Modulation of Alzheimer’s pathology by cerebro-ventricular grafting of hybridoma cells expressing antibodies against Aβ in vivo

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Abstract Accumulation in brain of the β-amyloid peptide (Aβ) is considered as crucial pathogenic event causing Alzheimer’s disease (AD). Anti-Aβ immune therapy is a powerful means for Aβ clearance from the brain. We recently showed that intravenous injections of anti-Aβ antibodies led to reduction, elevation or no change in brain Aβ42 concentrations of an AD mouse model. We report here, in a second passive immunization protocol, a different bioactivity of same antibodies to alter brain Aβ42 concentrations. Comparing the bioactivity of anti-Aβ antibodies in these two passive immunization paradigms underscores the potential of immune therapy for AD treatment and suggests that both the epitope recognized by the antibody and the mode of antibody administration are crucial for its biological activity.

Keywords: Alzheimer’s disease; Immunization; Therapy; Amyloid precursor protein; Transgenic mice; β-Amyloid peptide

1. Introduction

The β-amyloid peptide (Aβ), a cleavage product of the amyloid precursor protein (APP), is the primary component of senile plaques and a major player in Alzheimer’s disease (AD) pathology [1,2]. AD is an age-related neurodegenerative illness that is characterized by progressive cognitive deficits such as memory loss and a decline in mental abilities. High brain Aβ level is associated with impaired synaptic functions and the loss of neurons [2,3]. Therefore, both active and passive immunization approaches are being tested in experimental and clinical studies to remove Aβ from the affected brains (for a review see [3]).

Anti-Aβ immune therapy is shown to reduce the brain Aβ levels in human patients and in AD mouse models that express high levels of Aβ and exhibit an age-dependent AD-like pathology [4–8]. In addition, anti-Aβ immunizations ameliorated the cognitive deficits of transgenic mouse models of AD [9,10] and AD patients [11].

Three mechanisms have been proposed to explain the therapeutic potential of the anti-Aβ antibodies for the treatment of AD. These mechanisms include the catalytic conversion of fibrillar Aβ to less toxic forms [6,12,13], the opsonization of Aβ deposits leading to microglial phagocytosis [6,12,14], and the promotion of the efflux of Aβ from the brain to the circulation [5]. Evidence exists supporting all three mechanisms, which are not mutually exclusive. Clinical trials of active immunization with vaccines against human Aβ42 were halted due to an unacceptable incidence of meningoencephalitic reactions. Alternative strategies to the vaccination of AD patients with the Aβ42 peptide are suggested to be active immunization with Aβ fragments or passive immunization.

Therefore, it is inevitable to investigate whether anti-Aβ antibodies exert a constant bioactivity determined solely by the recognized epitope or whether the bioactivity of a given antibody also depends on the administration method. In this study, the efficacy of intra-ventricular grafting of antibody producing cells to reduce Aβ levels in brain and periphery was tested. These data are compared to the results obtained in a parallel experiment subjecting the same AD model to intravenous injections of same antibodies [15]. Our accumulative data verify the bioactivity of anti-Aβ antibodies to different immunization protocols in vivo. We show that the efficacy of these antibodies to reduce brain Aβ42 levels is dependent on the epitope recognized by these antibodies and on the mode of the antibody administration.

2. Materials and methods

2.1. Isotyping of the antibodies

Monoclonal anti-Aβ antibodies used in this study (Table 1) were subtyped using the Mouse Monoclonal Antibody Isotyping kit (Biorhringer Mannheim) according to the manufacturer’s instructions. Briefly, the antibodies were diluted to 1 µg/ml and 150 µl of the sample was applied to the development tubes containing lyophilised latex beads, incubated for 30 s and then agitated briefly. The strips were inserted into the test tubes and the results were interpreted after 5–10 min.

2.2. Intra-ventricular implantation of hybridoma cell clones

Transgenic mice with neuronal expression of the Swedish double mutation of APP (SwAPP mice) were bred and housed as described [16] and experiments were performed in accordance to national guidelines. Two microliters of the hybridoma cell suspension was injected into the right lateral ventricle of 6–7 weeks old SwAPP or non-transgenic littermates (n = 4–9 per group) according to a stereotaxic mouse brain atlas [17] and mice were analyzed 1–4 weeks later.

Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer’s disease; APP, amyloid precursor protein; i.c.v., intracerebro-ventricular
2.3. Biochemical analysis of treated mice

For analysis of APP processing and measurement of Aβ levels in brains, mice were anesthetized and perfused transcardially with ice-cold PBS. Frontal parts of the brains reaching from the stereotaxic coordinates interaural 6–5 were homogenized and subjected to Western blotting as described [15]. This area lies outside the site of taxic coordinates interaural 6–5 were homogenized and subjected to Western blotting as described [15]. This area lies outside the site of

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All antibodies belonged to the IgG1 subtype with the light chain.

2.4. Statistical analysis

Data were collected by investigators blinded to the experimental setup and were analyzed by non-parametric Mann–Whitney U test. In all graphs, means ± S.E.M. are shown.

3. Results

The effectiveness of anti-Aβ antibodies to reduce the Aβ levels in vivo was studied by intracerebro-ventricular (i.c.v.) implantation of antibody producing hybridoma cells into SwAPP mice. Hybridoma clones were selected on the basis of the varying bioactivity of the anti-Aβ antibodies in another passive immunization paradigm infusing intravenously purified antibodies to age-matched SwAPP littermates [15].

A standard number of hybridoma cells for i.c.v. grafting was established in preliminary experiments by grafting various cell numbers of the 22C4 clone into the right ventricle of age-matched non-transgenic mice. Peripheral antibody titers were measured by analyzing blood sera before the grafting and at the time of perfusion (n = 4–5 per group), reflecting the values of brain antibody titers. Anti-Aβ antibody titers increased with the number of implanted cells reaching a maximum at 1–2 weeks after grafting of 2.5 × 10^5 cells (Fig. 1A), whereas longer survival times did not lead to higher antibody levels. Therefore, a point of three weeks after implantation was chosen for sacrifice of SwAPP mice in order to be close to peak antibody concentrations, while at the same time allowing enough time for possible removal of Aβ by the produced antibodies. Moreover, the duration of three weeks ensured the comparability of this experiment to our previous study, treating age-matched SwAPP mice with purified anti-Aβ antibodies for three weeks intravenously [15].

Three weeks after the implantation of the hybridoma cells serum anti-Aβ antibody levels were assessed by analysis of the blood samples taken at the time of perfusion and were compared to baseline antibody levels of 23 untreated age-matched SwAPP littermates (Fig. 1B). At the time of perfusion, all SwAPP mice implanted with the above number of different hybridoma clones showed significant elevated anti-Aβ antibody titers when compared to untreated SwAPP littermates (Fig. 1B). Animals implanted with clone 22C4, however, showed antibody titers roughly sixfold higher compared to titers assessed for animals implanted with the three other clones, even though the same number of cells were implanted (Fig. 1B). This variation may reflect the differences in productivity of the various hybridoma clones or may be due to a varying turnover rate of the antibodies in vivo.

Next we assessed whether the intracerebral production of the antibodies against Aβ caused a change of Aβ concentrations in the CNS and the periphery, and whether the effects depended on the implanted hybridoma clone. Quantification of the concentrations of Aβ1-42 in the sera and brains of SwAPP mice implanted with hybridoma cells revealed that only grafting the hybridoma clone 8G7 reduced the Aβ1-42 concentrations, both in the brain and in serum, when compared to the corresponding levels of untreated age-matched SwAPP littermates (Fig. 1C, P ≤ 0.01). In contrast to results obtained by intravenous administration of these antibodies (Fig. 2), all other clones did not cause any significant changes (Fig. 1C).

![Fig. 1](image-url) Intracerebral grafting of anti-Aβ-producing hybridoma cells. (A) Anti-Aβ antibody titers in serum of non-transgenic mice implanted with 2.5 × 10^5 cells of clone 22C4 reached a maximum at 1–2 weeks post-grafting and declined thereafter (w1–w4 indicate that serum was prepared 1–4 weeks after the i.c.v. implantation of antibody producing hybridoma cells into SwAPP mice). Anti-Aβ antibody titers in sera of SwAPP mice grafted i.c.v. with hybridoma clones at the time of perfusion. The values are expressed as fold increase, relative to the baseline value obtained from averaging preimplantation readings from 23 aged-matched SwAPP mice that was set as 100%. (C) Aβ1-42 concentrations in brain homogenates and sera of i.c.v. implanted SwAPP mice. The values are expressed in percent variation, relative to the baseline obtained by averaging the corresponding values from a control group of four age-matched, non-implanted SwAPP littermates that was set as 100% (**P ≤ 0.01). Note that 8G7 is the only antibody that leads to reduction of Aβ levels in brain and serum in the i.c.v. passive immunization paradigm.

### Table 1

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Recognized immunogene</th>
<th>IgG subtype (light chain)</th>
</tr>
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<tbody>
<tr>
<td>22C4</td>
<td>Aβ1(1-42)</td>
<td>IgG1 (κ)</td>
</tr>
<tr>
<td>10G8</td>
<td>Aβ1(1-17)</td>
<td>IgG1 (κ)</td>
</tr>
<tr>
<td>9G10</td>
<td>Aβ1(1-42)</td>
<td>IgG1 (κ)</td>
</tr>
<tr>
<td>8G7</td>
<td>Aβ1(1-40)</td>
<td>IgG1 (κ)</td>
</tr>
</tbody>
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All antibodies belonged to the IgG1 subtype with the κ light chain.
increase of cerebral Aβ levels. Note that intravenous infusion of 8G7 leads to an unchanged, elevated or reduced in the brains of mice depending on the

4. Discussion

We then tested whether the differences in the bioactivity observed may be attributed to different IgG subtypes, which are known to vary in their opsonization capacities. All antibodies used in this study, however, belong to the same IgG isotype (Table 1).

Consistent with data obtained for the passive immunization achieved by intravenous injection of purified antibodies [15,16], when compared to untreated brains, no consistent alterations in APP processing could be detected by Western blotting in brains of mice involved in the i.c.v. passive immunization paradigm, suggesting that anti-Aβ immune therapy would not interfere with normal APP functions.

4. Discussion

The aim of this study was to test the efficacy of several anti-Aβ antibodies to reduce brain Aβ42 concentrations in vivo and compare this bioactivity to the results of a recently published study using the same antibodies in a conceptually different passive immunization procedure [15].

To compare the efficacy at which Aβ42 levels were reduced in brain or serum of treated mice, Aβ42 concentrations were quantified by ELISA. Different antibodies raised against defined epitopes of the Aβ peptide varied in their activity to mediate Aβ clearance from the brain indicating that the kinetics of Aβ clearance by monoclonal antibodies depended on the recognized epitope by the antibody [15]. Because all antibodies used in this study belong to the same IgG subtype, the observed differences in the bioactivity of these antibodies cannot be attributed to different opsonization capacity that varies between the IgG isotypes.

Anti-Aβ immune therapy is a powerful means to prevent amyloid pathology in transgenic mouse models of AD overexpressing human mutant APP [5–7,9,10,13] and in human subjects [4]. It is an unresolved issue, however, why some anti-Aβ antibodies reduce brain Aβ levels without entering the brain, whereas others are ineffective even though they recognize and bind Aβ in vitro [5,6].

We tested here whether the bioactivity of antibodies against Aβ may be influenced by experimental set up. Therefore, in addition to passive immunization of a mouse model of AD expressing the SwAPP by intravenous injection of purified antibodies (Fig. 2) [15], we set on in this study to evaluate the bioactivity of the same antibodies by a second passive immunization protocol; implanting the hybridoma cells secreting same antibodies intra-ventricularly.

The concept of passive immunization has often been tested in AD mouse models but normally in one experimental paradigm [5,6,18], rendering the generalization as to the applicability of certain antibodies very difficult. Our study therefore aimed, as a first step, in testing different immunization protocols in a mouse model resembling an early stage of AD pathology. We chose young SwAPP mice without plaque pathology to assess antibody-mediated changes in soluble Aβ, because of significant correlation of soluble Aβ concentrations with the degree of cognitive impairment in AD patients. Moreover, we were most interested in Aβ42 because it represents the most amyloidogenic Aβ species, and its levels are massively increased in the progression of pathology in both AD patients and APP transgenic mouse models [19,20].

Blood samples of treated SwAPP mice were taken at perfusion and assessed for peripheral antibody titers. All mice showed a significant elevation of anti-Aβ antibody levels in their blood indicating that grafted hybridoma cells produce the antibodies during the course of the study. After i.c.v. grafting of hybridoma cells, significant reductions in brain and serum Aβ42 concentrations were observed only in SwAPP mice implanted with hybridoma clone 8G7. The antibodies produced by the other three grafted hybridoma clones exerted no significant effect on Aβ42 levels in the i.v.c. passive immunization paradigm, a result that varies considerably from the outcome of the intravenous passive immunization study [15].

In a previous study, only 8G7 promoted a continuous uptake of Aβ42 by primary microglial cells in vitro [15]. All antibodies used in both studies recognize and bind the transgenically expressed Aβ as was shown by Western blotting and have facilitated Fc receptor-mediated microglial uptake of fibrillar Aβ42 in vitro [15]. Furthermore, SwAPP mice treated in both protocols were littermates and the duration of treatment was identical for both immunization protocols. Therefore, we conclude that the variations of the bioactivity of these antibodies to reduce cerebral Aβ concentrations in one immunization protocol must be attributed to the epitope they recognize. More importantly, however, the varying effect of the same antibody between the two immunization protocols should be caused by the different methods of antibody application to the mice.

The absence of an effect on brain levels of soluble Aβ can be explained by the failure of the antibodies to bind Aβ effectively in the periphery and thus exert the effect of a peripheral sink, or by the inability to induce enhanced microglial uptake of Aβ in the brain [21,22]. Only the 8G7 antibody could reduce the Aβ levels in brain and serum of i.c.v. treated mice. This finding is in line with data that mostly antibodies directed against the midportion of Aβ were able to bind the soluble peptide and prevent its oligomerization and fibrillogenesis [5], and the fact

![Fig. 2](image-url)
that the above antibodies (except for 8G7) were directed either against the N- or C-terminal epitopes of Aβ.

Moreover, the biological effects of the 8G7 antibody depended on the routes of antibody application. We speculate that one explanation for this varying bioactivity may be that the intravenous injection of 8G7 may have changed the blood–brain barrier permeability for Aβ. Because the transport of Aβ through the blood–brain barrier is bidirectional [15], it is possible that the peripheral administration of 8G7 caused a shift of Aβ concentrations between the periphery and the brain. In contrast, following the cerebro-ventricular production, when 8G7 is produced in the brain, it seems to be able to interact with Aβ and to alter Aβ catabolism leading to a significant reduction of Aβ levels in the brain and serum compartments.

Even though the concept of passive immunization was tested in AD mouse models before, no study has yet compared the consequences of different application procedures on the bioactivity of the same antibodies in vivo. The present study, in combination with our recently published results [15], offers a comparison of different strategies for the passive immunization in a mouse model mimicking AD pathology for several anti-Aβ antibodies. We show that the bioactivity of anti-Aβ antibodies not only depended on the epitope recognized, but also on the application mode of the antibody. A given antibody can exert an effect in one application strategy, whereas being ineffective or exhibiting opposite effects in another mode of application. These data emphasize the importance of validation the bioactivity of anti-Aβ therapy in several experimental passive immunization set ups prior to use in human subjects. They may be therefore valuable to prevent side-effects that may accompany future immune therapy strategies against AD.

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References


