens. As this approach may have brought about the most promising therapeutic approach for AD, it also challenged our previous knowledge of autoimmunity, immune tolerance and brain-immune interactions.

Amyloid-β-specific immunotherapy can considerably reduce amyloid burden and improve cognitive functions in animal models of AD. Although pre-clinical studies have proved successful, an initial clinical trial of active Aβ vaccine (AN-1792 trial performed by Elan) was halted because of the development of severe inflammatory reactions in the brains of some vaccinated AD patients. The severe side-effects were attributed to the use of the full length of the Aβ peptide together with QS21, a very strong adjuvant, the combination of which presumably led to the occurrence of pathogenic T cells at the brain vasculature and parenchyma. Nevertheless, the study of Aβ-reactive T cells is key to unravelling their occurrence and characteristics in healthy humans as well as in patients with AD, and hence a key to designing safer immune-based approaches for AD therapy.

Although the general dogma would not anticipate the occurrence of effector Aβ-reactive CD4 T cells in the circulation of human subjects, not only were they detected in almost all individuals tested but significantly more elderly subjects and AD patients showed strong Aβ-reactive T-cell responses compared with middle-aged subjects. The Aβ T-cell responses were primarily HLA-DR-dependent, and the presented T-cell epitopes derived primarily from residues 15–42 of Aβ (see Table 1). About 20% of all the subjects were found to bear HLA-DR alleles, which either did not stimulate Aβ-reactive T-cell lines or induced only a mild response. The great variability of HLA-DR alleles in humans, which is associated with multiple Aβ T-cell epitopes, presumably reflects a great variability in the magnitude of T-cell activation in humans and therefore the variations in specific Aβ-antibody titres evoked in AD patients following Aβ42 vaccination.

<table>
<thead>
<tr>
<th>Strain/MHC II or HLA</th>
<th>Aβ1–42 T-cell responsiveness</th>
<th>T-cell epitope within Aβ residues</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJL / I-Aβ</td>
<td>+</td>
<td>16–30</td>
<td>30–32,113</td>
</tr>
<tr>
<td>BALB/c / I-A^d</td>
<td>+</td>
<td>1–28</td>
<td>113</td>
</tr>
<tr>
<td>NOD / I-Ab^d</td>
<td>+</td>
<td>10–24</td>
<td>30</td>
</tr>
<tr>
<td>Congenic mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6 / I-A^b</td>
<td>+</td>
<td>10–24</td>
<td>31</td>
</tr>
<tr>
<td>NOD / I-Ab^b</td>
<td>+</td>
<td>16–30</td>
<td>30</td>
</tr>
<tr>
<td>Humanized mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR15</td>
<td>+++</td>
<td>25–42</td>
<td>27</td>
</tr>
<tr>
<td>DR4</td>
<td>+</td>
<td>16–33</td>
<td>27,32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–16, 1–28</td>
<td>113</td>
</tr>
<tr>
<td>DRB1*0101</td>
<td>++</td>
<td>1–28</td>
<td>32</td>
</tr>
<tr>
<td>DR3</td>
<td>+</td>
<td>1–16</td>
<td>32</td>
</tr>
<tr>
<td>DQ8</td>
<td>+</td>
<td>1–42</td>
<td>32</td>
</tr>
<tr>
<td>Human subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*0101/1301/1001</td>
<td>ND</td>
<td>15–35</td>
<td>27</td>
</tr>
<tr>
<td>DRB1*0401/0404</td>
<td>ND</td>
<td>18–32</td>
<td>27</td>
</tr>
<tr>
<td>DRB1*1501</td>
<td>ND</td>
<td>25–42</td>
<td>27</td>
</tr>
</tbody>
</table>

ND, not determined.

In mice, Aβ-reactive T cells were analysed following Aβ1–42 immunization and re-stimulation with Aβ1–42 or with shorter Aβ peptides in vitro. In human subjects, Aβ T-cell epitopes were analysed in isolated peripheral blood mononuclear cells stimulated initially with Aβ1–42 and thereafter with 15-residue-long overlapping peptides between 1 and 42 residues of Aβ. T-cell responsiveness was measured by the magnitude of antigen-driven T-cell proliferation and cytokine production following immunization. In humanized mice bearing the DRB1 1501 and 0401 alleles, peptides between residues 25 and 42 and between residues 18 and 32 served as the dominant T-cell epitopes, as observed also for T-cell lines derived from human subjects with these HLA genetic backgrounds. Aβ42 immunization of humanized HLA-DR4 and HLA-DR3/DQ8 transgenic mice evoked Aβ-reactive T-cell responses which could be partially stimulated by Aβ1–16, and DRB1 0101 humanized mice elicited T-cell responses to an epitope between residues 1–28. Since overlapping peptides between residues 15 and 42 of Aβ were not used in these studies, it is unclear whether additional weak T-cell epitopes are located at the N-terminus of Aβ or whether a truncated portion of the epitope was presented to the T cells.
Animal models allow one to more accurately investigate the contribution of an MHC class II genetic background to Aβ immunogenicity associated with the dominant epitope presented to T cells. They also allow a more efficient examination of the effect of various vaccination paradigms (i.e. route of administration and choice of adjuvant) on the dynamics and characteristics of the immune response elicited (i.e. antibody isotype and titres, and the profile of T-cell cytokines). In mice, Aβ immunogenicity markedly differs between strains; for example, Aβ is highly immunogenic in NOD and SJL mice, which have a dominant T-cell epitope between residues 10 and 24 of Aβ, whereas the peptide evokes only weak T-cell responses in C57BL/6 mice in which the epitope is between residues 16 and 30. NOD congenic mice bearing the I-A\(^b\) class II allele also fail to elicit a strong T-cell response, suggesting that the low immunogenicity of Aβ 16–30 in C57BL/6 mice is primarily a result of a low-affinity epitope selected by the I-A\(^b\) MHC class II. However, both C57BL/6 and B6.H-2\(^b\) congenic mice, but not SJL mice, exhibit enhanced Aβ-specific T-cell responses upon the depletion of regulatory T (Treg) cells, suggesting that under certain genetic backgrounds, Treg cells can significantly affect Aβ immunogenicity. As no differences in thymic expression of APP are observed between C57BL/6 and SJL mice, the mechanism behind the effect of Treg cells on Aβ immunogenicity in C57BL/6 mice and the reason it is not effective in the more Aβ-immunogenic SJL mice are yet to be revealed.

Overall, T-cell epitopes markedly vary between mice and humans, with multiple epitopes located primarily between Aβ residues 10 and 30 and between 15 and 42, respectively. Both MHC class II alleles and Treg cells are crucial for determining the strength and phenotype of the adaptive immune response to Aβ following immunization. The fact that almost all human subjects possess Aβ-reactive T cells in their circulation and that these tend to expand with age and with the progression of AD raises a number of questions that are yet to be answered. (i) Are these Aβ-specific T cells positively selected in the thymus or do they simply ‘escape’ negative selection? (ii) Do Aβ-reactive T cells play a role in the progression of AD and, if so, how? (iii) Can they be externally stimulated to beneficially halt the progression of AD? Clearly, Aβ-reactive T cells are activated upon immunization and induce Aβ antibody production, however, one should consider the great variability in T-cell responses that can be anticipated in humans; in fact, this variability may perhaps be translated to personalized medicine.

**Aβ-based vaccines**

Since Aβ T-cell epitopes are located primarily between residues 10 and 42 of Aβ in mice and in humans, N-terminal portions of Aβ, namely fragments within residues 1–15 comprising dominant Aβ B-cell epitopes, have been used to generate active Aβ vaccines (Fig. 1). These peptides were conjugated to carriers such as albumin or the promiscuous foreign T-cell epitope PADRE and were shown to elicit effective Aβ antibody responses without stimulating an Aβ-specific T-cell response. These vaccination studies have led to pre-clinical studies using the N-terminal portion of Aβ presented on the surface of virus particles or liposomes, or administered as Aβ-coding DNA plasmids or viral vectors and current clinical trials using such N-terminus Aβ peptides conjugated to diphtheria toxin or tetanus toxin are being carried out. The non-self carriers in these vaccines, although they avoid the T-cell response against Aβ, presumably evoke a strong T-cell response against the foreign epitopes and high titres of Aβ-specific antibodies (Fig. 1). In contrast to non-self carriers, our group generated a conjugate of Aβ1–15 and heat-shock protein 458 (hsp 458), a 17-amino-acid residue peptide derived from hsp 60. Compared with Aβ1–42, Aβ–hsp 60 immunization of humanized mice carrying the HLA-DR allele DRB1*1501 evoked a very mild T-cell response, evident by a significantly lower production of interferon-γ (IFN-γ) and interleukin-17 (IL-17) by draining lymph node-derived T cells. Notably, the mild T-cell response induced by Aβ–hsp 60 induced a gradual increase in specific Aβ antibody titres, which were sufficient for effective clearance of Aβ plaques from the brain of aged APP-transgenic mice. In addition to its function as a T-cell epitope, hsp 458 also activates the Toll-like receptor 4 pathway and so T-cell-independent antibody production evoked by Aβ–hsp 60 immunization is plausible.

Clinical trials using either Aβ42 active vaccination or anti-Aβ passive vaccination have so far failed to show treatment efficacy, so eliciting a beneficial adaptive immune response to Aβ appears to be more complicated than was originally thought. Indeed, clearance of Aβ plaques in mouse models of AD may be partially misleading because it may not accurately represent key pathological features of the disease. This could have several explanations. (i) Most animal models of AD are treated prophylactically (i.e. in a prevention mode) or following the initial Aβ deposition in the brain. They are rarely, if at all, conducted in ages and disease stages equivalent to human AD patients, in which immunity declines and brain inflammation is markedly enhanced. (ii) The increase in Aβ42/40 ratio in some mouse models of AD induces a more condensed form of plaques where the capacity of Aβ clearance in the brain is considerably reduced. This may represent a shift towards a fast-progressing form of AD where Aβ antibodies, either naturally occurring or generated following vaccination, are insufficient to promote a therapeutic effect. (iii) The inflammatory reaction at the vasculature and parenchyma...
in AD patients may be facilitated by the Aβ-specific antibodies depending on their titers, epitope specificity, the Fc glycosylation pattern or the type of Fc receptor.\(^{50-52}\) In addition, a robust expansion of Aβ-specific B cells occurs, which may lead to ectopically enhanced activation of pathogenic Aβ-specific T cells (Fig. 1). (iv) The loss of synapses and neurons, which leads to progressive cognitive decline throughout the course of AD, is moderate in most mouse models of the disease, so the impact of Aβ clearance on this key aspect of the disease is unclear. Stimulating an immune response that promotes Aβ clearance as well as neuronal repair (e.g. via cytokines and neurotrophic factors\(^{53-55}\)), which may be administered in a prevention mode, may therefore be considered a future goal for AD immunotherapy. Factors such as the vaccine carriers (either derived from self or non-self proteins), the routes and timing of vaccine administration and the choice of adjuvants may substantially decrease some of the risks described above and therefore improve treatment efficacy.

**Aβ-reactive CD4 T cells in brain inflammation and repair**

Given the immunogenicity of Aβ as demonstrated in humans and mice, it is clear that Aβ-reactive T cells can...
be boosted to promote pathogenic autoimmunity. In the following section we discuss the molecular and cellular setting that drives the homing of Aβ T cells to the brain and whether such a process can be used to enhance neuronal repair mechanisms in the aging and diseased brain.

The role of T cells in the brain has been widely studied in recent years. Trafficking T cells to the brains of APP-transgenic mice over-expressing transforming growth factor-β or IL-1β did not cause cellular or behavioural abnormalities and brain-specific T cells have been shown to play beneficial roles in murine models of brain injury, amyotrophic lateral sclerosis and stroke. Such specific T cells, or the cytokines they produce, participate in numerous activities such as increasing the uptake of Aβ plaques, releasing regulatory cytokines, increasing the expression of neurotrophic factors, increasing the buffering capacity of glutamate and enhancing neurogenesis. Our group has recently demonstrated that Aβ-reactive T cells are able to effectively target Aβ plaques in the brains of APP-transgenic mice and enhance the phagocytic activity of adjacent microglia (see Fig. 2 and refs 30 and 62), at least partially via IFN-γ-induced TREM2 and SIRP/β1 expression, which were recently suggested as DAP12-associated phagocytic receptors on microglia. Amyloid-β may be presented to T cells via co-localized MHCI Hhigh antigen-presenting cells, which either differentiate from brain-endogenous microglia or are recruited from the blood as a result of increased CCL2 expression. Interferon-γ emerges as a unique cytokine, which on one hand facilitates T-cell migration into and within the brain parenchyma, and on the other hand promotes immunoregulatory processes and neuronal repair in the brain.

Provided that IFN-γ signals to all neural populations, further research is required to determine how IFN-γ orchestrates its various effects in the brain. Clearly, the overall amounts of IFN-γ in the brain are crucial to shift its function from devastating at high levels to beneficial at low levels. Additional cytokines such as IL-10 and transforming growth factor-β, together with a profile of chemokines and neurotrophic factors secreted by the T cells, may prove therapeutic for the AD brain.

The specific mechanisms underlying the migration, activation and survival of the T cells within the brain parenchyma are yet to be identified. The model illustrated in Fig. 2 demonstrates that following Aβ immunization, Aβ-specific T cells target the brain vasculature in which Aβ is deposited. Expression of IFN-γ in the brain of a mouse AD model in limited amounts, which cause no spontaneous infiltration of bone marrow-derived cells, abnormal glial activation or neurological deficits, is required for the migration of T cells within the brain parenchyma. Three conditions can therefore promote Aβ-specific T-cell entry to the brain parenchyma: (i) depo-
Aβ plaque

From circulation:

Inflammation

Parenchymal basement membrane

Blood vessel

CXCR3

CD11c+

CD11c+

CCR5

Neurotrophic factors

IFN-γ

IFN-γ

MHCII/co-stimulation

Hippocampus

Lateral ventricle

From choroid plexus

Vessel

Cerebrospinal fluid

Figure 2. Migration of Aβ-specific T cells towards Aβ plaques in the brain parenchyma. (1) T cells may undergo activation following Aβ immunization or following drainage of Aβ or antigen-presenting cells (APCs) that carry Aβ to peripheral lymph nodes. Aβ-reactive helper T (Th) cells adhere and transmigrate into the perivascular space of Aβ-deposited vasculature in the brain (a, b). To cross the glia limitans, Th cells need to be re-stimulated by dendritic cells or, possibly, by other competent APCs located at the perivascular space and/or juxtavascular with processes sent into the perivascular space. Similar T-cell infiltration processes may occur at the choroid plexus (2), and/or the leptomeninges followed by their dissemination in the central nervous system subarachnoid space. Low levels of interferon-γ (IFN-γ) promote the infiltration process. Adhesion molecules such as P-selectin, vascular cell adhesion molecule-1 or intercellular adhesion molecule 1 (interacting with P-selectin glycoprotein ligand-1, integrin α4 and lymphocyte function-associated antigen-1, respectively, on the T cells) and chemokine signalling (such as via CCR5 and CXCR3) play a key role in mediating the extravasation of the T cells through the blood–brain barrier (BBB) or the blood–cerebrospinal fluid barrier. (3) Leucocytes accumulate at the subarachnoid and perivascular spaces and may impact on the overall inflammatory reaction at both the vasculature and parenchyma. (4) Once Aβ Th cells cross the glia limitans they migrate and accumulate around Aβ plaques, possibly interacting with APCs (i.e. microglia, or peripheral monocytes or dendritic cells recruited towards CCL2) that present Aβ T-cell epitopes. Cytokines such as IFN-γ are secreted by the T cells and facilitate Aβ clearance either by brain endogenous microglia or by infiltrating microglia-like cells. (5) T cells secreting IFN-γ and/or neurotrophic factors stimulate neural precursor cell proliferation and differentiation.
aspects that may be crucial to achieve treatment efficacy in patients with AD.

Disclosures
The authors declare that they have no conflict of interests.

References
12 Meyer EP, Ullmann-Schuler A, Staufenbiel M, Krucker T. Altered morphology and 3D aspects that may be crucial to achieve treatment efficacy in patients with AD.

Immunotherapy of Alzheimer’s disease

2013 John Wiley & Sons Ltd, Immunology
A. Monsonego et al.


Immunotherapy of Alzheimer’s disease

113 Kutszer MA, Cao C, Bai Y et al. Mapping of immune responses following wild-type and mutant Aβ42 plasmid or peptide vaccination in different mouse haplotypes and HLA Class II transgenic mice. Vaccine 2006; 24:4630–9.

© 2013 John Wiley & Sons Ltd, Immunology