Dynamics in the cytokine profile of beta-amyloid-specific human T cells

DISSERTATION

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABBREVIATIONS</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Hypothesis</td>
<td>12</td>
</tr>
</tbody>
</table>

## MATATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISPOT assays</td>
<td>15</td>
</tr>
<tr>
<td>Cell Separation</td>
<td>16</td>
</tr>
<tr>
<td>Elisa assays</td>
<td>17</td>
</tr>
<tr>
<td>Statistics</td>
<td>18</td>
</tr>
</tbody>
</table>

## RESULTS

| T cells of young individuals produce IFN-γ in response to Aβ1-42 | 19 |
| The endogenously primed Aβ1-42-specific T cell response in young individuals is Th1 polarized | 22 |
| Aβ1-42 induces IL-1β and IL-6 in cells of the innate immune system | 24 |
| In ageing individuals, Aβ1-42-specific T cells display decreased Th1 cytokine production combined with regulatory IL-10 production | 26 |
| In Alzheimer patients and in individuals with Trisomy 21, Aβ1-42-specific T cells exclusively produce regulatory IL-10 | 27 |
| Total conversion to regulatory IL-10 production does not occur in the immune response of elderly to two control antigens, mumps and tetanus toxoid | 29 |
| The Aβ1-42 specific IL-10 production in Alzheimer patients is CD4+ T cell derived | 32 |

## DISCUSSION

| Dynamics in the Aβ1-42-specific immune response in humans | 34 |
| Impact of the Aβ1-42-specific immune response on the disease course | 38 |
| The Aβ1-42-specific immune response in the light of autoimmunity | 38 |
| The outcome of the human Aβ1-42 vaccination trial | 40 |
4.5 The importance of inflammation and innate immunity for the pathogenesis of Alzheimer’s disease ............................................................... 43
4.6 \( \textit{A} \beta_1-42 \) as an intrinsic adjuvant ................................................................. 45
4.7 Mechanisms of beneficial autoimmunity in Alzheimer’s disease ................. 49
4.8 Regulatory functions in the immune response to \( \textit{A} \beta_1-42 \) .......................... 63
4.9 Site-specificity of the \( \textit{A} \beta_1-42 \) specific immune response ....................... 67
4.10 The impact of immune ageing on the response to \( \textit{A} \beta_1-42 \) ....................... 69
4.11 Conclusional remarks .................................................................................. 72

5 SUMMARY 79

6 ZUSAMMENFASSUNG 81

7 REFERENCES 83

8 ACKNOWLEDGEMENTS 108

9 CURRICULUM VITAE 109
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ&lt;sub&gt;1-42&lt;/sub&gt;</td>
<td>Beta-Amyloid 1-42</td>
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<tr>
<td>ABRA</td>
<td>Aβ-related Angiitis</td>
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<tr>
<td>APC</td>
<td>Antigen Presenting Cell</td>
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<td>APP</td>
<td>Amyloid Precursor Protein</td>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumine</td>
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<tr>
<td>CAA</td>
<td>Cerebral Amyloid Angiopathy</td>
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<tr>
<td>CD</td>
<td>Cluster of Differentiation</td>
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<tr>
<td>CFA</td>
<td>Complete Freud’s Adjuvant</td>
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<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CpG</td>
<td>Cytidine-phosphate-Guanosine</td>
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<tr>
<td>CSF</td>
<td>Cerebro-spinal Fluid</td>
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<tr>
<td>EAE</td>
<td>Experimental Allergic Encephalomyelitis</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
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<td>ELISPOT</td>
<td>Enzyme-linked Immuno Spot</td>
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<tr>
<td>FACS</td>
<td>Flow Cytometric Analysis</td>
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<tr>
<td>IFA</td>
<td>Incomplete Freud’s Adjuvant</td>
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<td>IFN-γ</td>
<td>Interferon Gamma</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>i.c.</td>
<td>intracutaneous</td>
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<td>i.p.</td>
<td>intraperitoneal</td>
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<td>i.v.</td>
<td>intravenous</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MAC</td>
<td>Membrane Attack Complex</td>
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<td>MBP</td>
<td>Myelin Basic Protein</td>
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<td>MMSE</td>
<td>Mini Mental State Exam</td>
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<td>MS</td>
<td>Multiple Sclerosis</td>
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<td>NK cells</td>
<td>Natural Killer Cells</td>
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<tr>
<td>PAMP</td>
<td>Pathogen-associated Molecular Pattern</td>
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<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cells</td>
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<td>Abbreviation</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<td>PLP</td>
<td>Proteolipid Protein</td>
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<tr>
<td>PTX</td>
<td>Pertussis Toxin</td>
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<tr>
<td>QS&lt;sub&gt;21&lt;/sub&gt;</td>
<td>Quillaja Saponaria 21</td>
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<tr>
<td>sAP</td>
<td>Serum Amyloid Protein</td>
</tr>
<tr>
<td>TCR</td>
<td>T Cell Receptor</td>
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<tr>
<td>Th cell</td>
<td>T Helper Cell</td>
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<tr>
<td>TGF-β</td>
<td>Tumor Growth Factor Beta</td>
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<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
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<tr>
<td>TNF-α/β</td>
<td>Tumor Necrosis Factor Alpha/Beta</td>
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<td>Tr-1 cells</td>
<td>T regulatory 1 cells</td>
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<td>TT</td>
<td>Tetanus Toxoid</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

“As she was unable to understand any particular situation, she got upset any time a doctor wanted to examine her. Only after several efforts it was possible to obtain any data. She suffered from serious perception disorders. When the doctor showed her some objects she first gave the right name for each one, but immediately afterwards she had already forgotten everything. … She evidently did not understand many questions. She did not remember the use of particular objects. …

After four and a half years of illness the patient died. She was completely apathetic in the end, and was confined to bed in a fetal position (with legs drawn up), was incontinent and in spite of all the care and attention given to her she suffered from decubitus.” (Alzheimer A, 1907)

This first case study of a 51-year-old female patient with Alzheimer’s disease, presented by Alois Alzheimer himself in 1907, is a striking example of the tragic consequences of the disease for the affected patients. Not only is Alzheimer’s a fatal disease, but it also imposes years of declining cognitive capacities, distorted personality traits and progressive dependency on nursing care on the patients. In being the first to link the psychoorganic symptoms of the disease with the typical histological findings, Alzheimer was able to establish that Alzheimer’s disease is a pathology distinct from other age-related psychiatric disorders.

In the meantime, the disease represents a difficult challenge to modern health care. Since the risk of getting the disease increases sharply with age, modern health care systems face a growing number of patients who cannot benefit from their increased life expectancy because of loosing mental and cognitive capacities that are the presupposition to maintain a high quality of life.

Today, in Germany 7,2% of the population above 65 years of age suffers from age-related dementia, a rate that increases to 34% for the population above 90 years of age [16]. In line with the changing demographics, the total number of
patients with Alzheimer’s disease in Germany is expected to double until the year 2050 [16].

Although extensive efforts are made to find treatment options for the disease, treatment methods so far available can merely improve the symptomatic suffering of the patients and are of very limited, if any, impact on the disease progression. Therefore, all emerging new treatment concepts for this disease are met by a high level of attention from both the scientific community and the public. This also holds true for the concept of active vaccination in Alzheimer’s disease, which has emerged since the late 90s as a treatment option that might have the potential not only to prevent the onset of the disease but even to cure it [130].

One of the most prominent histological hallmarks of Alzheimer’s disease is the accumulation of beta-amyloid-containing plaques and associated neural degeneration in the central nervous system [21]. Beta-amyloid 1-42 (Aβ1-42) as one main component of these plaques is a cleaving product of the intrinsically produced beta-amyloid-precursor protein (APP). While Aβ1-42 is constitutively produced in considerable quantities throughout life, its aggregation in the CNS is favored by overproduction and length of exposure [18]. Thus, mice with a transgenic and humans with an inherited overexpression of this protein show early plaque formation and pathology typical of Alzheimer’s disease [18, 67]. The transgenic mice either carry human mutations of the APP gene and/or mutations of the presinilin 1 and 2 locations, encoding one of the APP cleaving enzymes. Both these different transgenic mutations result in simple overproduction of Aβ1-42, and the clinical presentation of these animals shows that this overproduction is sufficient to cause the onset of Alzheimer-like pathology.

Since APP is encoded on chromosome 21, individuals with Trisomy 21 also overproduce APP and are prone to develop early onset Alzheimer’s disease at ages where the disease rarely occurs in the normal population [70].

In addition to overproduction, the biodegradation rate of Aβ1-42 is likely to influence its tendency to cause pathology-inducing accumulations. A key mechanism of Aβ1-42 biodegradation is thought to be the uptake or phagocytosis by cells in the brain.
that are responsible for tissue- and metabolic homeostasis, such as microglia or astrocytes [14, 158, 22].

One of the core functions of antibodies of different isotype classes and binding specificities/densities is the facilitation of phagocytosis by opsonization, resulting in the increased clearance of the targeted structure from the organism. Most healthy individuals have been shown to harbor spontaneous titers of anti-Aβ1-42 antibodies, but their role in Aβ1-42 turnover in humans is unknown [40, 52, 162, 161]. It is also unclear whether such “natural” anti-Aβ1-42 antibodies arise by random cross-reactive environmental stimulation or as a consequence of a spontaneous autoimmune response to Aβ1-42.

However, immunization studies of Alzheimer-prone transgenic mice with Aβ1-42 have been able to demonstrate how dramatically Aβ1-42–specific antibodies as part of an adaptive immune response can alter the dynamics of Aβ1-42 metabolism that otherwise results in the onset of the disease in these animals. Antibodies induced by active vaccination have been shown to prevent both plaque formation and Alzheimer-like pathology in transgenic mice [2, 14, 73, 125, 130]. Upon successful initiation of an adaptive immune response against Aβ1-42, plaques in the brains of vaccinated mice decreased significantly or disappeared entirely, depending on the vaccine composition and time of vaccination during the disease course [130, 6, 48, 73, 80, 163, 85]. More importantly, vaccinated animals have also been shown to be spared from the clinical manifestations of the disease, being able to maintain their cognitive capacities that non-vaccinated animals loose as their plaque deposition progresses [5, 73, 109, 110, 22].

In contrast to these clear results from transgenic animals, very little information is available on the impact of spontaneous anti-Aβ1-42 antibodies (and of a possibly accompanying Aβ1-42–specific T cell response) on Aβ1-42 homeostasis in humans. Only one recent study, using polyclonal immunoglobulins containing anti-Aβ1-42 antibodies as a treatment for Alzheimer patients, was able to show that spontaneously occurring antibodies in humans have an impact on Aβ1-42
homeostasis in the means of altering the CSF/plasma Aβ1-42 ratio and result in the clinical stabilization of treated patients [39].

It remains a matter of debate whether spontaneous autoimmune responses can constitutively contribute to tissue homeostasis in general. Originally, it has been postulated by Grabar in the 50s that autoantibodies may have the function of helping the clearance of unwanted cellular material and debris (“Grabar antibodies”) [56, 149]. However, it has remained very challenging to find clinical examples of the physiologic importance of such protective autoimmune antibody production. In the light of the extensive number of sometimes devastating autoimmune diseases the concept of protective autoimmune responses has remained marginal to the understanding of the meaning of autoimmunity. Autoimmune activity is commonly understood to be a potentially hazardous byproduct of the much needed multispecific immune repertoire in antipathogenic host defense. This autoimmune reactivity needs to be effectively controlled in order to avoid the onset of autoimmune pathology [86, 90, 103]. Especially in the case of an ubiquitously expressed autoantigen such as Aβ1-42, tolerance induction could be expected to take place by inactivating T cells (and to a lesser extent B cells) that are specific for the autoantigen.

Autoreactive T cells are orders of magnitude more sensitive to the induction of tolerance than autoreactive B cells, therefore representing a key factor in the establishment of self-tolerance [105]. In order to better understand why in the case of Aβ1-42 an autoimmune antibody production can be detected in many individuals, the understanding of the nature of a potentially accompanying T cell response appears to be crucial. T cells with high affinity for an autoantigen are eliminated in the thymus in a process called negative selection; in contrast, low affinity autoreactive T cells can escape thymic selection [20]. Such T cells are of naïve phenotype, apparently “ignorant” of the endogenous autoantigen. These dormant autoimmune T cells carry the potential to initiate an autoimmune response, but in most cases these T cells need to be activated by experimental immunization or by a crossreactive infection in order to become memory/effector
cells [148, 29]. Only then these differentiated T cells can mediate effector functions such as the production of cytokines that stimulate the phagocytotic activity of macrophages or promote the production of antibodies by antigen-specific B cells [127].

One of the main reasons why autoreactive T cells stay ignorant to their autoantigen is the fact that most autoantigens are presented in the body in the absence of so called “danger” signals. Such signals, called pathogen-associated molecular patterns (PAMPs), are primarily of microbial origin and stimulate Toll-like receptors (TLRs) on antigen presenting cells (APCs) [50, 118, 31, 89]. Normally, an immune response is only initiated if antigen presentation occurs in the context of simultaneous TLR engagement. Thus, the additional requirement of the recognition of PAMPs for the priming of an adaptive immune response can be understood as an additional safety check in order to ensure that potentially hazardous immune effector mechanisms such as respiratory burst can only be activated if there really is a pathogenic “non-self” infection [29].

With Aβ1-42 being an ubiquitous autoantigen, and expressed in the assumed absence of such “danger” signals, it remains to be understood why it does not behave like most other autoantigens. The continuous presentation of autoantigens normally induces profound T cell tolerance and not effector mechanisms such as the above-mentioned intrinsic antibody production [69, 121].

Besides this, another aspect stays in conflict with the assumed interactions between Aβ1-42 and the adaptive immune system, as well as with the beneficial effects that have been illustrated by the vaccination studies with transgenic animals. Adaptive immune responses are target-specific, and an important feature of this target-specificity is the requirement of physical contact of the involved cells with the antigenic structures of the target. Thus, in almost all examples of relevant adaptive immune responses, a direct presence of T and B cells and/or antibodies in the target structure can be put into evidence.
This also holds true for autoimmune responses that attain clinical relevance with regard to their disease-mediating properties, such as in multiple sclerosis or lupus erythematoses \[83\]. In these cases, cells or products like target-specific antibodies can be found with such regularity in the attacked organic structures that the impact of the adaptive response is unquestionable. In the case of lupus erythematoses the intradermal deposition of antinuclear antibodies is so characteristic that it is regarded as a diagnostic feature with pathognomic importance \[9\].

Not so in Alzheimer’s disease. In sharp contrast to the above mentioned examples of clinically significant autoimmune responses, in the Alzheimer inflicted brain no or only very little remarkable presence of activated B or T cells or antibodies can be found \[14, 160, 46\]. Therefore, before the striking results of the vaccination studies in transgenic mice became known, no impact was assumed for the adaptive immune system on the pathologic process in the brain. Even if the clinical outcome of $A\beta_{1-42}$ vaccination is very impressive and humoral mechanisms seem to have a key function, it is still a matter of debate how the initiated autoimmune response exerts its beneficial effect on $A\beta_{1-42}$ turnover. In order of this response to show its repetitively verified beneficial clinical impact, neither a marked local intracerebral T or B cell infiltration nor antibody deposition seems to be a mandatory presupposition \[35, 14, 157\].

Adaptive immune responses, that mediate antigen-specific host defense or are responsible for autoimmune diseases, are unique to the mammalian immune system. In addition to that a more simple, unspecific defense system exists, called innate immune system, whose components have been phylogenetically preserved much longer than those of the adaptive immune system \[46\]. However, this more archaic innate system is of importance for the host defense in all complex organisms, regardless of the existence of an additional adaptive immune system. One of the central defense mechanisms of innate immune reactions is the mediation of inflammation, basically creating an environment which is hostile to the survival of intruding pathogens \[11, 43, 82, 93\]. In addition of this, inflammation and other mechanisms of innate immunity have been shown to be of central
importance for many chronic degenerative diseases, including such an ubiquitous process as atherosclerosis [30, 161].

While it remains challenging to understand the impact of the Aβ1-42 specific adaptive immune response on the disease course, the central role of chronic inflammatory mechanisms and cells of the innate immune system is much more evident.

At the sites of so called dense plaque formation an associated inflammatory response can always be observed. In the Alzheimer brain, and in transgenic mice, microglia (representing cells of the innate branch of the immune system) can be found in the proximity of Alzheimer plaques and are in an activated state [42, 55, 91, 95]. Moreover, it has been shown in vitro that Aβ1-42 alone leads to the activation of microglia resulting in the secretion of proinflammatory cytokines such as IL-1β and IL-6 [75, 96, 100, 28]. A participation of almost all other effector mechanisms of innate immune responses could be put into evidence in the surroundings of amyloid plaques, such as complement activation, release of active oxygen derivates, production of prostaglandines and the local upregulation of acute phase proteins such as CRP or sAP [93].

Inflammation can be understood as an organism’s general response to injury. As such, an association of inflammatory mechanisms with a degenerative disease like Alzheimer’s appears not unusual but as something that could be expected. However, as mentioned above, the high level of inflammation found in the case of Alzheimer’s disease seems not only to be a mere response to the injury caused by the accumulation of Aβ1-42 and the subsequent neurodegeneration, but it seems to be induced by Aβ1-42 itself. Just as the deposition of Aβ1-42–containing plaques is the morphological hallmark of Alzheimer’s disease, the involvement of inflammation and innate immune effector mechanisms can be understood to be a pathophysiological hallmark of the disease.

In situations where both inflammation and the activation of the innate immune system become chronic, the process of amyloidosis can be associated [46].
Amyloidosis is caused by the accumulation of oligomeric peptides in lamellar cross-beta-sheet structure. Differences in amyloidogenicity of such peptides can arise from genetic polymorphisms, from alterations in their production, metabolic turnover or conformation, or from their involvement in chronic inflammatory processes [25, 66]. Alzheimer’s, in addition to being an inflammatory pathology, can be viewed as such an amyloidotic disease, since the histological accumulation of Aβ1-42-containing plaques closely resembles the one in other amyloidotic diseases in the periphery [46, 81]. In line with this, as mentioned, other typical components of classic amyloidotic depositions, such as CRP and sAP, can be found in Alzheimer plaques [93].

Taking into consideration all the recently available information on the impact of the immune system on Alzheimer’s disease, the disease process seems to interact at two different points with the immune system. The much more imminent point of reciprocal effects is the pathological process in the brain, where the involved chronic inflammatory innate immune mechanisms contribute to disease progression and neural degeneration [93]. A second, apparently distinct point of interaction appears to be the initiation of an adaptive immune response, that in contrast to the innate inflammatory immune mechanisms in the CNS can favor disease protection by the facilitation of Aβ1-42 clearance as shown in vaccinated transgenic animals [73, 109, 130].

At a first glance, no interaction between the intrinsic adaptive immune activity and the pathology-related inflammatory mechanisms in the brain seems to take place. As mentioned, although a constant adaptive immune activity in the periphery can be shown in the means of antibody production in humans [40, 161, 162], no or only very few cells or components of the adaptive immune system can be found at the sites of CNS plaque deposition [14, 160, 46].

However, the unfortunate outcome of a first human vaccination trial with Aβ1-42 in combination with a strong Th1 adjuvant (QS21) points into a different direction. The trial had to be halted in early 2001 because of the onset of
meningoencephalitis in as many as roughly 6% of the vaccinated patients [44, 108, 116, 128, 160]. Even if before this trial no such side effects were observed in vaccinated transgenic mice, in patients this complication seemed to be the consequence of the onset of an adaptive autoimmune T cell response, since the clinical presentation of meningoencephalitis corresponded to a local infiltration by activated CD4+ T cells [44, 114].

Thus, the notion that the Aβ1-42–specific immune response in the periphery and the inflammatory innate immune response are two distinct and largely unrelated entities seems to be an oversimplified one. The onset of autoimmune encephalitis is a hint for the fact that Aβ1-42–specific cells, depending on their activational state, have access to the brain, can express effector functions, and potentially influence the pathological process beyond the simple provision of Aβ1-42–specific antibodies. In the light of the obvious capacity of Aβ1-42–specific cells to access the brain it has to be expected that there is a potential interaction with the local intracerebral presentation of Aβ1-42 and the inflammatory environment in which this presentation occurs, even without additional vaccination or accompanying clinical signs of autoimmunity.

Another hint for a constant interaction of the Aβ1-42–specific adaptive immune response and the changing dynamics of Aβ1-42 presentation comes from the largely varying results from the murine vaccination studies, and from the comparison of the response in wildtype and transgenic, and in young and old transgenic animals [32, 37, 105, 122, 140, 151, 163]. Generally speaking, if there was no constant interaction with the changing dynamics in the presentation of this autoantigen before vaccination, no variations at this scale should appear in the observed post-vaccinational responses.

The existing differences point out that due to the constant interaction with the autoantigen Aβ1-42, the Aβ1-42–specific cell pools must have been altered before vaccination in a way that they harbor different capacities to mount a response.

Little information is available on the level of interaction that occurs between an Aβ1-42–specific adaptive immune response in the periphery and the local
pathological mechanisms in the brain. Information is also scarce on what could be the basis of the intrinsic adaptive $\text{A\beta}_{1-42}$–specific immunity in humans and the observed alterations to which the $\text{A\beta}_{1-42}$–specific response is object both in mice and humans.

In the light of the potentially harmful effects of an $\text{A\beta}_{1-42}$–specific autoimmune activity and the varying beneficial effects of animal vaccination it seems to be crucial to further understand the underlying dynamics of this response. The use of a vaccination in the context of a probably preexisting adaptive immune response to $\text{A\beta}_{1-42}$ means that a preexisting balance between this activity and $\text{A\beta}_{1-42}$ as the target is altered. This can result both in protection from the disease (as shown in murine studies), but also in autoimmune aggravation for the vaccinated individuals (as happened in the human vaccination trial and later has been reproduced in a murine study) [49].

It only seems appropriate to further follow the strategy of influencing this balance by active vaccination if the impact of the $\text{A\beta}_{1-42}$–specific adaptive immune response on the disease course is understood much more precisely.

T helper cells, by the secretion of a characteristic cytokine profile, set the direction in which an adaptive immune response evolves [166, 34]. The two well-established distinct T helper cell subsets, Th1 and Th2 cells, the first characterized by the production of IFN-$\gamma$, IL-2, and IL-12, the second by the production of IL-4, IL-5 and IL-13, can be solely responsible for the appropriateness and outcome of an adaptive immune response. This is illustrated by the fact that immune deviation from a Th1 to a Th2 profile can be sufficient to halt autoimmune pathology (as already clinically exploited in the case of multiple sclerosis) [99, 111]. At the same time, the successful clearing of an infection, as extensively studied for the case of murine leishmania infection, is equally dependant on the orientation of the involved specific T helper cell population [59].

In addition to above cited immune deviation, the induction of peripheral tolerance is another important mechanism to be prevent autoimmune disease. As in the
case of the overall effector functions of an adaptive immune response, T cells play a prominent role in the mechanisms that help to maintain autoimmune tolerance. As mentioned, CD4+ Th cells are known to be the cell population in adaptive immunity that is the most sensitive to the induction of peripheral tolerance [105]. Since without T helper functions priming and maintenance of effector functions of an adaptive immune response is impaired, this high T cell sensitivity for the induction of tolerance has a limiting effect on the initiation or maintenance of any antigen-specific immune response [64]. But in addition to that, T helper cells are not only known to be the object of tolerance induction but also to harbor a subpopulation of regulatory T cells that is not silenced but rather actively involved in the maintenance of peripheral tolerance and in controlling the scale of adaptive immune responses [27, 57, 115, 141, 156]. Therefore, in order to better understand the effector profile of the adaptive immune response to Aβ1-42, the knowledge of the impact of the antigen-specific T cells appears to be essential.
1.1 Hypothesis

The aim of this study was to examine whether there are $\text{A}\beta_{1-42}$–specific T cells in humans and what their cytokine signature consists of.

In using ELISPOT assays we analyzed T cells derived from human PBMCs for the $\text{A}\beta_{1-42}$–specific secretion of an array of model Th1 (IFN-$\gamma$ and IL-2) and Th2 (IL-4 and IL-5) cytokines.

We also were interested whether regulatory T cell functions might play a role in the alterations of the autoimmune response to $\text{A}\beta_{1-42}$, as it is suggested by the varying levels of antibody titers in humans of different ages or the varying outcomes of animal vaccination.

We therefore tested for the $\text{A}\beta_{1-42}$–specific secretion of IL-10, a cytokine known to be central to the in vivo function of different regulatory T cell populations.

Furthermore we were interested whether the effector mechanisms of $\text{A}\beta_{1-42}$-specific T cells undergo similar changes as have been described for $\text{A}\beta_{1-42}$-specific antibody production. Therefore, we compared the cytokine profiles of different age groups (20-30, 40-50 and > 60 years) with a group of patients with possible or probable Alzheimer’s disease.

It is important to understand whether the observed changes in the $\text{A}\beta_{1-42}$-specific adaptive immune response are a distinct characteristic of this immune response or whether they rather are an expression of the overall alteration of adaptive immune responses with age. To address this, we also tested for two model non-self antigens, mumps and tetanus toxoid, to see whether other antigen-specific responses undergo the same alterations at the response to $\text{A}\beta_{1-42}$.

To further understand the mechanisms that underlie the dynamics of this antigen-specific response, we also tested the adaptive immune response in a group of individuals with Trisomy 21. This group was studied, because APP is encoded on chromosome 21, and individuals with Trisomy 21 reveal an overproduction of $\text{A}\beta_{1}$. 
resulting in the early onset of Alzheimer's disease at ages where the disease rarely occurs in the normal population [70].

Last, we were interested in the mechanisms that could be the basis for the regular priming of an adaptive immune response against the autoantigen Aβ_{1-42}. Considering the fact that Aβ_{1-42} deposition in the brain results in local inflammation, we were interested in whether the upregulation of inflammatory cytokines could also be mediated by Aβ_{1-42}-challenged antigen-presenting cells (APCs) derived from PBMC. Such upregulation could in turn provide the necessary "danger" signal for antigen presentation of Aβ_{1-42} in the periphery that results in the priming of an adaptive immune response.
2 MATERIALS AND METHODS

2.1 Study subjects

Participating volunteers were recruited through the Research Registry of the Center of Memory and Ageing of University Hospitals of Cleveland in Cleveland, OH. All patients (mean age 73±8.53, n=19, 9 female, 10 male) fulfilled the criteria of either possible (n=9) or probable (n=10) Alzheimer’s disease. The mini mental status exam score of the participating patients ranged between 6 and 23 (mean 16±6.31). None of the patients was receiving steroids, 6 of the patients were under non-steroidal anti-inflammatory medication.

Old healthy control individuals (mean age 67.43±2.51, n=7, 4 female, 3 male) were either recruited through the center of Memory and Ageing of University Hospitals of Cleveland, often being the spouses of the participating patients, or through the Golden Age Group, Cleveland, OH, a group of retired persons volunteering in social work. Three of the participating old healthy control donors were under non-steroidal anti-inflammatory medication.

Middle aged individuals (mean age 49.56 ± 3.28, n = 9, 5 female, 4 male) and young healthy individuals (mean age 24.65 ± 2.60, n = 17, 7 female, 10 male) were both recruited on-site at the Biomedical Research Building of Case Western Reserve University, Cleveland, OH. While none of the young healthy individuals was under non-steroidal anti-inflammatory medication, 2 of the donors of the middle-aged groups were under such medication.

Individuals with Trisomy 21 (mean age 32.17 ± 13.32, n = 6, 3 female, 3 male) were contacted through the practice of Charles Tyler, MD, Cleveland, OH. Human studies were performed with the approval of the Institutional Review Board of Human Studies at the University Hospitals of Cleveland.
2.2 ELISPOT assays

ELISPOT assays were performed as previously described [97]. Briefly, PBMCs from the donors were purified from venous blood samples collected in heparinized 10 cc blood tubes by centrifugation on Isoprep density gradients (Robbins Scientific, Sunnyvale, CA).

Small-volume ELISPOT plates (BioWhittaker, Walkersville, MA) were coated overnight at 4°C with 50 μl capture antibody in PBS: IFN-γ M700A-E (2 μg/ml; Endogen, Woburn, MA), IL-2 5334.21 (8 μg/ml; R&D Systems, Minneapolis, MN), IL-4 8D4-8 (2 μg/ml, PharMingen, San Diego, CA), IL-5 TRFK5 (3 μg/ml, PharMingen, San Diego, CA) and IL-10 JES39D7 (4 μg/ml, PharMingen, San Diego, CA) respectively. Plates were then blocked with bovine serum albumin (10 g/l in PBS) for 60 minutes and subsequently washed three times with PBS. PBMCs were plated in complete RPMI medium (94% RPMI, 5% ABO, 1% L-glutamine) at 75x10^5 cells per well (unless specified differently in the Figure legends). RPMI used was from BioWhittaker, Walkersville, MA and human AB serum from Gemini Bioproducts, Calmasas, CA, which was heat inactivated at 56°C for 30 minutes before usage. The following antigens or mitogens were added in the indicated concentrations (unless specified differently): Human beta-amyloid peptide 1-42 (25 μg/ml; California Peptide, San Diego, CA), Mumps antigen (final dilution 1:16; BioWhittaker, Walkersville, MA), Tetanus toxoid (final dilution 1:120; Accurate Chemical and Scientific, Westbury, NY), and Phytohemaglutinin (10 μg/ml; Sigma, St. Louis, MO). The cells were cultured for 24 hours in an incubator at 37 °C in the case of IFN-γ, IL-2 and IL-10 assays and for 48 hours in the case of IL-4 and IL-5 assays, unless specified differently under Results or in the Figure Legends. After incubation plates were washed three times with PBS, three times with PBS-TWEEN (0.5%), and 50 μl/well of secondary biotinylated detection antibodies were added diluted in PBS-BSA-TWEEN to the subsequent concentrations: IFN-γ B 133.5 (2 μg/ml; Endogen, Woburn, MA), IL-2 BG5 (0.06 μg/ml; Endogen, Woburn, MA), IL-4 8D4-8 (2 μg/ml, PharMingen, San Diego, CA), IL-5 JES1-5A10 (2 μg/ml, PharMingen, San Diego, CA) and IL-10 JES3-12G8 (2 μg/ml, PharMingen, San Diego, CA) respectively. After overnight incubation at 4
\(^{\circ}\text{C}\) plates were again washed three times with PBS-Tween and another incubation period of 120 minutes followed the addition of 50\(\mu\text{l/well}\) of Streptavidin-AP (Dako, Carpentia, CA) at 1:1000. Development solution (BCIP/NBT Phosphatase Substrate, KPL, Gaithersburg, MA) was used, and the reaction was stopped after spots became visible. The plates were air-dried overnight before subjecting them to image analysis on a Series 3 ImmunoSpot Image Analyzer (Cellular Technology, Cleveland, OH).

ELISPOT assays have two advantages in studying low-frequency antigen-specific T cell effector functions as they are characteristic to autoimmune responses. First of all, T cells are tested directly ex vivo without further in vitro culturing. Therefore, an alteration of effector functions, as it has been shown to occur during in vitro culturing of T cell populations, is avoided, allowing a very direct assessment of the cytokine profile displayed in vivo [97]. Secondly, specific autoreactive T cell populations often only occur in very low frequencies. ELISPOT assays are very helpful in overcoming this limitation since they have a sensitivity of as high as 3x10\(^{-5}\) cells [97].

### 2.3 Cell Separation

Cell samples for assays working with purified populations of CD3\(^{-}\), CD4\(^{+}\), CD8\(^{+}\) and B\(^{+}\) cells were obtained by using cell depletion cocktails (RosetteSep; Stemcell Technologies, Vancouver, BC, Canada). The efficacy of enrichment was controlled by FACS analysis. Flow cytometry was performed as previously described [65] using a Becton Dickinson FACScan, staining with labeled anti-CD4\(^{+}\), CD8\(^{+}\), and CD3\(^{+}\) antibodies (all from PharMingen, San Diego, CA). 5000-10000 live cells were analyzed per sample. The enrichment for the desired phenotypes was between 87\% and 94\%.

ELISPOT assays testing for IFN-\(\gamma\) or IL-5 secretion on purified T cell populations were performed using 35x10\(^{5}\) CD4\(^{+}\) or CD8\(^{+}\) cells with 35x10\(^{5}\) of T cell depleted
PBMCs as APCs per well. Testing T cell specific IL-10 production we used $25 \times 10^5$ CD4$^+$ or CD8$^+$ cells with $10 \times 10^6$ purified B cells as APCs per well [58].

2.4 Elisa assays

ELISA assays were performed as previously described [166]. We pulsed PBMCs or T cell depleted PBMCs in standard flat bottom 96 well plates at 37°C for 24h with the antigens and mitogens diluted in complete RPMI (94% RPMI, 5% ABO, 1% L-glutamine), after which 150 µl of supernatants were harvested. ImmunoMaxisorb 96 well microtiter plates (Nalge-Nunc International, Denmark) were precoated overnight at 4°C with IL-1β ILB1-H67 (1.5 µg/ml; Endogen, Woburn, MA) or IL-6 MP5-20F3 (1.5 µg/ml; Endogen, Woburn, MA) in 0.1 M bicarbonate buffer. The plates were then blocked by adding gelatin working solution (0.1 % gelatin in PBS/Tween). 50µl of supernatants was added to the precoated ELISA plates containing 50 µl of gelatin working solution for a minimum of 16 h at 4°C. As a standard, serial dilutions of recombinant IL-6 RIL650 (2.5 ng to 27 pg in increments of 8; Endogen, Woburn, MA) were added in gelatin working solution. Plates were incubated overnight and washed 3 times with PBS/Tween and biotinylated secondary antibodies IL-1β ILB1-H6 (2 µg/ml; Endogen, Woburn, MA) or IL-6 M621B (1 µg/ml; Endogen, Woburn, MA) were added in gelatin working solution. The plates were incubated at room temperature for 4 h and washed 3 times with PBS/Tween; then, 100 µl of alkaline-phosphatase-conjugated streptavidin (Dako, Denmark) diluted 1:2000 in gelatin working solution were added. Plates were incubated for 2 h at room temperature, washed 3 times with PBS/Tween and 4 times with final washing medium (50 mM Tris base, 67 mM NaCl, 3 mM NaN₃) before adding the substrate solution (1.6 mg/ml of p-Nitrophenyl phosphate di-sodium salt x 6H₂O, Research Organics, Cleveland, OH) in 500 mM NaHCO₃, 137.5 mM MgCl₂ x 6H₂O, 3 mM NaN₃, (pH 10.3). The reaction products were quantified by measuring absorbance at 405 nm using an ELISA plate reader.
2.5 Statistics

Statistical analysis was conducted using SigmaStat 7.0 (SPSS, Chicago, IL). Statistical significance was defined as $p < 0.05$, and differences with a $p$-value of $< 0.01$ were defined as highly significant. Significance of differences between different study groups was assessed using the paired t-test or the Mann-Whitney rank-sum test.
3 RESULTS

3.1 T cells of young individuals produce IFN-γ in response to Aβ1-42

PBMCs were isolated from peripheral venous blood samples from 17 young healthy individuals, 20-30 years of age (mean age 24.65 ± 2.60, n = 17, 7 female, 10 male). The PBMCs were tested for Aβ1-42-induced production of IFN-γ in a 24h ex vivo ELISPOT assay. Under these conditions, only T cells that have already differentiated in vivo into a memory state can produce IFN-γ; naïve T cells require more than 72 h of antigen-driven differentiation before they achieve the ability to secrete this cytokine. Therefore, this test system measures for in vivo primed antigen-specific T cell memory. 14 of the 17 tested subjects displayed a highly significant increase (p = 0.003) of IFN-γ spots in the Aβ1-42 stimulated cultures relative to the medium control wells (Fig. 1A). This induction of IFN-γ was not seen with two neural control self antigens, myelin basic protein (MBP) and proteolipid protein (PLP) (Fig. 1C). Both neuroantigens are major constituents of the myelin sheet, and immunization with either is suited to induce the autoimmune disease of experimental encephalomyelitis (EAE). This means that both MBP and PLP can be potent antigens when coinjected with an adjuvant providing an appropriate “danger” signal. However, confirming previous observation, our testing for IFN-γ did not reveal evidence for spontaneous in vivo priming of MBP-PLP-specific T effector/memory cells (p = 0.522), in contrast to Aβ1-42.

The data in Fig. 1A and 1C were obtained testing unseparated PBMCs. While peptide-induced production of IFN-γ is a strong indicator for the presence of a T cell memory response, cells of the innate immune system such as NK cells are also known to be capable of IFN-γ production. To address this, cell separation experiments were performed to define the cellular origin of the Aβ1-42-induced IFN-γ cytokine production. The experiments could not detect IFN-γ secretion in T cell (CD3)-depleted PBMCs, suggesting a T cell origin for the present IFN-γ secretion (p = 0.450, Fig 1B).
Beta-amyloid 1-42 (Aβ1-42)-specific interferon gamma (IFN-γ) producing T cells are present in young healthy subjects.

A, B, and C. Peripheral blood mononuclear cells (PBMCs) were collected from young healthy donors ranging from 20 to 30 years of age. The cells were stimulated with beta-amyloid 1-42 (Aβ1-42) before (A) and after (B) depletion of T cells (cluster of differentiation (CD) 3 positive cells). (C) PBMCs were stimulated with myelin basic protein – proteolipid protein fusion protein (MBP-PLP).

The number of interferon gamma (IFN-γ) spots was measured after a 24h ex vivo culture. For each subject, the spontaneous spot formation in the medium containing wells was compared with the numbers of spots in antigen containing wells; the two data points for each individual are connected with a line. Each data point represents the mean of quadruplicate wells. In 14 of the 17 tested young healthy individuals the increase in IFN-γ spots upon stimulation with Aβ1-42 was highly significant (p = 0.003, A) when compared to the medium wells. In contrast, no significant cytokine production was found upon the stimulation of PBMCs depleted of CD3+ cells (T cells) with Aβ1-42 (n = 7, p = 0.450, B) or of whole PBMCs with MBP-PLP (n = 7, p = 0.522, C).
To determine whether the CD4+ or CD8+ subpopulations of T cells produces the cytokine, purified CD4+/CD8+ cells from 4 young healthy donors (mean age 24.5±2.5, n = 4, 1 female, 3 male) were exposed to Aβ1-42 in the presence of purified T cell depleted PBMCs functioning as APCs. IFN-γ production was induced in both CD4+ and CD8+ cells in significant scale (p = 0.047, p = 0.043). The data establish that endogenously primed Aβ1-42-specific CD4+ and CD8+ cells are present in healthy young individuals. The presence of such T cells suggest that the Aβ1-42-specific autoantibodies producing B cells in young healthy individuals are backed by cognate T cell help, and are not the product of random crossreactive antigen encounters.

Figure 1.2
The beta-amyloid 1-42 (Aβ1-42)-specific interferon gamma (IFN-γ) production is both cluster of differentiation (CD) 4+ and CD8+ T cell derived.

A and B. Cluster of differentiation (CD) 4+ (C) and CD8+ (D) cells were isolated from peripheral blood mononuclear cells (PBMCs) of 4 random young donors, and T cells were plated with antigen presenting cells (APCs) (T cell depleted PBMC) in a 1:1 ratio, in the presence of beta-amyloid 1-42 (Aβ1,42). The patients chosen for this experiment were not pretested for their Aβ1-42-specific cytokine profile. An interferon gamma (IFN-γ) Enzyme-linked Immuno Spot (ELISPOT) assay of 24h duration was performed. For each subject, the spontaneous spot formation in the medium containing wells was compared with the numbers of spots in the Aβ1,42-containing wells; the two data points for each individual are connected with a line. Each data point represents the mean of quadruplicate wells. The induction of IFN-γ was found to be significant for both CD4+ (p = 0.047) and CD8+ (p = 0.043) cells.
3.2 The endogenously primed Aβ1-42-specific T cell response in young individuals is Th1 polarized

While Th1 polarized memory cells produce IFN-γ and IL-2 and Th2 polarized cells secrete IL-4 and IL-5, T memory cells can also produce an unpolarized mixed Th1/2 cytokine set. In contrast, an immunoregulatory subset of T cells (T reg) is characterized by the production of regulatory cytokines like IL-10. Therefore, the detection of IFN-γ alone is not sufficient to fully illustrate the effector functions of the endogenously primed Aβ1-42 specific T cells since IFN-γ production might occur as a component of unpolarized mixed immunity. To comprehensively characterize the cytokine polarization of the Aβ1-42-specific T cells, Aβ1-42-induced production of IL-2, IL-4, IL-5 and IL-10 in addition to IFN-γ was measured in all tested donors. In young healthy individuals, supplementary to the production of IFN-γ, IL-2, and to a lesser extent IL-5 production was detected in a highly significant manner (p < 0.01); In contrast, there was no significant production of IL-4 and IL-10 (p > 0.05). Therefore, the endogenously primed Aβ1-42-specific T cells were Th1 polarized with a minor Th2, and essentially no regulatory cytokine component. No significant cytokine secretion could be detected in PBMCs stimulated with the control autoantigen MBP-PLP (p > 0.05 for all cytokines).
Peripheral blood mononuclear cells (PBMCs) were collected from 17 young healthy donors ranging from 20 to 30 years of age. The cells were stimulated with beta-amyloid 1-42 (Aβ1-42) (hatched bars) or with myelin basic protein – proteolipid protein fusion protein (MBP-PLP) (solid bars) and the number of interferon gamma (IFN-γ), interleukin 2 (IL-2), IL-4, IL-5 and IL-10 spots was measured in a 24h ex vivo culture, except for IL-4 and IL-5 where 48 h cultures were performed to account for the delayed secretion kinetics. The mean and standard deviation of spot numbers induced in the 17 donors is represented for each cytokine. The scale for IL-10 has been adjusted to the subsequent figures for better comparability. In addition to the highly significant IFN-γ production, induction of IL-2, and to a lesser extend of IL-5, was found to be of high significance (p < 0.01) in response to stimulation with Aβ1-42; the production of both IL-4 and IL-10 did not reach significant levels (p > 0.05). No significant production of any of the tested cytokines was found upon the stimulation with MBP-PLP (p > 0.05). Significant levels of cytokine induction are marked with a star (*) in the above graph.
3.3 $\mathrm{A\beta_{1-42}}$ induces IL-1$\beta$ and IL-6 in cells of the innate immune system

The presence of an endogenously primed autoimmune Th1 response in young healthy individuals is an uncommon finding. This, along with the known activating properties of $\mathrm{A\beta_{1-42}}$ on microglia makes it possible that $\mathrm{A\beta_{1-42}}$ may also activate professional APCs of the innate immune system, providing an intrinsic danger signal for T cell priming. To test this hypothesis, it was examined whether $\mathrm{A\beta_{1-42}}$ would trigger the production of IL-6 and IL-1$\beta$ in T cell depleted PBMCs. $\mathrm{A\beta_{1-42}}$ induced IL-6 (Fig. 3A) and IL-1$\beta$ (Fig. 3B) in a dose dependent manner. Unlike MBP-PLP, $\mathrm{A\beta_{1-42}}$ induced a highly significant IL-1$\beta$ response ($p = 0.002$) over medium background, as well as an IL-6 response ($p = 0.014$). In contrast to MBP-PLP ($p = 0.427$; $p = 0.932$), $\mathrm{A\beta_{1-42}}$ therefore strongly activates cells of the innate immune system, explaining why only the latter autoantigen constitutively primes an autoimmune T cell response. Apparently, $\mathrm{A\beta_{1-42}}$ not only acts as an autoantigen, but also has intrinsic adjuvant properties, and therefore is able to provide “danger” signals to functional APCs.
Legend to Figure 3

PBMCs of healthy donors were depleted of CD3+ cells and exposed to Aβ1-42 and MBP-PLP in the concentrations specified. After 24h, the culture supernatants were collected and subject to ELISA analysis in the dilutions specified, measuring IL-1β (A) and IL-6 (B). Upon stimulation with 25 µg of Aβ1-42, the induction of IL-1β was highly significant (p = 0.002); The induction of IL-6 was weaker, but still highly significant (p = 0.014). In contrast, induction of neither IL-1β nor IL-6 was significant upon the stimulation with the equivalent amount of MBP-PLP (p = 0.427; p = 0.932).
3.4 In ageing individuals, Aβ₁₋₄₂-specific T cells display decreased Th1 cytokine production combined with regulatory IL-10 production

Autoantibody levels to Aβ₁₋₄₂ are known to be decreased in Alzheimer patients. Moreover, the induction of Aβ₁₋₄₂-specific antibodies by vaccination strategies is impaired in APP-transgenic and aged mice. Since the data presented here suggest that the Aβ₁₋₄₂-specific antibodies arise by cognate T cell help, the question arises whether this age- and disease-related decline in the ability to produce Aβ₁₋₄₂-specific antibodies results from a change in the Aβ₁₋₄₂-specific T cell compartment.

To test this hypothesis, the cytokine signature of Aβ₁₋₄₂-specific T cells was measured in individuals of age 45-55 (mean age 49.56 ± 3.28, n = 9, 5 female, 4 male), and above 65 (mean age 67.43±2.51, n = 7, 4 female, 3 male). Relative to young individuals (mean age 24.65 ± 2.60, n = 17, 7 female, 10 male) in whom the mean frequency of Aβ₁₋₄₂-induced IFN-γ spots was 63/3x10⁵ PBMCs (Fig. 2), the frequency of IFN-γ producers was reduced to 17/3x10⁵ and 19/3x10⁵ for the middle aged and the elderly, respectively. The reduction for both groups was statistically significant when compared with the young healthy group (p = 0.034; p = 0.039). For IL-2 (p = 0.061; p = 0.980), IL-4 (p = 0.517; p = 0.577) and IL-5 (p = 0.205; p = 0.081) production, no significant frequency changes were noted in middle aged and elderly individuals, respectively, when compared with the young healthy group. In contrast, however, the frequency of Aβ₁₋₄₂-induced IL-10 spots increased from 7/3x10⁵ PBMCs in young individuals to 36/3x10⁵ (p < 0.001) and 234/3x10⁵ cells (p < 0.001) in middle aged and elderly, respectively. The frequency increase of the Aβ₁₋₄₂-induced IL-10 producers from middle aged to elderly was also highly significant (p = 0.010). Therefore, we found evidence for a decrease in Th1 immunity and an increase of Aβ₁₋₄₂-specific regulatory IL-10 production with progression in age. Unlike Th1 cells that are helper cells for antibody production, T cells producing regulatory cytokines like IL-10 can act as “suppressor cells” for effector functions. The observed switch from a Th1 cytokine set to regulatory IL-10 production could explain the decreased propensity of elderly to display Aβ₁₋₄₂-specific effector mechanisms, such as the provision of
cognate T cell help for specific antibody production. These results are presented in figure 4 A + B on page 28.

3.5 In Alzheimer patients and in individuals with Trisomy 21, Aβ1-42-specific T cells exclusively produce regulatory IL-10

The cytokine lineage of PBMCs derived from patients with Alzheimer’s disease is displayed in figure 4D. In these subjects, the Aβ1-42-induced IFN-γ and IL-2 recall responses were close to undetectable, and thus decreased with high significance when compared with the elderly (p < 0.001 for IFN-γ and IL-2). IL-4 and IL-5 responses continued to be at the background level. In contrast, a vigorous IL-10 response was detected in Alzheimer patients that was of comparable magnitude as in the elderly controls (p = 0.756). IL-10, being induced in the absence of Th1 (IFN-γ and IL-2) or Th2 (IL-4 and IL-5) cytokines suggests total regulatory polarization of the effector functions of Aβ1-42-specific T helper cells. IL-10 producing T cells can arise as the consequence of chronic immune stimulation. It therefore seems conceivable that in addition to age-related changes in Aβ1-42-metabolism the congenital overexpression of APP could contribute to the emergence of this response.

To address this hypothesis, Aβ1-42 recall responses were tested in subjects with Trisomy 21 who do not only bear APP overexpression, but also have an increased risk of developing early-onset Alzheimer’s disease. The age of our Trisomy 21 study group ranged from 15 to 45 years (mean age 32.17 ± 13.32, n = 6, 3 female, 3 male), therefore corresponding to the young and middle aged control groups. Unlike the unafflicted subjects of similar age, individuals with Trisomy 21 displayed a cytokine signature in conformity with the one of patients with Alzheimer’s disease: Vigorous IL-10 production in the close to complete absence of any other of the tested cytokines (IFN-γ, IL-2, IL-4, IL-5) cytokines (Fig. 4C).
Figure 4

**Aβ_{1-42}**-specific cytokine profile in middle aged and elderly, and in individuals with Trisomy 21 or Alzheimer’s disease

Legend to figure 4 (part I)

PBMCs from healthy donors of different ages (A, B), as well as PBMC from individuals with Trisomy 21 and Alzheimer’s disease (D, E) were tested for their cytokine profile upon stimulation with Aβ_{1-42}. Individuals with Alzheimer’s disease (D) or Trisomy 21 (C) showed marked IL-10 production to Aβ_{1-42}, but strongly decreased IFN-γ and IL-2 production when compared to middle aged (A) or old healthy (B) individuals. IFN-γ, IL-2 and IL-10 were measured after a 24-hour incubation period, IL-4 and IL-5 after 48 hours, taking into account the differences in cytokine secretion kinetics.
Figure 4 (part II)
Beta-amyloid 1-42 (Aβ1-42)–specific cytokine profile in middle aged and elderly, and in individuals with Trisomy 21 or Alzheimer’s disease.

In contrast, Aβ1-42-induced IL-10 spots increased from 7/3x10^5 PBMC in young individuals to 36/3x10^5 (p < 0.001) and 234/3x10^5 cells (p < 0.001) in middle aged and elderly, respectively (marked above with one star (*), and to compare with figure 2). The frequency increase in the Aβ1-42-induced IL-10 production from middle aged to elderly was also highly significant (p = 0.010, marked above with two stars (**)). The absence of any Aβ1,42-specific IFN-γ and IL-2 production in individuals with Trisomy 21 or Alzheimer’s disease was highly significant (p < 0.001) when compared with the production of the specified cytokines in middle aged (marked with three stars (**)) and old healthy individuals (marked with four stars (****)), respectively.

3.6 Total conversion to regulatory IL-10 production does not occur in the immune response of elderly to two control antigens, mumps and tetanus toxoid

It was addressed whether the loss of Th1 activity in favor of a regulatory response is unique for Aβ1,42-specific T cell reactivity in Alzheimer’s disease or a general consequence of immune senescence of the aged immune system. Mumps and tetanus toxoid (TT) were chosen as control antigens, because most individuals can be expected to have had exposure to these antigens early in life. In contrast to the “autoantigen” Aβ1,42, both of them are “foreign” antigens to which the T cell system is not continuously exposed.

Even though the cytokine lineage was found to be altered between young healthy and elderly individuals, differences were not significant (p > 0.05), and none of the cytokines totally disappeared as in the response to Aβ1,42. The comparison of the recall response for aged individuals and Alzheimer’s patients is shown in Fig. 5.1 and 5.2 B + C: no significant differences were seen (p > 0.05).

Therefore, the “disappearance” of the Aβ1,42-specific IFN-γ and IL-2 reactivity in Alzheimer’s patients and individuals with Trisomy 21 does not seem to be a general age-related phenomenon, but appears to be a result of the chronic immune stimulation by the autoantigen Aβ1,42.
Figure 5.1 + 5.2

Total conversion to regulatory IL-10 secretion with age does not occur in the immune response to two control antigens, mumps and tetanus toxoid.

Legend to figure 5.1 + 5.2 (part I)

For two other antigens, no significant difference in cytokine signature could be detected between young healthy and old healthy individuals and patients with Alzheimer’s disease (p > 0.05). PBMCs from young and old healthy individuals and from patients with Alzheimer’s disease were compared for the memory cytokine profile to two control antigens.
Legend to figure 5.1 + 5.2 (part II)

Tetanus toxoid and mumps antigen were chosen, being two antigens to which most people have had exposure early in life. The cytokine signature to mumps antigen is illustrated in 5.1 A for young healthy, in 5.1 B for old healthy individuals, and in 5.1 C for Alzheimer patients. In 5.2 A, B, and C, the cytokine profile of the three groups to tetanus toxoid is shown.
3.7 The Aβ₁-₄₂ specific IL-10 production in Alzheimer patients is CD4+ T cell derived

Since IL-10 can be produced by an array of different cells, cell separation experiments were performed with PBMCs from Alzheimer patients (mean age 70±7.51, n = 3, 3 female, 0 male) to verify the T cell origin of the Aβ₁-₄₂-specific IL-10 production. Purified CD4+ and CD8+ cells were challenged with Aβ₁-₄₂ in the presence of purified B cells functioning as APCs as described before. The IL-10 recall response was detected in the CD4+ cell population (p = 0.024, Fig. 5), but not in the CD8+ cell population (p = 0.383, Fig. 5) or APCs alone (data not shown). The detection of Aβ₁-₄₂-specific IL-10 secretion by CD4+ T helper cells underlines the T cell-mediated nature of the observed changes in the Aβ₁-₄₂-specific immune response of patients with Alzheimer’s disease.
To determine the origine of the interleukin 10 (IL-10) production against beta-amyloid 1-42 (Aβ₁-42) as shown in figure 4, the response of peripheral blood mononuclear cells (PBMCs) from three patients with Alzheimer’s disease was compared with the response of purified cluster of differentiation (CD) 4+ and CD8+ cells from the same persons. The patients chosen for this experiment were not pretested for their Aβ₁-42-specific cytokine profile. Single wells from one patient, containing medium or Aβ₁-42, are shown in figure 6. The testing for Aβ₁-42 mediated cytokine secretion was performed in quadruplicate wells, and the results from the patient shown here are representative of the response found in the other tested patients. The numbers below the wells represent the mean spot numbers from all four wells, including the standard deviation. In this assay a cell ratio of 25x10⁵ CD4+ or CD8+ cells with 10x10⁶ purified B cells as antigen presenting cells (APCs) was used per well. Induction of IL-10 secretion was significant when purified CD4+ cells were challenged with Aβ₁-42 (p = 0.024), but no significant induction was seen when purified CD8+ cells were challenged (p = 0.383).

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<th>PBMC</th>
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<td>19.5 ± 3.53</td>
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<tr>
<td>Aβ₁-42</td>
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<td>62.5 ± 12.34</td>
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Figure 6
Cluster of differentiation (CD) 4+ T cells of individuals with Alzheimer’s disease produce interleukin 10 (IL-10) when stimulated with Beta-amyloid 1-42 (Aβ₁-42)
4 DISCUSSION

This study shows for the first time that Aβ_{1-42}-specific T cells obtained directly ex vivo from young healthy individuals become regularly and spontaneously primed to a Th1 state. Moreover, this T cell response is shifted to a regulatory profile in elderly, and a total conversion to a regulatory cytokine profile occurs in patients with Alzheimer’s disease and Trisomy 21.

Such an intrinsic adaptive T cell response to Aβ_{1-42} in humans seems to be the rule and not the exception since it could be verified by a highly significant cytokine production in 14 out of 17 tested young healthy individuals.

4.1 Dynamics in the Aβ_{1-42}-specific immune response in humans

Aβ_{1-42}–specific cytokine secretion appears to be a highly dynamic phenomenon, as illustrated in this study by the varying cytokine profile of individuals of different ages, having Trisomy 21 or suffering from Alzheimer’s disease (Fig 2,4).

An array of murine studies using transgenic animals, as well as some human studies suggest that the observed changes in the Aβ_{1-42}–specific immune response are closely related to Aβ_{1-42} production, deposition and turnover.

In line with the findings presented here on Aβ_{1-42}–specific cytokine secretion in humans, it has been established that there is an intrinsic specific IgG antibody production against Aβ_{1-42}, and that Aβ_{1-42}-specific antibody titers lower with age and onset of Alzheimer’s disease [40, 161, 162, 52]. The same phenomenon has been reported in Aβ_{1-42} overproducing transgenic mice that also show an age-related decline of antibodies against Aβ_{1-42} occurring without prior vaccination [145]. The comparability of results of the latter study with results from human subjects underline the usefulness of Aβ_{1-42} overproducing transgenic mice in understanding
the interaction of the adaptive immune system with the disease-inducing accumulations of $\alpha\beta_{1-42}$. More importantly, the comparability of the $\alpha\beta_{1-42}$-specific antibody levels in transgenic mice which have been housed in a controlled, pathogen-free environment makes it also rather unlikely that the antibody levels measured in humans are the product of random, crossreactive antibody production and not of an intrinsic adaptive immune response against $\alpha\beta_{1-42}$. In conclusion, the studies on $\alpha\beta_{1-42}$-specific antibody production in humans of various ages and in Alzheimer patients imply that B cell effector functions, as expressed by the production of antibodies, are subject to similar dynamics as the $\alpha\beta_{1-42}$-specific T cell response studied here.

Another study, focusing on the proliferative behavior of $\alpha\beta_{1-42}$-specific human T cells, reveals that $\alpha\beta_{1-42}$-specific T cell proliferation in lymphocytes from young healthy individuals is significantly stronger than the proliferation of T cells from elderly individuals and Alzheimer patients [152].

The data presented in this study may provide an explanation for observed differences in $\alpha\beta_{1-42}$-specific T cell proliferation. It was found in this study that IL-2 production is an important part of the $\alpha\beta_{1-42}$-specific Th1 profile in young individuals (Fig 2). Such IL-2 production by specific T cells is a major component of the autocrine proliferative signal for T cells during the initiation of an adaptive immune response.

In contrast to the marked IL-2 secretion in young individuals, $\alpha\beta_{1-42}$-specific T cells in elderly and Alzheimer patients have a different cytokine profile, producing IL-10 but little IL-2 in elderly and no IL-2 but only IL-10 in Alzheimer patients (Fig 4, 6).

In the presence of a cytokine signature switched to such regulatory cytokine production, lymphocyte proliferation can be expected to be impaired, just as it was observed in the above cited study upon stimulation with $\alpha\beta_{1-42}$ for T cells derived from individuals of different ages or with Alzheimer’s disease.
The results from vaccination studies with transgenic, Aβ1-42-overproducing animals reveal dynamics similar to the ones in the intrinsic Aβ1-42-specific response in humans.

Immunization studies with Aβ1-42 in both wild type and APP-transgenic mice unanimously support the notion that the development of immune tolerance to Aβ1-42 is related to increased and constant Aβ1-42 challenge in transgenic or aged mice [32, 37, 105, 109]. Not only are transgenic animals more difficult to vaccinate than their wildtype counterparts and show a later and lower rise of Aβ1-42-specific antibody titers and cytokine production [105, 163], but also show old animals a much weaker response to Aβ1-42 vaccination than young animals [32, 122, 145]. Thus, vaccination efforts in mice, in relation to age and/or overproduction of Aβ1-42, reflect the results of the study presented here for the intrinsic specific immune response in humans: Transgenic animals, suffering from an increased burden of Aβ1-42, show an impaired Aβ1-42-specific Th1 profile, as expressed by lower IgG 2a antibody titers and lower levels of IFN-γ and IL-2 production to Aβ1-42 [105, 163]. This corresponds to the results presented in this study from individuals with Trisomy 21 who also overproduce the antigen Aβ1-42 and present an impaired intrinsic Th1 response with literally no IFN-γ and IL-2 production (Fig 4).

All these results taken together imply that the differences observed in the human and murine adaptive immune response to Aβ1-42 and differences in Aβ1-42 metabolism are not two unrelated events but that there are close interactions between the two.

However, two other studies, testing T cell lines generated from individuals of different age groups and Alzheimer patients for their Aβ1-42-specific activity, came to results that differ from the results of the above-cited studies and of the study presented here [106, 101]. Contradicting other findings, it was found that T cell lines generated from Alzheimer patients and elderly control subjects displayed an increased frequency and strength of proliferation when compared with an adult
control group. No comparison was carried out in both studies with a young control group comparable to the one presented in this study. Furthermore, it was also suggested that the detected activated T cell lines rather had a Th2 cytokine profile, illustrated by IL-5 and IL-13 secretion, in contrast to the Th1-orientated activity as presented here and as observed in the animal studies [106].

These different findings could be explained by the different experimental approach of using in-vitro generated T cell lines in the cited publication instead of the use of directly ex vivo harvested T cells in this study. It is known that in vitro culture can modify the original cytokine commitment of T cells and, in addition to that, favor the outgrowth of certain T cell lineages over others, changing the original in vivo frequencies and cytokine lineage [142, 77]. Moreover, it even has been shown that by culturing T cells with an antigen, antigen-specific T cell lines can be grown from individuals, who have not had any in vivo exposure to the antigen. For example, HIV antigen-reactive T cells can be generated from HIV-negative blood donors [142].

The results presented in this study on the Aβ1-42-specific cytokine signature in young healthy individuals also reveal the existence of Aβ1-42-specific IL-5 producing T cells directly ex vivo (Fig 2). However, while IL-5 producing cells were more frequent in vivo than IL-4 producing ones, they only constituted a small population relative to the IFN-γ and IL-2 producing cells in young individuals, and the IL-10 producing cells in elderly and Alzheimer patients. Thus, in the results presented in this study the IL-5 component of possible Th2 immunity is only a very minor element of the overall Aβ1-42-specific cytokine repertoire. It seems possible that the split-well-assays performed in the above mentioned publication were able to detect low frequency IL-5 clones in Alzheimer patients that do not show major activity in young healthy individuals in the context of the predominantly Th1-orientated Aβ1-42-specific T cell response as detected by the study presented here. Overall, results from ex vivo studies, such as ELISPOT assays, and in vitro studies can be difficult to compare, and the reflection of the in vivo situation by long-term in vitro culturing might be very limited.
4.2 Impact of the Aβ1-42–specific immune response on the disease course

Whereas a hint for an impact of the intrinsic adaptive immune response against Aβ1-42 on the disease course in humans has only been generated by one study, the results from the vaccination studies in animals speak a clear language [39]. Whenever transgenic animals have been vaccinated in time to mount a vigorous adaptive immune response to Aβ1-42, they are not only protected against the typical pathological hallmarks of Aβ1-42 plaque deposition, but also against the onset of clinical symptoms as shown by their remarkably improved performance in memory and cognitive testing in comparison to unvaccinated animals [5, 73, 109, 22, 110].

These results give the interactions of the adaptive system and the metabolism of Aβ1-42 in Alzheimer’s disease a new dimension: Not only seems an adaptive immune response to Aβ1-42 to be both in humans and mice a regular and highly dynamic phenomenon, but also seems this response able to influence the Alzheimer-like pathology in mice in a way that both typical histopathological features of plaque deposition and clinical symptoms can be halted. However, the success of active vaccination is not guaranteed. If animals do not rise a vigorous enough response to Aβ1-42, for e.g. if vaccinated at higher ages and at a progressed disease stage, the vaccination can lose its clinical effectiveness in improving the cognitive performance of the animals or lowering plaque deposition [7, 32, 6, 105].

4.3 The Aβ1-42–specific immune response in the light of autoimmunity

An intrinsic adaptive immune response against Aβ1-42 is a model autoimmune response against an ubiquitously expressed „self“ antigen. In general, autoimmune responses can be understood as a price that has to be paid for the multispecificity of the adaptive immune system, needed for the specific recognition of infectious pathogens. The high level of specificity is necessary to
eliminate or effectively control intruding pathogens, and at the same time to make sure that only minimal harm is caused to the organism itself [86, 90, 103]. This major task can only be assured by the distinction between intruding pathological „non-self“ that needs to be attacked and eliminated and „self“ that should not be targeted by the adaptive immune system. This is ensured by the mechanisms of central and peripheral tolerance, by which autoreactive B and T cell clones are either eliminated (depletion, activation induced cell death), silenced (anergy) or controlled (regulation) [29, 115, 27, 90, 11, 51, 168].

It is generally accepted that autoimmune diseases arise when such autoreactive cell clones escape this controlled state and “mistakenly” initiate an immune response against „self“-antigens whose presence should be tolerated [156, 10, 27, 90]. In many examples of classic autoimmune diseases, such as type I diabetes, multiple sclerosis or lupus erythematoses, the initiation of an immune response against a „self“-antigen leads to clinical pathology, in the consequence of which the function of the target organ or tissue can be severely impaired.

In the case of Alzheimer's disease, two aspects of the autoimmune response to Aβ1-42 are not in conformity with this basic concept of autoimmunity. Firstly, the general concept of the detrimental effects of autoimmunity does not hold true. Upon the vaccination of the transgenic animals a potent autoimmune response evolved, including the activation of many effector mechanisms such as antibody production or the activation of T cells to produce cytokines typical of a Th1 signature like IFN-γ or IL-2. But instead of causing autoimmune pathology, this autoimmune response against Aβ1-42 has been extensively shown in transgenic mice to be highly beneficial by preventing the clinical onset of Alzheimer's disease. [32, 37, 105, 109, 125, 74]. Thus, the autoimmune response against Aβ1-42 stands in sharp contrast to many other examples for the detrimental effects of autoimmune responses.

A part from this, another aspect remains unusual. Both this study focusing on the cytokine profile of Aβ1-42-specific T cells and other studies focusing on lymphocyte proliferative behavior or antibody production illustrate that an adaptive immune
response against Aβ1-42 in humans seems not to be the exception but rather the rule.
In a direct comparison with the cytokine secretion to another model autoantigen, MBP-PLP fusion protein, this study could show that this level of autoimmune activity is not a general phenomenon, but unique to the response to Aβ1-42 (Fig 1.1, 2). In this study in all tested individuals, no cytokine secretion could be detected to MBP-PLP fusion protein, whereas 85% of tested young healthy individuals showed a highly significant secretion of Th1 cytokines upon the stimulation with Aβ1-42. Therefore, not only the potentially beneficial impact of autoimmune responses against Aβ1-42 is very different from other known autoimmune responses, but also its high frequency in healthy individuals.

### 4.4 The outcome of the human Aβ1-42 vaccination trial

Because of the very promising results of the animal studies and the lack of any apparent side effects, a human vaccination trial was initiated in 2000 using a vaccine called AN1792. In concordance with the murine vaccination protocols, QS21 as a potent Th1 adjuvant was used.

The outcome of this trial was at the same time promising but also very discouraging. The trial had to be halted after the onset meningoencephalitis in roughly 6% of the vaccinated patients, and subsequent complications resulted in the death of so far two patients [44, 108, 116, 128, 160]. Preliminary histopathological studies of the brains of the two deceased patients and serological results from one cohort of the patients support the following interpretation of the onset of these serious complications:

The cause of the onset of meningoencephalitis seemed to be an autoimmune inflammatory response initiated by intracerebral infiltration of activated CD4+ Th cells [44, 114]. This is a pathological feature that is also a hallmark of another T cell-mediated autoimmune disease, multiple sclerosis. Before the human trial, in vaccinated animals neither intracerebral CD4+ Th cell infiltration had been
observed, nor the onset of meningoencephalitis [84, 130]. In the two examined human brains, in contrast to the presence of CD4+ T cells, no accompanying B cell infiltration could be found. Also, no correlation seemed to exist between serum levels of Aβ1-42–specific antibodies in the patients and the onset of meningoencephalitis. This lead researchers to the conclusion that the onset of meningoencephalitis was solely CD4+ Th cell mediated without any contribution of Aβ1-42–specific antibodies or B cells [68, 124].

But in spite of these very serious side effects, there also were some potentially beneficial aspects to the arising immune response in the vaccinated patients. Aβ1-42–specific antibodies were produced, and antibodies in the sera from vaccinated patients were able to bind Aβ1-42 in plaques of both human and transgenic mouse brain slides [68]. Moreover, the histopathological analysis of the brain of one of the deceased patients revealed that typical Alzheimer plaques were resolved in some brain regions [44, 114].

A preliminary cognitive analysis of patients from the Zurich cohort of the study might provide evidence that the vaccination was also clinically effective; The vaccinated patients were able to maintain their cognitive performance as evaluated by using the Mini Mental State Exam (MMSE), whereas their unvaccinated counterparts showed a medium-one-year decline of 6.1 points. 21% of the treated patients were even able to improve their MMSE scores [21].

In summary, this first human vaccination trial points out the following aspects:

On the one hand, Aβ1-42:QS21 vaccination in humans is capable of initiating an adaptive immune response resulting in the activation of T helper cells and the production of antibodies. These antibodies, in turn, are able to bind the pathology-mediated Aβ1-42 fibrils, a process that might prevent plaque formation or facilitate their resolution [116]. Also, vaccinated patients might be able to maintain their cognitive capacities [21].

On the other hand, Aβ1-42 vaccination in humans presents itself as being a double-edged sword: Autoimmune intracerebral inflammation can result, sharing such an
important feature as activated CD4+ Th cell infiltration with multiple sclerosis as an example of a typical T-cell-mediated autoimmune disease of the CNS.

Even if prior to the initiation of the human trial no such autoimmune complications were known from animal studies, a murine study that used the same adjuvant preparation for vaccination that is commonly used to induce the animal model of multiple sclerosis (EAE), consisting of CFA and PTX, showed different results. This vaccination protocol resulted in the onset of autoimmune pathology in the Aβ1-42-vaccinated animals that resembled the clinical presentation of classical EAE [49].

Another murine study (initiated after the human vaccination trial), could show that, as suspected, the CNS level of Th1-type cytokines such as IFN-γ can throw the Aβ1-42–specific immune response out of its balance and cause autoimmune meningoencephalitis [104]. It also has been cautioned that vaccinated mice might show intracerebral inflammation in the course of vaccination more often than so far observed, because their brains were mostly only examined at the end point of the studies and not at different time points after the initial vaccination [116].

Also, new insights in the pathological processes in the Alzheimer brain defeat the earlier notion that no autoimmune pathology is involved in the natural course of the disease. Recently, a subclass of Alzheimer-associated Cerebral Amyloid Angiopathy (CAA) has been defined, so called Aβ-related Angiitis (ABRA). In this new disease, lymphocytotic (mostly T cells) and giant cells, busy with the clearance of perivascular Aβ1-42, mediate an autoimmune angiitis [150]. Therefore, even in unvaccinated humans, the intrinsically present Th1-directed Aβ1-42-specific immune response, as shown in this study, habours the potential to cause autoimmune pathology under certain conditions.

Thus, the adaptive response against Aβ1-42 seems to be one of the few examples of a potentially beneficial autoimmune response, but at the same time it can take a direction that leads to the same detrimental self-aggression as known from many other autoimmune responses.
4.5 The importance of inflammation and innate immunity for the pathogenesis of Alzheimer’s disease

In order to understand the different parameters that contribute to the pathogenesis of a disease, genetic polymorphisms and their impact on the risk profile of an individual can be studied. As exploited in the design of the transgenic mouse models for Alzheimer’s disease, primordial risk factors for Alzheimer’s disease are all polymorphisms that result in the increased accumulation of extracellular Aβ₁-42 [67]. In addition to that, there is a second body of polymorphisms with impact on the risk profile for Alzheimer’s. All studied polymorphisms, that favor the onset or increase the strength of inflammatory pathways, result in a significant increase of the risk to suffer from Alzheimer’s disease [93]. Thus, besides the overproduction of Aβ₁-42 and inhibition of its clearance, a second factor seems to be crucial for the pathogenesis of Alzheimer’s disease: The readiness to initiate and maintain inflammatory responses.

The importance of inflammation is strongly supported by the histopathological analysis of the sites of intracerebral plaque formation: Wherever Alzheimer-typical plaques arise in the brain, the surrounding tissue reacts with a typical inflammatory reaction. This includes the migration and activation of microglia and astrocytes, the upregulation of inflammatory cell surface markers, MHC complexes and intracellular inflammatory signal transduction pathways, the secretion of proinflammatory cytokines and prostaglandines and the activation of the complement cascade [1, 18, 21, 26, 93, 100, 45, 143].

A very general definition of inflammation could be „response to injury“. Therefore, inflammation in the context of Alzheimer’s disease could be a simple consequence of the „injury“ caused by Alzheimer plaque deposition. However, in comparison to other neurodegenerative diseases, Alzheimer’s involves a much higher level of inflammation [100], and this seems to have a more substantial importance to the pathogenesis of this disease than of other
neurodegenerative diseases. Several studies allow the conclusion, that inflammation is not only reactive, but that Aβ₁₋₄₂ itself plays the role of an inflammatory peptide that is strongly interwoven with the activation and control of central inflammatory mechanisms.

It has been shown that Aβ₁₋₄₂ induces the migration of the main cellular mediators of intracerebral inflammation, microglia and astrocytes [100]. In addition, Aβ₁₋₄₂ stimulates intracellular pathways such as the NFκB pathway, which in turn leads to the expression of proinflammatory cytokines like IL-6, IL-1β and TNF-α, or the release of reactive oxygen species [100, 28, 95].

As in most innate immune or inflammatory reactions, in Alzheimer's disease a central effector mechanism is the complement cascade [93]. Neurons adjacent to plaques are decorated with membrane attack complexes (MACs), and core acute phase proteins, CRP and sAP, are sharply upregulated in the regions of plaque deposition [21]. Not only the latter two, CRP and sAP, can bind to the collagen tail of C1q, which is the first component of the classical complement pathway, but also Aβ₁₋₄₂ itself [21]. Because of Aβ₁₋₄₂ being such a predominant component of the plaques, it can be assumed that it itself is a main inductor of the complement cascade as one of the effector mechanisms that results in neural degeneration [93].

The same inflammatory pathways that have been shown to be directly upregulated by Aβ₁₋₄₂, can in turn increase the release of Aβ₁₋₄₂. The release of Aβ₁₋₄₂ by neurons was found to be increased by IL-1β and IL-6 or a combination of the two, and by primary astrocytes when treated with a combination of IFN-γ and TNF-α or IL-1β [19, 33].

Taking this information together, a kind of vicious cycle could be suggested for the accumulation of Aβ₁₋₄₂-containing plaques in the CNS; Aβ₁₋₄₂ induces and maintains an array of inflammatory pathways, and some of these pathways in turn upregulate the molecule that is their own inductor, Aβ₁₋₄₂.

Although Aβ₁₋₄₂ is such an ubiquitously expressed peptide, efforts to attribute a clear function to this peptide have remained difficult. The strong involvement in
inflammatory pathways might be a clue to the peptide's function. It has been suggested that A\(\beta\)\(_{1-42}\), regarding its physiologic function, is closely linked to other members of the Pentraxin family that have been structurally preserved from horseshoe crabs to higher vertebrates [46]. CRP and sAP also belong to the family of Pentraxins and are, as mentioned before, part of Alzheimer plaques and interact with inflammatory pathways in a way similar to A\(\beta\)\(_{1-42}\) [18, 26]. Members of the Pentraxin family have been shown, in addition to their ability to activate the classical complement pathway, to readily aggregate in fibrillar structures and to play a role in the encapsulation of infected sites by amyloidosis. This can be regarded as an effector mechanism of unspecific innate immune defense [46]. It has been suggested, that A\(\beta\)\(_{1-42}\) deposition, as found in Alzheimer’s disease, could function as a “cell’s last defense” for a cell that is exposed to constant stress due to a chronic inflammatory setting [26]. The encapsulation of an infected or chronically inflamed site by amyloidotic depositions could represent a defense strategy to limit the extension of a locally harmful process that cannot be cleared from the organism.

It seems that the disease-mediating amyloidosis characteristic for Alzheimer’s disease is based on similar mechanisms as the amyloidotic encapsulation as a defense strategy in innate immunity. The strong interaction of A\(\beta\)\(_{1-42}\) with inflammatory cascades in the pathogenesis of Alzheimer’s disease could be a remnant of its archaic physiologic function, in regards of which it is similar to other acute-phase-proteins [46]. Interestingly, this close link is also supported by the finding that the expression of its precursor, APP, is regulated by the same transcription factors as the expression of most other acute-phase-proteins [18].

4.6 A\(\beta\)\(_{1-42}\) as an intrinsic adjuvant

The high frequency of intrinsic autoimmune activity against A\(\beta\)\(_{1-42}\) remains a striking difference in comparison to the low level of activity found against other autoantigens. This typical low level of activity was shown in this study for the model autoantigen MBP-PLP (Fig 1, 2).
To avoid autoimmune pathology, simple epitope presentation by APCs is normally not sufficient to activate cells of the adaptive immune system strongly enough to initiate a full-scale immune response [31, 118, 71].

A full-scale activation of an adaptive immune response can only take place if additional general „danger“ signals are provided by APCs to involved T and B cells. These danger signals, that provide sufficient costimulation, cytokine secretion and other activational signals for cells of the adaptive immune system, are provided by APCs upon the recognition of so called pathogen-associated molecular patterns (PAMPs) [11, 50, 118, 76]. PAMPs are mostly unique to intruding pathogens and are not expressed by the host organism itself. Thus, the recognition of PAMPs by APCs, such as LPS, CPG DNA motives or flagellin, represents another measurement to allow the immune system to differentiate between self and non-self [112].

In most cases where autoantigens are presented to autoreactive T or B cells, the full activation of these cells and the outbreak of an autoimmune disease can be prevented by the fact that PAMPs and other accessory costimulatory signals are missing, resulting in the deprivation of the adaptive immune system from the mandatory secondary activational signals [118]. This helps to ensure that potentially harmful effector mechanisms, such as phagocytosis, respiratory burst and complement activation, are only initiated if there is an appropriate threat to the organism [11].

Because Aβ1-42 has been shown to be such a key element in the initiation of inflammatory pathways in the CNS, it is tempting to postulate that Aβ1-42 itself could contribute to the „danger“-signal-rich environment in the periphery that is mandatory for the initiation of an adaptive autoimmune response. Therefore in this study, Aβ1-42 and MBP-PLP, both being autoantigens, were compared in their capability to induce the proinflammatory cytokines IL1-β and IL-6 in APCs derived from PBMCs (Fig 3).

As already extensively studied for the intracerebral setting we could show that Aβ1-42 initiates an equally inflammatory cascade in APCs in the periphery resulting in
high levels of IL1-β and IL-6 secretion (Fig 3). In contrast, no such secretion could be found upon the stimulation of APCs with MBP-PLP.

These results illustrate that a possible reason for the ubiquitous adaptive immune response against Aβ1-42 could be based on the fact that Aβ1-42 itself provides the secondary „danger“ signal that is the presupposition for the priming of an adaptive immune response, for e.g. by inducing the secretion of IL1-β and IL-6. Other autoantigens, such as MBP-PLP, lack this ability, and consequently the priming of an immune response against them is not such a frequent event as in the case of Aβ1-42.

It has been established that there is an array of specific receptors for such PAMPs, so called Toll-like receptors (TLR). These receptors induce upon the recognition of above-mentioned PAMPs specific intracellular pathways that result in the upregulation of „danger“ signals [11, 139, 118]. The receptors have specificities for different molecules, for e.g., TLR-4 recognizes bacterial LPS, TLR-5 recognizes flagellin, and TLR-9 recognizes CPG motives [11].

It remains questionable whether a self-peptide like Aβ1-42 could be an activating ligand to such receptors whose main function is to recognize non-self pathogenic patterns and to provide a differentiative signal between non-self and self [112]. However, there are a number of examples that the engagement and activation of TLRs might be more complicated than the simple recognition of “non-self” PAMPs. TLRs have been first identified in drosophila flies [3]. In contrast to humans, in these animals TLRs do not directly bind PAMPs, but a self-protein, the so called Spatzle protein, which leads to the dimerization and activation of TLRs upon the recognition of PAMPs. Thus, the main activational ligand in drosophila flies is a self protein and not a foreign one.

A number of self-molecules have been found to bind and activate TLRs in vertebrates, too, especially TLR-4 [31]. These ligands consist of molecules that are upregulated or exposed in situations where tissue damage occurs, such as heat-shock-proteins, HSP60 and HSP70, extracellular matrix components like fibronectin, fibrinogen or hyaluronan, or the self-protein β-defensin, which is expressed at mucosal surfaces in response to bacterial infections [31]. Excintingly,
it recently could be shown that $A\beta_{1-42}$ itself binds to TLR-4 and mediates some of its activating properties on APCs via this receptor [154].

In line with this it has been suggested that in addition to “non-self” PAMPs, a second set of apoptotic cell-associated molecular patterns (ACAMPs) exists, which activates the innate system in situations where debris of damaged and apoptotic cells needs to be cleared [43].

Therefore, a more precise definition of TLR functioning could be the recognition of all kinds of “self” and “non-self” danger signals, instead of a restriction to PAMPs, representing signals for infectious danger only.

In being a TLR ligand, $A\beta_{1-42}$ could contribute both to the expression of danger signals by APCs necessary for effective antigen presentation, and to the chronic inflammation as a core element of Alzheimer pathology in the CNS. This hypothesis is supported by the finding that the intracerebral injection of a typical TLR ligand, LPS, can entirely replace the $A\beta_{1-42}$ induced inflammatory cascade in the CNS. It could be shown that the sole injection of the TLR ligand LPS into rat brains induced chronic inflammation and histopathological changes that closely resembled the ones characteristic for Alzheimer’s disease [63, 62]. This illustrates that mechanisms downstream to TLR engagement are a substantial part of the pathological cascade in Alzheimer’s disease.

Adjuvants, such as CFA or CPG, engage TLRs, and are used in vaccines to cause an inflammatory setting and to overcome the lack of costimulatory signals [31]. $A\beta_{1-42}$, in contrast to other autoantigens, appears to act as its own intrinsic adjuvant, and to constitutively provide the danger signal that enables effective antigen presentation for the priming of an adaptive $A\beta_{1-42}$-specific immune response.
4.7 Mechanisms of beneficial autoimmunity in Alzheimer's disease

It has been difficult to find examples for beneficial effects of adaptive autoimmune responses, in contrast to the devastating effects of autoimmune diseases. Nevertheless, already the founders of immunological research hypothesized that antibodies as part of antigen-specific immune responses were not only directed against foreign antigens to provide host protection, but that they also function as cell receptors and are involved in intrinsic physiologic processes (for e.g., P. Ehrlich). Quickly it was shown that antibodies could not only be formed against bacterial toxins, but also against erythrocytes. It then was hypothesized, that hemolytic autoantibodies might exist and be of beneficial effect in the destruction of senescent erythrocytes. However, they could not be experimentally proven at that time, and Ehrlich concluded, that autoantibodies either did not exist, or were functionally impaired because of a „horror autotoxicus“ of the body based on the potentially harmful effects of such a response. Below, in table Ia an overview is given of historic theories of potentially beneficial effects of autoimmunity. In table Ib some of the increasing number of examples of beneficial autoimmune activity is depicted that have attracted increasing interest in recent immunological research.
Table 1a

Theories on beneficial or protective functions of autoimmunity in the course of historical immunological research [144]

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Humoral immunity</th>
<th>Cellular immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1897-</td>
<td>Ehrlich P</td>
<td>• Antibodies are not only specific for foreign antigens, but also serve as receptors in intrinsic physiologic processes</td>
<td></td>
</tr>
<tr>
<td>1901</td>
<td></td>
<td>• Antibodies take part in the normal economy of the body</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• The great risk of autoimmunity (horror autotoxicus) leads to the functional impairment of existing autoantibodies, no matter whether of potentially beneficial or detrimental impact</td>
<td></td>
</tr>
<tr>
<td>1900</td>
<td>Morgenroth J and Ehrlich P</td>
<td>• Autoimmunity is prevented by a complex network of auto-anti-antibodies, specific for the antigen-binding sites of antigen-specific antibodies</td>
<td></td>
</tr>
<tr>
<td>1884</td>
<td>Metchnikoff E</td>
<td></td>
<td>• Immune responses have evolved from primitive digestive functions, initially being involved in the digestion and disposal of all (foreign or intrinsic) unwanted substances</td>
</tr>
<tr>
<td>1901</td>
<td>Besredka A</td>
<td>• Autoantibodies take an important role in the clearance of worn out aged erythrocytes and other body cells</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Author</td>
<td>Humoral immunity</td>
<td>Cellular immunity</td>
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<tr>
<td>------</td>
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<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1904</td>
<td>Gershon B and Kondo F</td>
<td></td>
<td>• Autoantigen-specific „suppressor“ T cells protect against autoimmunity by regulation of autoimmune T helper cells</td>
</tr>
<tr>
<td>1978</td>
<td>Jerne NK</td>
<td>• Immunregulation happens by an Idiotype-Antiidiotype network, where immune responses are favorably controlled and autoimmune pathology is inhibited by a network system of autoantibodies specific for the antigen binding site of other antibodies</td>
<td></td>
</tr>
</tbody>
</table>
### Table Ib

*Examples of protective or beneficial autoimmune responses in current research*

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Humoral immunity</th>
<th>Cellular immunity</th>
</tr>
</thead>
</table>
| 1997 | Haury M [61] | • Natural (auto)antibodies:  
Soluble IgM antibodies produced by a distinct B cell subset (B1 cells) spontaneously *without* antigen stimulation  
• Natural (auto)antibodies possess an epitope repertoire that is distinct from the one of *antigen-induced* B2-cell-derived antibodies and that has been highly preserved between individuals and species  
• Believed to always be specific for both a *self* and a *foreign* antigen  
• Physiologic role in both the maintenance of intrinsic tissue homeostasis by having a „housekeeper function”, and in the provision of an early host protection layer before antigen-induced adaptive antibody production kicks in | |
<p>| 1991 | Avra-meas S [8] | | |
| 1991 | Avra-meas S [8] | | |
| 1992 | Stewart J [147] | | |</p>
<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Humoral immunity</th>
<th>Cellular immunity</th>
</tr>
</thead>
</table>
| 2001 | Schwarz [137, 138] |                  | • Brain damage:  
  |       |                   | • Site-specific autoimmune Th1 cells limit secondary degeneration after an intracerebral lesion by positive regulation of microglia function during the process of secondary degeneration |
| 2004 | Forger F [47] | • Lupus erythematoses:  
  |       | | • Natural autoantibodies specific for dsDNA lower the severity of lupus-induced glomerulonephritis, possibly by epitope masking |
| 2003 | Wildbaum G [164] | • Rheumatoid arthritis  
  |       | | • In rats, spontaneous and beneficial autoantibody production against proinflammatory cytokines, such as TNF-α (including many others) |
| 2004 | Bagnato F [13] | • Myasthenia gravis  
<p>|       | | • Natural autoantibodies against IFN-α and -β could be detected in Myasthenia gravis patients and were of favorable impact on the disease course |
| 2003 | Jambou F [72] | | • Presence of natural anti-TCR antibodies in patients against the TCR of Th cells providing help in anti-acetylcholine receptor antibody production |</p>
<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Humoral immunity</th>
<th>Cellular immunity</th>
</tr>
</thead>
</table>
| 1996 | Palinski W [117] | • Atherosclerosis  
• Natural IgM autoantibodies against oxLDL limit  
atherosclerosis in atherosclerosis-prone mice  
• Vaccination-induced Th2-directed IgG 1 antibody production against oxLDL lowers atherosclerosis | |
| 2004 | Binder CJ [17] |                                                                                   | • Atherosclerosis:  
• Autoreactive, oxLDL-specific Th2 cells favor protection against atherosclerosis by providing with IL-5 secretion a proliferative signal to anti-oxLDL natural autoantibody producing B1cells |
| 2000 | Harrison LC [60] | • Type I diabetes:  
• Autoantigen-specific (Insulin) T regulatory cells can be induced by mucosal or oral vaccination and inhibit the onset of the disease in NOD-mice | |
| 1992 | Miller A [98] | • EAE  
• Oral antigen administration results in the appearance of MBP(autoantigen)-specific tolerance inducing T regulatory cells | |
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<th>Year</th>
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<th>Humoral immunity</th>
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<td>Schenk D [130]</td>
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<td>• <strong>Alzheimer's disease</strong></td>
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<td>Nicoll JA [114, 124]</td>
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<td>Th1-adjuvant QS21 (vaccine AN1792) resulted in T-cell mediated meningoencephalitis</td>
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<td>DeMattos RB [35]</td>
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<td>• The effect can be achieved by passive vaccination with an (A\beta_{1-42})-specific</td>
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The success of vaccination against Aβ1-42 in the prevention and amelioration of Alzheimer’s disease in transgenic animals is perhaps so far the strongest support for the concept of beneficial autoimmunity. Autoimmunity could not only be a risky side-effect of the necessity to have a potent adaptive immune repertoire against „non-self“, but advantages could lie in autoimmune activity itself [134, 136].

Another example of where autoimmune activity is of survival-determining importance is antitumor immunity [41, 87]. Constant autoimmune antitumor responses might be a key mechanism in eliminating potentially malign cell clones that can be expected to arise frequently during the lifespan of an organism.

The finding of an adaptive immune response against Aβ1-42 in young healthy individuals being such a common phenomenon could be an expression of similar importance of the autoimmune response in humans in the prevention of Alzheimer pathology.

Aβ1-42–specific antibody production might be the clue to the beneficial effects on the disease course because the protective effects can be attained by passive vaccination with monoclonal Aβ1-42–specific antibodies without that the recipient’s immune system needs to be specifically activated against Aβ1-42 [14, 35]. A recent study using polyclonal immunglobulins containing Aβ1-42–specific antibodies in the treatment of Alzheimer patients with the intention to compensate the known deficit in humoral Aβ1-42–specific immunity of Alzheimer patients was able to shift the CSF/serum Aβ1-42 equilibrium and to achieve some improvement of patients in cognitive testing [39]

If antibody production has the major beneficial effect in Alzheimer-specific immunity by promoting the clearance of Aβ1-42, it remains striking that both in many vaccination trials and in the intrinsic human immune response a Th1 phenotype prevails, as shown in this study (Fig 1, 2).

Unlike Th1 cells, being potentially autotoxic by their inflammatory properties, Th2 cells are anti-inflammatory, and organ-specific autoimmune Th2 responses have been shown in many cases to be not only non-pathogenic but also capable of preventing Th1 cell-mediated organ-specific autoimmune diseases [27]. It could be expected that for the simple antibody-mediated clearance of an extracellularly
acumulating peptide like Aβ1-42, a Th2-orientated adaptive immune response without the proinflammatory profile of a Th1 response would be appropriate. Th2-type immunity can be experimentally induced by using a vaccine in an adjuvant without the addition of microbial products, that otherwise would provide an associated „danger“ signal to the environment in which the priming of the immune response occurs. As a consequence of the adverse events in the human vaccination trial with the Th1-directed vaccine AN1792, subsequent studies were able to show that Aβ1-42-specific Th2 responses also induce antibody production [54, 78, 80, 157]. Although some of these studies report clearing of Aβ1-42-containing plaques from the brains of Aβ1-42-overproducing transgenic animals, a clinical effect on the cognitive capacities of vaccinated animals with a Th2-response has not yet been reported [80, 85, 157].

Apart from the attempts to induce a Th2-directed Aβ1-42-specific immune response by vaccination, the intrinsic proinflammatory adjuvant properties of the self protein Aβ1-42 (Fig 3) provide an explanation why Th1- and not Th2-type immunity arises spontaneously; Aβ1-42 seems to act as a Th1-adjuvant. Three different models have been put forward for the effect of Aβ1-42-specific antibodies on Aβ1-42 accumulation and clearance. Firstly, such antibodies might be able to prevent pathologic plaque formation by blocking determinants on Aβ1-42 that are required for fibrillar aggregation [12]. Secondly, antibodies have been shown to enhance phagocytosis by opsonization directly in the CNS [14]. Thirdly, absorption and elimination of Aβ1-42 in the immune periphery has been suggested to create an Aβ1-42-concentration gradient between periphery and CNS with Aβ1-42-draining qualities [35, 36]. While all three mechanisms could possibly act in parallel, the latter two are directly dependant on the phagocytotic uptake of Aβ1-42.

In addition to the above mentioned facilitation of Aβ1-42 turnover by Aβ1-42-specific antibodies, it has also been hypothesized that Aβ1-42-specific antibodies can halt the neurotoxic effects of soluble Aβ1-42 [131].
One of the primary functions of Th1 cells is to activate macrophages at sites of antigen encounter to become engaged in defense reactions, including enhanced phagocytosis [153]. In addition, a Th1-type cytokine profile has been found to be more effective in inducing Fcγ-mediated phagocytosis than a Th2-type profile [123]. Furthermore Th1 responses seem to be equally potent as Th2 responses in providing cognate T cell help to antigen-specific B cells for antibody production [127].

The spontaneous priming of Aβ1-42-specific Th1 cells in humans can therefore contribute to the antibody-driven homeostatic purpose of an Aβ1-42-specific immune response both by providing cognate T cell help for the priming of Aβ1-42-specific B cells and by stimulating phagocytic clearance of opsonized Aβ1-42 by scavenger cells such as macrophages. In order to effectively fulfill these two beneficial effector functions of an Aβ1-42-specific immune response, no Th2-type immunity is necessary, and Th1-type immunity might be rather helpful. Furthermore, the beneficial effects of Aβ1-42-specific Th1 cells seem to go beyond priming and providing help to antibody-producing B cells and phagocytosis; Recently, transgenic mice were vaccinated with the T cell epitope of Aβ1-42 only [104]. In the absence of any measurable antibody production, this vaccination protocol resulted in the clearance of Aβ1-42-containing plaques from the brains of vaccinated animals comparable to the usage of passive vaccination protocols or vaccination with full-length Aβ1-42, but also caused transient meningoencephalitis. A second study reported increased cerebral Aβ1-42-clearance upon vaccination of B-cell deficient animals [48]. Therefore, Aβ1-42-specific Th1 cells alone can mediate Aβ1-42 clearance in an antibody-independant manner.

In addition to the above-mentioned beneficial effects of Aβ1-42-specific Th1 cells, other protective functions have been suggested for autoreactive Th1 effector cells that could be responsible for the favorable effects of Aβ1-42-specific T cells in absence of Aβ1-42-specific B cells.
Antigen specific Th1 cells have been shown to be of great importance for the beneficial regulation of inflammatory reactions after tissue injury [113, 135, 133, 134].

After an injury only part of the finally resulting damage is caused by the injury itself. The damage is often worsened by the subsequent reaction to the injury in a process called secondary degeneration [138]. Mostly, the resulting inflammatory reaction is viewed as a major cause of secondary degeneration. In line with this, antiinflammatory medication is given in many diseases involving chronic inflammatory cascades with the purpose to limit the supposed detrimental effects of inflammation on the regenerative capacity of tissue. In spite of this, there is an increasing body of information indicating that inflammation is not only an unfavorable side effect of injury, but that it also is the unreplacable presupposition for successful regeneration. Although some epidemiological studies suggested that antiinflammatory treatment might provide protection against Alzheimer’s disease in limiting local inflammation, controlled clinical trials using Cox-2 inhibitors failed to show a protective effect of antiinflammatory treatment [22, 92]. It is known that a systemic inflammatory stimulus can help the regeneration after an unrelated injury by causing increased local inflammatory activity [102]. In line with this, unrelated intracerebral inflammation caused by a viral infection increased plaque clearance in Aβ1-42-overproducing transgenic mice [146]. The fact that inflammation as such is not necessarily harmful is supported by other findings. For e.g., anti-inflammatory treatment after CNS insult is not in general helpful, but only in the first few hours after the incident [138]. In an optic nerve model of multiple sclerosis in rats, anti-inflammatory steroid therapy can result in more damage than when no anti-inflammatory treatment is given [38]. Installation of macrophages as one of the key mediators of inflammatory effector mechanisms favored regeneration in optic nerve or spinal cord injury [138]. Therefore, not inflammation per se seems to be detrimental, but some inflammatory modalities out of a spectrum of possible inflammatory responses.
Also, site- and antigen-specific Th1 cells have been found to be crucially involved in steering the secondary reactions after CNS injury to a favorable outcome [136]. For e.g., both adoptive transfer of MBP-specific T cells and active vaccination against MBP protect rats better against secondary degeneration caused by mechanisms subsequent to an injury than their non-treated counterparts. T cell depleted rats even suffer from more severe secondary degeneration than unvaccinated animals [138].

Moreover, such T cells need to be target-specific; T cells specific for retinal antigens can only protect against secondary degeneration of the retina and not of the optic nerve, and T cells specific for the myelin sheet can influence favorably optic nerve damage but not retinal damage [136].

T cells actively carrying out these functions need to be of Th1 effector phenotype, and not of a regulatory or a Th2 phenotype: Protective properties in steering secondary inflammatory reactions could only be verified for effector Th1 cells, secreting proinflammatory cytokines such as IFN-γ or IL-2 [138]. In contrast, regulatory T cells, in charge of downregulating other T cell effector mechanisms, have been found to negatively impact the regeneration from CNS injury in rats [136].

There exists further evidence that Th1-mediated autoimmunity might be involved in regenerative processes. For e.g., IFN-γ induces the secretion of growth factors after CNS injury or promotes bone regeneration due to the activation of osteoblasts [11, 15]. Additionally, it has been found that Th1 cells cannot only be reduced to the function of a producer of potentially proinflammatory cytokines, but that they also release regulatory cytokines like IL-10, which might be helpful in finetuning the scale of the inflammatory component of a Th1 response so that its different aggressive and regenerative components are carefully adapted to the unique challenges in different adaptive immune responses to both “self” and “non-self” antigens [11].

In summary, these results allow two novel conclusions that are of great importance for the possible impact of the intrinsic adaptive T cell response against Aβ1-42 in humans.
Firstly, inflammation after CNS injury is not generally harmful but also the physiological basis of successful regeneration, as illustrated by the beneficial effects of macrophage infiltration after injury.

Secondly, an adaptive autoimmune response involving effector Th1 cells is involved in the efficient and favorable control of the inflammatory reaction after CNS injury. If this immune response is missing (in T cell depleted rats) or downregulated (by adoptive transfer of regulatory T cells), the secondary reactions to injury seem to be poorly controlled and to induce further damage.

Recently, it has been possible to show that the regulation of innate inflammatory responses is also crucial in the overall immune response to $\alpha\beta_{1-42}$. In these studies, T-cell mediated release of IFN-γ and IL-4 determined the phenotype of microglia, induced dendritic cell migration to the brain, and decided on whether the resulting inflammatory response was neurotoxic or plaque clearance and regenerative activiy could take place [24, 22, 23].

The $\alpha\beta_{1-42}$ specific T cells that were identified in this study in young healthy individuals were of clearly Th1-polarized lineage, whereas this response vanished in individuals with Alzheimer’s disease or with Trisomy 21 (Fig 4). Besides providing above-mentioned help in the antibody-mediated clearance of $\alpha\beta_{1-42}$, Th1-type $\alpha\beta_{1-42}$-specific T cells exactly fulfill the presuppositions of autoimmune cells with beneficial controlling functions of inflammation that were identified in the reaction to other CNS injuries [133].

Taking these findings into consideration, it remains difficult to imagine that the adaptive Th1 response to $\alpha\beta_{1-42}$ as found in young healthy individuals has no impact on the pathogenic process in the CNS.

Since $\alpha\beta_{1-42}$ itself induces and sustains inflammatory reactions, $\alpha\beta_{1-42}$–specific Th1 cells could have a key function in keeping the control of $\alpha\beta_{1-42}$ homeostasis and its inflammatory properties in healthy individuals.

The unfortunate outcome of the human vaccination trial underlines that the effects of the adaptive immune response cannot be reduced to the favorable impact that
antibodies alone might have on the clearance of $A\beta_{1-42}$. Recent information indicates that a change in the vaccine formulation during the trial by adding polysorbate 80 induced a Th2-Th1 shift in the resulting response, and authors attribute the responsibility for the onset of meningoencephalitis to this shift [124]. Therefore it remains crucial to consider that autoimmune vaccination in the case of a preexisting immune response means to shift a preexisting balance between the potentially beneficial or detrimental effects of autoimmunity.

Despite the onset of meningoencephalitis in some patients, histological analysis of the patients’ brains and clinical follow-up gave promising signs that the vaccination also had beneficial effects on the patients in the trial. In order to better control the effects active vaccination has on the preexisting response against $A\beta_{1-42}$, more information has to be gathered on what the impact of this preexisting immune activity against $A\beta_{1-42}$ is and what the regulatory mechanisms of this immune response are.

At this point, based on the very diverse functions that have been suggested to autoimmune Th1 activity in the process of tissue de- and regeneration, it remains speculative what impact the observed Th1-type autoimmune activity to $A\beta_{1-42}$ has on the course of Alzheimer’s disease. Although, as mentioned, studies have shown that vaccination strategies inducing an $A\beta_{1-42}$-specific Th2 response or a humoral response in absence of an accompanying T cell response are effective in the provision of $A\beta_{1-42}$-specific antibodies and plaque clearance, no systematic comparison has been carried out between different $A\beta_{1-42}$-specific T-cell mediated immune responses and isolated humoral responses. This holds especially true for the effectiveness in plaque clearance and regarding the clinical outcome.
4.8 Regulatory functions in the immune response to Aβ_{1-42}

The observation in this study that T cell-mediated Aβ_{1-42}-specific effector mechanisms are replaced by IL-10 secretion, having a predominantly downregulatory impact on inflammatory immune responses, can be understood as an example of active immune regulation (Fig 4).

However, IL-10 is a cytokine which is expressed by a broad array of cells and which has been shown to have a variety of functions that exceeds its role in the regulation of immune responses [107]. Therefore, it seems important to understand by which cell population IL-10 is produced in the context of Aβ_{1-42}-specific immunity. Using an assay for T cell-derived IL-10 secretion, it could be shown in this study that the observed Aβ_{1-42}-specific IL-10 secretion in Alzheimer patients is CD4+ T cell derived (Fig 6) [58]. In contrast, no such T cell-derived Aβ_{1-42}-specific IL-10 secretion could be put into evidence in young healthy individuals (data not shown).

However, several mechanisms can induce peripheral tolerance, and not all of them involve active control mechanisms. For example, anergy (the loss of the ability of an antigen-specific cell population to express effector functions), depletion (the disappearance of an antigen-specific cell population), activation-induced cell death and immune deviation (the alteration of the effector mechanisms expressed) are all mechanisms of peripheral tolerance but do not necessarily depend on the active downregulation of the immune activity by another cell population [29, 10, 115, 27, 90]. In contrast, the identification of an IL-10 secreting T cell population, as in the case of Alzheimer immunity, allows the assumption that the total silencing of the otherwise identifiable Aβ_{1-42}-specific Th1-orientated effector mechanisms in Alzheimer patients and individuals with Trisomy 21 is an active process. The function of IL-10 to control and downregulate Th1-orientated effector mechanisms has been shown in autoimmune diseases...
such as type I diabetes or infections such as Cryptococcus neoformans infection [165, 126].

In general, regulatory T cells have remained difficult to characterize [10, 94, 115, 156]. This is related to the fact that they neither dispose of a unique marker that clearly classifies their population nor that they have functional properties that are limited to their population [94, 156]. These difficulties in studying regulatory T cells have even lead to a period in immunological research where their existance in general has been cast doubt on [51].

However, many studies show that regulatory T cells can be of decisive importance in the fate of an adaptive immune response and in the prevention of autoimmune diseases. It has been found that the abortion of the T regulatory cell compartment in EAE-prone rats leads to the onset of the disease, and that in parallel the adoptive transfer of regulatory T cells can stop autoimmune pathology [10, 27, 115].

Autoimmunity remains a double-edged sword, and so far the examples of beneficial effects are far outweighed by the high prevalence of autoimmune diseases. If autoimmunity is such a regular and even potentially beneficial phenomenon, the importance of tight regulation becomes very evident [115, 156]. It is difficult to imagine that such crucial regulation should only be based on the passive mechanisms of peripheral tolerance, such as depletion, anergy, or activation-induced cell death of repetitively activated autoreactive cell clones. Regulatory T cells, even if difficult to characterize, should have the pivotal role when it comes to balancing beneficial immune activity and or detrimental autoimmunity, being the „safety valve“ in the maintenance of this sometimes fragile balance [136, 159].

Not only in autoimmune but also in chronic infectious immune responses the importance of tight regulation becomes very obvious [94, 115]. It often remains an
open question whether the prevailing immune activity is of beneficial impact or not [90]. For e.g., in chronic hepatitis C, the destruction of the liver is not solely the result of the viruses’ virulence, but rather of the impact of the resulting immune response against the virus [94].

Regulatory mechanisms are crucial in chronic immune activity, and it is little surprising that active regulatory mechanisms such as T-cell-derived IL-10 production can be identified in a disease like Alzheimer’s, involving both chronic inflammation and innate immune activity.

So far, three different types of regulatory T cells could be characterized, partly by their phenotypic characteristics and partly by their functional properties [10, 90, 94, 115]. Little information, however, is available on the relation of these cell populations to each other and to other T cell populations, especially to naive or anergized cells. It is not clear, whether these cells have to be viewed as separated entities, whether their populations overlap, or whether one phenotype evolves from another [94, 156].

In the case of a first class of regulatory T cells, naive CD4+CD25+ T regulatory cells, some of the regulatory functions seem to be dependant on cell-cell contact, and some on the secretion of cytokines, depending on whether the cells are examined in an in vitro or in vivo setting [90, 115]. However, in the in vivo situation the secretion of IL-10 and TGF-β, two cytokines that have evolved to be of primordial importance for the regulation of immune activity, seems to be central to the function of this regulatory cell population.

A second T regulatory cell population, so called Tr-1 cells, has been primarily described in human immune responses [10]. This cell type has also been shown to secrete both key regulatory cytokines, IL-10 and TGF-β, but in this case the regulatory function of Tr-1 cells seems to be centrally dependant on the IL-10 secretion [10, 94].
Furthermore, a third type of T regulatory cells, so called Th3 cells, has been characterized. As in the case of Tr-1 cells, this cell type has been found to have the potential to produce both IL-10 and TGF-β, but here TGF-β seems to be of more functional impact than IL-10 [94, 156].

Even if so called “natural” T regulatory cells have been described that fulfill their regulatory function without having a defined antigen specificity, for all above described classes of T regulatory cells antigen-specificity has been found with outcome-determining impact on disease course [156].

In this study, a T cell-derived Aβ1-42-specific IL-10 secretion was identified that appears with increasing age and persists in individuals with Alzheimer’s disease or Trisomy 21 in the total absence of the Th1 cytokine profile that is present in young healthy individuals (Fig 4,6). IL-10 secreting T cells, as identified here, could belong to the previously characterized regulatory Tr-1 population, which has been best described for the human immune system.

However, further information is necessary to fully describe this Aβ1-42-specific regulatory subset, especially in regards of its functional profile. Unfortunately, up to date no assay exists to verify antigen-specific T cell derived TGF-β secretion in humans using the ELISPOT technique, which allows to identify such low-frequency antigen-specific T cell clones.

It therefore remains an open question which other effector functions the observed Aβ1-42-specific IL-10 secreting CD4+ T cells express and which of the three established T regulatory cell types they could be part of.

An immune deviation from a Th1 effector phenotype to an IL-10 secreting regulatory phenotype, depending on both age and antigen load, as found in this study, could also be found in repeatedly vaccinated Aβ1-42-overproducing transgenic animals. With increasing age and numbers of Aβ1-42 vaccination (representing repeated antigen challenges), these animals also display a decrease
in Aβ_{1,42}-specific IFN-γ secretion, and a simultaneous increase in IL-10 secretion [150].

### 4.9 Site-specificity of the Aβ_{1,42}–specific immune response

In the context of the suggested interaction between the Aβ_{1,42}–specific immune response and the disease course, it remains surprising that local participation of cells of the adaptive immune system is not very prevalent in Alzheimer-inflicted brains [84, 93].

Besides antigen specificity, another important characteristic of an adaptive immune response is a certain site-specificity. In order to mount an immune response against an infectious pathogen, cells have to become primed in a local setting in draining lymph nodes, and to fulfill their effector functions upon priming, they migrate to the site of infection [118, 156]. The type of immune response that arises after antigen encounter is strongly dependant on the local environment where the antigen encounter occurs and can be very different, depending on whether an antigen is presented via oral route, via inhalation, or subcutaneously [27].

This implies, that even if the immune system as a total is a model of a decentralized network, it fulfills its effector functions upon activation in a rather localized manner.

In order to understand the discrepancy between the obviously effective Aβ_{1,42}–specific immune activity (as demonstrated in vaccinized transgenetic animals) and the little evidence of any local contribution, it could be hypothesized that this might be caused by the antigen itself.

It has been shown that Aβ_{1,42}, regardless of its site of production, can freely cross the blood brain barrier, and that there is a constant equilibrium between serum and CNS Aβ_{1,42} levels [36]. As mentioned, the presence of Aβ_{1,42}-specific antibodies in the periphery alone is sufficient to shift the CNS-serum-equilibrium in a way that
plaque formation can be inhibited in the brains of transgenic animals [36]. As mentioned, the same shift could also be induced in Alzheimer patients by a treatment with polyclonal immunoglobulins [39]. Should this impact on the concentration equilibrium between CNS and periphery be sufficient to explain the beneficial effects of Aβ1-42 vaccination, no physical presence of cells of the adaptive immune system in the CNS would be needed.

Considering the free flotation of Aβ1-42 between the central nervous system and the immune periphery, it could even be possible that the priming of this antigen-specific response does not have to occur in the CNS or its draining lymph nodes, as it normally would be expected for a site-specific immune response. As suggested, Aβ1-42 might have its own adjuvant properties due to its ability to upregulate inflammatory “danger” signals. As a consequence, this antigen with its dispersed presentation could present a “systemic” and not a localized challenge to the immune system and thus initiate “systemic” rather than localized immune activity.

Time dynamics of the Aβ1-42-specific adaptive immune response throughout life could provide another explanation for the lack of B or T cells in the Alzheimer-afflicted brain. The hallmark of the Aβ1-42-specific immune response in Alzheimer patients and individuals with Trisomy 21 is the replacement of a Th1 effector profile by a T cell subset producing regulatory IL-10 (Fig 4). Since one of the core functions of this cytokine is to downregulate adaptive Th1 immune activity [126, 165], the predominance of Aβ1-42-induced IL-10 production in Alzheimer patients could explain why it is difficult to show remarkable CNS infiltration by T or B cells at the end of life.

Another important aspect to be kept in mind is the difference in dynamics in comparison to other autoimmune responses such as multiple sclerosis.
In MS, in most cases the disease course is a relapse-remitting one, implicating that there is a shift between phases of high immune activity and phases of a more dormant immune response.

In contrast to this, in Alzheimer’s both the in vivo stimulation of the adaptive immune system and later the disease course are of chronic nature. That means that there rather is a constant low-level stimulation of the \( A\beta_{1-42} \)-specific cell pool, never high enough to cause clinical signs of autoimmune pathology. Such a constant low-level activity can explain why the \( A\beta_{1-42} \)-specific immune activity collapses with increasing age.

In summary, these findings allow the following conclusions: Firstly, the decrease of both B and T cell effector responses and their replacement by T cells with a regulatory cytokine profile in patients with Alzheimer’s disease helps to explain why no major B or T cell presence can be found in the Alzheimer-inflicted brain at advanced stages of the disease.

Secondly, this lack of physical presence does not allow the conclusion that the autoimmune activity in younger individuals, who do not have any clinical pathology, has no impact on \( A\beta_{1-42} \) metabolism and future risk of disease onset.

4.10 The impact of immune ageing on the response to \( A\beta_{1-42} \)

This study establishes that the cytokine signature of the intrinsic T cell response to \( A\beta_{1-42} \) undergoes profound changes according to age and differences in \( A\beta_{1-42} \) metabolism (Fig 4). These changes lead to the appearance of a T cell subset secreting regulatory IL-10 (Fig 6), whereas all other effector functions vanish that otherwise could have influenced the disease course.

The appearance of such a regulatory cytokine signature has been linked to repeated antigen stimulation, and hence could be a general consequence of age
[4, 69], and not a unique characteristic of a certain antigen-specific immune response. If all antigen-specific immune responses changed with age in the same way, it would be difficult to interpret these changes as having a major pathogenetic impact on a single disease.

Research focussing on the alterations in the ageing immune system comes to a variety of sometimes contradictory conclusions, but in general T cell populations are supposed to shift from naïve to memory phenotype [132, 119]. The finding, that immune responses upon vaccination of old mice are slower and weaker both in cytokine secretion and antibody production, is an illustration of this and could be related to the decreased naive cell pool that builds the basis for the initiation of novel immune responses [122, 105, 64].

Secondly, aged lymphocytes seem also to be impaired on a functional level; Among other functions, the following have been shown to be impaired: The expression of costimulatory molecules, lipid draft, functioning of the "immune synapse" and the readiness to proliferate upon increased costimulation or mitogen stimulation [132, 155, 120, 64].

In contrast to the information available on general changes of the immune system in ageing individuals, little information is available on changes of antigen-specific immune responses and their cytokine signatures in humans of different ages.

It therefore presented a central question in this study whether the changes found in the Aβ1-42–specific T cell response are unique to the response against this specific antigen or rather a common feature of all ageing antigen-specific immune responses.

In order to address this, the Aβ1-42–specific cytokine signature was compared with the signature against tetanus toxoid and mumps, two antigens most people have been challenged with during their lifetime either due to an infection (mumps) or due to repetitive vaccination (tetanus toxoid).

In line with the changes already shown for other functions of the ageing immune system in humans, this study found that the antigen-specific cytokine profiles to tetanus toxoid and mumps undergo changes with age, too (Fig 5.1, 5.2).
Similar to the response to Aβ1-42, the response to the other two antigens, tetanus toxoid and mumps, was altered in the means of an increasing IL-10 secretion.

However, in contrast to the Aβ1-42-specific response, there was a marked difference in that the initial Th1 component persisted in the case of the two control antigens only and not in the case of Aβ1-42.

While elderly and Alzheimer patients alike retained comparable numbers of mumps- and tetanus-specific Th1 cells and the changes between all age groups concerning these two antigens did not reach significance, the Aβ1-42-specific Th1 component was the only one to be significantly reduced with age, and to completely be lost in Alzheimer patients (Fig 4).

As in Alzheimer patients, a complete loss of Th1 reactivity to Aβ1-42 was seen in individuals with Trisomy 21.

These data suggest that the changes observed in the Aβ1-42-specific T cell population are not a mere consequence of the general ageing of the adaptive immune system, but that they are the result of the unique dynamics of the interaction between the Aβ1-42-specific T cell population and the differences in Aβ1-42 expression, metabolism and deposition.

An explanation for the antigen specificity of the changes occurring in the ageing immune system could be found in the different dynamics of each specific antigen challenge [129].

In the context of this, the Aβ1-42-specific T cell population could become more profoundly tolerized in comparison to other antigen-specific T cell populations due to the more chronic stimulation with the endogenous autoantigen Aβ1-42. This chronic stimulation shows its effect on the immune system with age and, more markedly, with the altered antigen presentation in Alzheimer patients or individuals with Trisomy 21 (Fig 4).
The functional limitations of aged lymphocytes and their partial unresponsiveness are closely related to the shortening in telomerase length in these cells, which represents a key regulator of the cellular lifespan [129]. Therefore, a cell population exposed steadily to its antigen like the Aβ1-42-specific one, can be expected to undergo more proliferation cycles and subsequent telomerase shortening than a population that only encounters its antigen occasionally; Thus, the first cell population can be expected to age more quickly than the latter one.

The fact that the much younger individuals with Trisomy 21 (mean age 32.17 ± 13.32) show the same total replacement of the Th1-polarized effector response by a downregulatory IL-10 one than the older Alzheimer patients (mean age 73±8.53), underlines that the changes in the Aβ1-42 specific immune response are more related to the dynamics in the presentation of this endogenous autoantigen than to the age of the individual of interest.

This is further supported by the preservation of the initial Th1 component to the other two control antigens, which shows that the disappearance of the Aβ1-42-specific activity is not a imperative consequence of age-related immune senescence (Fig 4, 5.1, 5.2).

### 4.11 Conclusional remarks

Recapitulating the general knowledge on autoimmunity, the outcome of Aβ1-42 vaccination in mice and humans, and the findings of this study, the following could be a working model for the effects of an autoimmune Aβ1-42-specific response on Alzheimer’s disease.

Instead of understanding autoimmunity as a mere byproduct of a multispecific adaptive immune system and as a threat of autoimmune disease, evidence has been found that it can be beneficial to the organism [133, 136].
In the light of the beneficial effects of the murine vaccination studies with $A\beta_{1-42}$, it is tempting to postulate that the intrinsic $A\beta_{1-42}$-specific Th1 response in humans presented in this study has the potential to exert beneficial effects on the pathology of Alzheimer’s disease, too.

These beneficial effects could be based on the promotion of the clearance of an otherwise harmful metabolic product, on the regulation of inflammatory mechanisms, or on the active support of tissue regeneration, for e.g. by promoting the release of growth factors or allowing neurogenesis [22].

Both the regulation of the chronic inflammatory cascade, being sustained by $A\beta_{1-42}$ itself, and the production of clearance-inducing antibodies could mediate protective effects of an $A\beta_{1-42}$-specific adaptive immune response in Alzheimer’s disease.

In contrast to other autoantigens, the intrinsic inflammatory properties of $A\beta_{1-42}$, allowing it to function as its own adjuvant, and its ubiquitous distribution offer an explanation why a Th1-type $A\beta_{1-42}$-specific immune response is regularly primed to an effector state in young healthy individuals [154].

The limited success of vaccination efforts in elderly transgenic animals and altered cytokine signatures show that mechanisms of peripheral tolerance and immune regulation gain in impact in this immune response with increasing age and duration of antigen exposure.

In line with this, with increasing age, the intrinsic T cell activity in humans is profoundly shifted to regulatory IL-10 secretion, accompanied by the loss of the Th1 effector profile.

This leads to the paradox situation, that at the stage where the accumulation of $A\beta_{1-42}$ becomes pathogenic, no or little T or B cell-mediated effector mechanisms
are left in order to exert beneficial effects such as the regulation of the chronic inflammation or the production of antibodies for enhanced clearance of Aβ\textsubscript{1-42}.

The unexpected outcome of the human vaccination trial, however, underlines that the autoimmune response to Aβ\textsubscript{1-42}, as all autoimmune responses, has the potential to induce autoimmune pathology. At the same time, histological brain examination and clinical follow-up revealed potentially protective effects for the vaccinated patients [21, 68, 114]. In order to keep the balance between the two poles of beneficial and detrimental outcomes of autoimmune activity, tight regulation is mandatory, a function that intrinsically could be fulfilled by T cell-derived production of regulatory IL-10, serving as a „safety valve“ [165].

Histological analysis of the two deceased patients’ brains with meningoencephalitis in the vaccination trial showed local CD4+ T cell infiltration, leading to the widely accepted interpretation that meningoencephalitis was caused by autoreactive CD4+ T cells.

Subsequent studies focussed on the development of safer vaccination protocols that avoid a potentially harmful Aβ\textsubscript{1-42}-specific Th1 response and at the same time provide the desired intrinsic Aβ\textsubscript{1-42}-specific antibody production. For e.g., protocols using intranasal administration of a recombinant adenovirus vector encoding Aβ\textsubscript{1-42} and GM-CSF successfully initiated an Aβ\textsubscript{1-42}—specific Th2 response [78]. Using the B cell epitope of Aβ\textsubscript{1-42} in combination with an ubiquitous T cell epitope or an adenovirus encoding tandem repeats of Aβ\textsubscript{1-6} resulted in a robust provision of Aβ\textsubscript{1-42}-specific antibodies in absence of any Aβ\textsubscript{1-42}-specific T cells or the onset of meningoencephalitis in mice [79, 2, 167]. Different adjuvant preparations were used in order to achieve a Th2-directed Aβ\textsubscript{1-42}-specific immune response [6, 53, 85, 88].

However, knowledge on the impact of the adaptive immune response to Aβ\textsubscript{1-42} on Alzheimer’s disease still is limited. In order to better understand the underlying mechanisms, the following aspects seem to be essential:
1. The results from the passive vaccination studies in transgenic animals suggest that antibodies alone can positively influence the disease, probably by the interference with the plaque formation and/or by the induction of clearance in the CNS itself or the periphery. Another study shows that an $\text{A}\beta_{1-42}$–specific Th1 response in the absence of any B cell response can induce the clearance of $\text{A}\beta_{1-42}$–containing plaques as well [150]. No direct comparison exists between the beneficial effects of passive and active vaccination. Therefore, it is not known whether a full adaptive immune response has beneficial effects that go beyond the effects of antibodies provided by passive vaccination.

2. Both the beneficial effects of active vaccination and the detrimental effects of autoimmune inflammation have so far only been studied in the case of Th1-type immunity. The above cited studies postulate that Th2-type responses might be safer than Th-1 type responses, but only occasionally any information on the therapeutic effectiveness of Th2-orientated vaccination protocols is given [110]. Comparison of the effectiveness of Th1-type with Th2-type immunity would be helpful to understand what impact the effector mechanisms have that are characteristic either to Th1 or to Th2 immunity.

3. The regulatory IL-10 production by T cells as identified in this study in elderly individuals, in Alzheimer patients and individuals with Trisomy 21 needs to be further characterized. It needs to be investigated whether this IL-10-producing T cell subset shows other effector functions and phenotypic features of regulatory T cells.

4. Even though tolerance and regulation have been repetitively suggested to be responsible for the varying results of $\text{A}\beta_{1-42}$ vaccination in old and young, and
in transgenic and wildtype animals, these mechanisms have not been further characterized.

In order to understand this better, transgenic animals could be depleted of regulatory T cells before vaccination, or regulatory T cell subsets could be adoptively transferred upon successful vaccination.

Also, the disease course in immune-deficient transgenic animals could be observed in order to understand what impact a potential intrinsic immune response has on the disease course in unvaccinated transgenic animals.

5. More attention has to be paid to the fact that results from studies with transgenic animals cannot be simply transferred to the human situation. The expression of human Aβ1-42 in transgenic mice is regulated by a foreign promotor and therefore cannot interact with physiological processes as in the case of the expression of native Aβ1-42 in humans or non-transgenic animals.

It has been cautioned that transgenic animals do not copy the full human disease, and therefore an immune response to Aβ1-42 might take a different course in transgenic mice than in humans [128].

In order to gain better comparability between animal and human results, vaccination studies should also be carried out using wildtype murine Aβ1-42 as an antigen in wildtype animals, which have a physiological regulation of Aβ1-42 expression.

Furthermore, it could be helpful to understand whether the risk of autoimmune complications in mice is an age- and disease-related one.

In addition to the already known higher effectiveness of vaccination at younger ages it could also be safer to use active vaccination at a younger age where Aβ1-42 deposition and local inflammation has not yet advanced as much as in old individuals.
6. It seems that the onset of autoimmune encephalitis both in humans and animals is a T-cell mediated process. The above cited studies conclude that the lack of meningoencephalitis in vaccinated mice allows a prediction about the safeness of their protocols in humans. However, mice seem to be less prone to the onset of meningoencephalitis upon $\alpha\beta_{1-42}$ vaccination than humans. Mice tolerate $\alpha\beta_{1-42}$ vaccination using the potent Th1 adjuvant CFA without showing clinical and histological signs of meningoencephalitis; In contrast, humans develop meningoencephalitis after $\alpha\beta_{1-42}$ Th1-directed vaccination. The only vaccination protocol so far that induced meningoencephalitis in mice was a protocol using $\alpha\beta_{1-42}$ in CFA and PTX as adjuvants, otherwise commonly used in the induction of EAE.

In order to allow any conclusion about the safeness of an altered $\alpha\beta_{1-42}$ vaccine with an unrelated T cell epitope, the vaccine should be used with the only adjuvant preparation so far known to induce meningoencephalitis in mice in combination with $\alpha\beta_{1-42}$, CFA plus PTX; All other adjuvants seem to be tolerated by mice without any side effects no matter what epitope of $\alpha\beta_{1-42}$ is administered in them.

Due to the outcome of the human vaccination trial the strategy of Alzheimer vaccination received a major drawback. On the one hand, active vaccination, even if practical and economic, seems not to be a feasible approach if autoimmune complications occur in roughly 6% of the vaccinated patients.

On the other hand, the diagnosis of Alzheimer’s disease is especially devastating for the patient and his family because treatment options to halt or improve the disease are extremely limited. At the same time, in animal research vaccination against $\alpha\beta_{1-42}$ so far has been the therapeutic approach with the most impressive amelioration of the disease.

The human vaccination trial was initiated under a very limited understanding of the impact of the immune response to $\alpha\beta_{1-42}$ on the disease process.
This impact now seems to be by far more complicated than the simple provision of antibodies for opsonization and phagocytosis.

It has to be kept in mind that Aβ_{1-42} vaccination, besides antitumor vaccination, is the only example, where the initiation of a formerly dormant autoimmune activity is supposed to be of a beneficial effect.

It remains to be hoped that the strategy of active vaccination is not abandoned in the consequence of the discouraging outcome of the first human vaccination trial.

Once the fragile balance between autoimmune protection and autoimmune pathology is better understood, active vaccination may offer a safe and effective path to the prevention and treatment of Alzheimer’s disease. Current efforts to develop a vaccine for an active vaccination trial in humans consisting of a conjugate of the B cell epitope of Aβ_{1-42} and an unrelated carrier protein could be a promising approach to achieve an adaptive immune response that is both effective and safe [124].
5 SUMMARY

Spontaneous beta-amyloid 1-42 (Aβ1-42)-specific antibody production and T cell proliferation suggest that the adaptive immune system in humans is intrinsically activated against Aβ1-42. Enzyme-linked Immuno Spot (ELISPOT) assays were used in this study to assess directly ex vivo the cytokine lineage of T cells reactive to Aβ1-42 in healthy humans of different ages, in individuals with Alzheimer’s disease and Trisomy 21.

A constitutively primed Aβ1-42–specific T helper 1 (Th1) response could be established in young healthy individuals, consisting of interferon gamma (IFN-γ), interleukin 2 (IL-2), little interleukin 5 (IL-5) and no interleukin 4 (IL-4) or interleukin 10 (IL-10). In contrast to the intrinsic activity to the autoantigen Aβ1-42, no significant cytokine secretion could be detected to two other central nervous system (CNS) autoantigens, myelin basic protein (MBP) and proteolipid protein (PLP).

It could be shown that Aβ1-42 induces the release of the inflammatory cytokines interleukin 1β (IL-1β) and interleukin 6 (IL-6) in antigen presenting cells (APCs) derived from peripheral blood mononuclear cells (PBMCs) at a highly significant level. This could possibly induce the secondary “danger” signals necessary for the regular priming of the autoimmune response against Aβ1-42. No inflammatory cytokine secretion could be detected by PBMC-derived APCs when stimulated with the autoantigens MBP and PLP.

While with increasing age the Th1-orientated cytokine production was shown to decrease, an Aβ1-42-specific IL-10 component was found to arise simultaneously. In contrast to that, in Alzheimer patients or individuals with Trisomy 21 only Aβ1-42-specific IL-10 secretion could be detected, in the absence of any accompanying Th1- or Th2-type cytokine release. The IL-10 production in Alzheimer patients was found to be cluster of differentiation (CD) 4+ T helper cell derived.
The changes of the Aβ_{1-42}-specific immune activity with age could not be verified at the same scale for two common foreign antigens, mumps antigen and tetanus toxoid. Thus, the alterations of the Aβ_{1-42}-specific immune response seem to be a consequence of the unique circumstances of Aβ_{1-42} production, metabolism and clearance, and not only of the alterations taking place in the ageing immune system.

This is further supported by the finding that the much younger individuals with Trisomy 21 show the same total IL-10 shift of the Aβ_{1-42}–specific adaptive immune response as the much older patients with Alzheimer’s disease.
6 ZUSAMMENFASSUNG


Eine vorbestehende Aβ_{1-42}-spezifische und quantitativ bedeutsame T Helferzellen Typ I (Th1)-Antwort konnte in jungen gesunden Personen nachgewiesen werden, bestehend aus Interferon Gamma (IFN-γ), Interleukin 2 (IL-2), wenig Interleukin 5 (IL-5) und kein Interleukin 4 (IL-4) oder Interleukin 10 (IL-10). Im Unterschied zu der intrinsisch vorhandenen Immunantwort gegen Aβ_{1-42} konnte für Myelin-basisches Protein (MBP) und Proteolipid Protein (PLP) als zwei weitere zerebrale Autoantigene keine signifikante Zytokin-Produktion nachgewiesen werden.

Ferner zeigte sich, dass Aβ_{1-42} mit einem hochsignifikanten Niveau die Freisetzung von inflammatorischen Zytokinen IL-1β und IL-6 durch Antigen präsentierende Zellen, gewonnen aus peripheren mononukleären Blutzellen, hervorrufen kann. Dies könnte möglicherweise das sekundäre „Gefahrensignal“ bereitstellen, das für die Auslösung einer adaptiven autoimmunen Antwort gegen Aβ_{1-42} notwendig ist. Im Gegensatz dazu konnte eine Freisetzung von inflammatorischen Zytokinen durch Antigen-präsentierende Zellen nicht nachgewiesen werden, wenn diese mit den Autoantigenen MBP und PLP konfrontiert wurden.

Während in gesunden Probanden gezeigt werden konnte, dass mit ansteigendem Alter die Th1-gerichtete Aβ_{1-42}-spezifische Immunantwort abnahm, kam
gleichzeitig eine $\text{A}\beta_{1,42}$-spezifische IL-10 Produktion auf. Im Unterschied hierzu konnte in Personen mit Trisomie 21 oder Alzheimer Patienten eine ausschließliche Produktion von IL-10 bei volliger Abwesenheit von Th1- oder Th2-typischer Zytokinproduktion nachgewiesen werden. Die IL-10 Produktion in Alzheimer Patienten wurde durch Cluster of Differentiation (CD) 4+ T Helferzellen bereitgestellt.

Diese Veränderungen in der Signatur der $\text{A}\beta_{1,42}$-spezifischen Immunantwort konnten nicht im gleichen Umfang nachgewiesen werden für die Immunantwort gegen zwei häufige Fremdantigene, Mumps Antigen und Tetanus Toxoid. Dementsprechend scheinen die Veränderungen in der $\text{A}\beta_{1,42}$-spezifischen Immunantwort die Folge von den ganz speziellen Bedingungen der Produktion, des Stoffwechsels und der Beseitigung von $\text{A}\beta_{1,42}$ zu sein, und nicht nur von den Veränderungen, die ganz allgemein im alternden Immunsystem stattfinden.

Diese Annahme wird auch dadurch untermauert, dass die wesentlich jüngeren Probanden mit Trisomie 21 dieselbe vollständige IL-10 Polarisierung der $\text{A}\beta_{1,42}$-spezifischen Immunantwort zeigen wir die deutlich älteren Alzheimer Patienten.
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