Microglial activation facilitates Aβ plaque removal following intracranial anti-Aβ antibody administration

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The mechanisms by which anti-Aβ antibodies clear amyloid plaques in Aβ depositing transgenic mice are unclear. In the current study, we demonstrate that inhibition of anti-Aβ antibody-induced microglial activation with anti-inflammatory drugs, such as dexamethasone, inhibits removal of fibrillar amyloid deposits. We also show that anti-Aβ F(ab’)2 fragments fail to activate microglia and are less efficient in removing fibrillar amyloid than the corresponding complete IgG. Diffuse Aβ deposits are cleared by antibodies under all circumstances. These data suggest that microglial activation is necessary for efficient removal of compact amyloid deposits with immunotherapy. Inhibition of this activation may result in an impaired clinical response to vaccination against Aβ.

Keywords: Amyloid deposits; Anti-Aβ; Microglia

Introduction

Alzheimer’s disease (AD) is characterized clinically by progressive cognitive decline and characterized pathologically by amyloid plaques, neurofibrillary tangles and neuron loss (Hardy and Selkoe, 2002). Another pathological event in AD is an inflammatory response which involves the activation and proliferation of microglia and astrocytes (Akiyama et al., 2000). The amyloid hypothesis has targeted the Aβ peptide as the primary focus for therapeutic interventions in AD (Hardy and Selkoe, 2002). Amyloid plaques consist of amyloid-β protein fibrils, which are positively stained by Congo red and thioflavine-S. In addition, diffuse amyloid deposits can be identified using immunohistochemistry.

Vaccination using Aβ1-42 was first described by Schenk et al. (1999). That report showed that immunization with Aβ1-42 in the PDAPP transgenic mouse dramatically reduced Aβ deposit accumulation, both diffuse and compact. The vaccination was later shown to prevent cognitive decline in APP+PS1 (Morgan et al., 2000) and TgCRND8 (Janus et al., 2000) transgenic mice. Passive immunization with anti-Aβ antibodies was also demonstrated to have benefit pathologically (Bard et al., 2000) and cognitively (Dodart et al., 2002; Kotilinek et al., 2002). The Aβ vaccine advanced quickly to human clinical trials where, in Phase II, several patients developed cerebral inflammation, leading to a halt in further inoculations (Schenk, 2002).

The exact mechanism by which immunotherapy reduces Aβ deposition remains unknown; suggested mechanisms include Fc receptor-mediated phagocytosis via microglia (Schenk et al., 1999; Wilcock et al., 2001, 2003), dissolution of amyloid fibrils (Frenkel et al., 1999; Solomon et al., 1997) and sequestration of circulating Aβ resulting in an increased net efflux of Aβ from brain and plasma (DeMattos et al., 2001).

These competing hypotheses have led to disputes regarding the accessibility of circulating antibodies to the CNS, the role of systemic Aβ content in this process and the degree of requirement for specific Aβ epitopes to be targeted by the antibodies (Bard et al., 2003; Holtzman et al., 2002). Moreover, in AD patients, the blood–brain barrier is variably leaky (Hock et al., 2002). Bacskaï et al. (2001) were the first to demonstrate anti-Aβ antibody removal of amyloid deposits following direct application into the brain, therefore bypassing the blood–brain barrier. To identify the potential role of microglia in antibody-mediated removal of Aβ deposits, we have opted to avoid some of the complications regarding brain penetration and apply antibodies directly to the CNS by intracranial injections.

We have recently reported that following intracranial anti-Aβ antibody administration, there is a biphasic clearance of Aβ deposits (Wilcock et al., 2003). The first is a rapid removal of diffuse Aβ deposits occurring between 4 and 24 h after injection. The second is the removal of compact, thioflavine-S-positive amyloid deposits between 24 and 72 h following injection. This removal of fibrillar deposits is associated with a transient activation of microglia, detectable at 72 h, but not 7 days after the injection. Remarkably, by 7 days, both diffuse and compacted Aβ deposits are largely cleared, the microglial reaction has resolved, and the injected anti-Aβ antibody is almost completely removed.

In the current study, we further investigate the relationship between microglial activation and fibrillar amyloid removal. First,
we test the capacity of several anti-inflammatory agents to impair the microglial response and monitor their effect on Aβ clearance. We also investigate whether antibody fragments lacking the Fc domain can clear the fibrillar deposits, and monitor the effects on microglial activation. The results are consistent with the argument that microglial activation and Fc receptor-mediated phagocytosis are important steps in the rapid clearance of Aβ deposits by intracranially administered anti-Aβ antibodies.

Materials and methods

Anti-inflammatory drug study

Singly transgenic APP Tg2576 mice were obtained from our breeding program at USF started in 1996 (Holcomb et al., 1998). In the first experiment, 39 APP transgenic mice aged 16 months were assigned to one of five experimental groups. Four of these groups received intracranial anti-Aβ antibody injections (44-352; Mouse monoclonal anti-human Aβ1-16 IgG1; Biosource, Camarillo, CA) into the frontal cortex and hippocampus at a concentration of 2 μg/2 μl in each region. The remaining group received intracranial anti-HIV monoclonal antibody directed against gp120 (from Ken Ugen, University of South Florida) into frontal cortex and hippocampus at a concentration of 2 μg/2 μl in each region (N = 7) as a control for potential nonspecific activity associated with injecting IgG into the brain. Of the four groups receiving anti-Aβ antibody, one group received no further treatment (N = 8), one group received twice-daily intraperitoneal injections of dexamethasone (Sigma-Aldrich, St. Louis, MO) at a dose of 5 mg/kg (N = 9), one group received twice-daily intraperitoneal injections of minocycline (Sigma-Aldrich) at a dose of 45 mg/kg (N = 7) and one group received once-daily subcutaneous injections of NCX-2216 (nitro-ferulo-flurbiprofen; NiCox, S.A., Sophia-Antipolis, France) at a dose of 7.5 mg/kg (N = 8). All treatments following the intracranial injection were commenced immediately following a 30-min recovery from surgery. All mice were sacrificed at 72 h following surgery and treatments were continued through the morning of sacrifice.

Antibody fragment study

Twenty Tg2576 APP transgenic mice aged 19.5 months were assigned to one of four groups, all groups received intracranial injections into the frontal cortex and hippocampus. The first group received anti-Aβ antibody (2286; mouse monoclonal anti-human Aβ19-40 IgG1; Rinat Neurosciences, Palo Alto, CA) at a concentration of 2 μg/2 μl in each region. The second group received anti-Aβ, F(ab')2 fragments prepared from the anti-Aβ antibody at 2.2 μg/2 μl in each region. The third group received IgG directed against Drosophila anniesiac protein (Rinat Neurosciences) as a control for nonspecific aspects of intact IgG injection. The final group received control F(ab')2 fragments prepared from the IgG directed against Drosophila anniesiac protein to control for nonspecific effects of F(ab')2 injection. All mice survived for 72 h after surgery.

Preparation of F(ab')2 fragments

The ImmunePure IgG1 Fab and F(ab')2 preparation kit (Pierce Biotechnology, Rockford, IL) was used to prepare the F(ab')2 fragments from the anti-Aβ IgG and the control IgG against Drosophila protein. The instructions provided with the kit were followed (http://www.pierceen.com/files/0465jm5.pdf). Briefly, 0.5 ml of 1 mg/ml IgG was added to 0.5 ml mouse IgG1 mild elution buffer. This was applied to an equilibrated immobilized ficin column, allowed to enter the column and digested at 37°C for 20 h. A 4-ml elution was obtained and applied to an equilibrated immobilized protein A column for separation of the F(ab')2 from Fe complexes and undigested IgG. Four 1-ml fractions of product were obtained. As determined by running a gel electrophoresis, only the second and third elutions were found to contain F(ab')2 fragments and appeared of similar intensities on the gel. The two elutions containing F(ab')2 fragments were pooled and concentrated using centricron centrifugal filter devices (Millipore Corp., Bedford, MA) to a volume of approximately 200 μl. Preliminary experiments found that injections of the F(ab')2 fragments concentrated directly from the column caused seizures when injected into some mice. Thus, the initial concentrate was diluted in 4 ml of fresh PBS and reconstituted to dilute residual proprietary elution buffer components which may cause seizures. No seizures or neurotoxicity were found in the mice included here. The concentrated product was run on an SDS-PAGE. A Bradford assay was also performed to establish concentrations of the F(ab')2 fragments using Bradford protein assay reagent concentrate (Bio-Rad, Hercules, CA).

Surgical procedure

On the day of surgery, the mice were weighed, anesthetized with isoflurane and placed in a stereotaxic apparatus (51603 dual manipulator lab standard, Stoelting, Wood Dale, IL). A midsagittal incision was made to expose the cranium and two burr holes were drilled using a dental drill over the right frontal cortex and hippocampus to the following coordinates: cortex: AP +1.5 mm, L 2.0 mm; hippocampus: AP −2.7 mm, L −2.5 mm, all taken from bregma. A 26-gauge needle attached to a 10-μl Hamilton (Reno, NV) syringe was lowered 3 mm ventral to bregma and a 2-μl injection was made over a 2-min period. The incision was cleaned with saline and closed with surgical staples.

Tissue preparation

On the day of sacrifice, mice were weighed, overdosed with 100 mg/kg pentobarbital (Nembutal sodium solution, Abbott laboratories, North Chicago, IL) and intracardially perfused with 25 ml 0.9% sodium chloride followed by 50 ml freshly prepared 4% paraformaldehyde (pH 7.4). Brains were rapidly removed and immersion fixed for 24 h in freshly prepared 4% paraformaldehyde. The brains were then incubated for 24 h in 10%, 20% and 30% sucrose sequentially to cyroprotect them. Horizontal sections of 25-μm thickness were then collected using a sliding microtome.

Fig. 1. Anti-inflammatory drugs impaired fibrillar amyloid removal to roughly the same extent as they decreased microglial activation following anti-Aβ antibody injections. Panels A–E show CD45 immunohistochemistry in the hippocampus. Panels F–J show Aβ immunohistochemistry in the hippocampus. Panels K–O show thioflavine-S staining in hippocampus. Mice were injected intracranially with anti-Aβ antibody followed by no treatment (A, F, K), dexamethasone treatment (B, G and L), minocycline treatment (C, H and M) or NCX-2216 treatment (D, I and N). Mice shown in panels E, J, and O were injected with anti-HIV antibody as a control for nonspecific effects of IgG injection. Magnification ×40. Scale bar = 120 μm.
and stored at 4°C in DPBS buffer with sodium azide to prevent microbial growth.

**Immunohistochemical methods**

Six to eight sections approximately 100 μm apart were selected spanning the injection site and stained using free-floating immunohistochemistry methods for total Aβ (rabbit antiserum primarily reacting with the N-terminal of the Aβ peptide 1:100000) and CD45 (Serotec, Raleigh NC; 1:3000) as previously described (Gordon et al., 2002). For immunostaining, some sections were omitted from the primary antibody to assess nonspecific immunohistochemical reactions. Adjacent sections were mounted on slides and stained using 4% thioflavine-S (Sigma-Aldrich) for 10 min. It should be noted that there are a limited number of sections that include the injection volume. We have opted to measure a few markers reliably rather than a larger number of markers with fewer sections each.

**Data analysis**

The immunohistochemical reaction product on all stained sections was measured using a videometric V150 image analysis system (Oncor, San Diego, CA) in the injected area of cortex and hippocampus and corresponding regions on the contralateral side of the brain. Data are presented as the ratio of injected side to noninjected side for Aβ, thioflavine-S and CD45. Normalizing each injection site to the corresponding contralateral side diminishes the influence of inter-animal variability and permits reliable measurements of drug effects with a smaller number of mice. Importantly, there is no injected antibody detectable in the contralateral side. To assess possible treatment-related differences, the ratio values for each treatment group were analyzed by ANOVA using StatView software version 5.0.1 (SAS Institute Inc., NC) followed by Fischer’s LSD means comparisons.

**Results**

Following intracranial injection of anti-Aβ antibody 44-352 into the hippocampus and frontal cortex, there was a significant activation of microglia detectable by CD45 immunohistochemistry. In the hippocampus, the most intense area of activation appeared in the granule cell layer of the dentate gyrus close to the site of injection within the hilus/CA4 region. However, there was a much more diffuse activation that filled the remainder of the dentate gyrus (Fig. 1A). In the frontal cortex, the activation formed a gradient surrounding the injection site without a clear laminar profile (not shown). Following the intracranial injection of anti-Aβ antibody, treatment with the steroidal anti-inflammatory agent dexamethasone completely inhibited the microglial activation with only several small cells faintly stained for CD45 in hippocampus (Figs. 1B and 2A; P < 0.05) and in frontal cortex (Fig. 2A, P < 0.001). The staining pattern and values observed in this group matched that of the group administered a control IgG antibody alone.

Minocycline, a drug previously shown to inhibit microglial activation in several CNS inflammation models, appeared relatively ineffective at inhibiting the microglial activation observed as a result of intracranial anti-Aβ antibody administration. In the hippocampus, the intense area of activation in the granule cell layer was still present, as was the more diffuse activation in the remainder of the dentate gyrus (Fig. 1C). In the frontal cortex, although there was a significant difference between the minocycline-treated mice and the untreated mice (P < 0.01), the microglial activation in the minocycline-treated mice was still significantly greater than in the dexamethasone-treated mice (P < 0.05; Fig. 2A).

NCX-2216 combines a nitric oxide generating moiety with the typical NSAID drug flurbiprofen. In the hippocampus, NCX-2216 treatment following the intracranial injection of anti-Aβ antibody partially inhibited the activation of microglia (Fig. 1D). This drug did not inhibit the intense activation observed in the granule cell layer of the dentate gyrus but did diminish the more diffuse activation. The quantification from the frontal cortex found a significant inhibition of microglial activation (P < 0.01; Fig. 2A). Thus, with respect to inhibiting microglial activation follow-
ing anti-Aβ antibody injection, dexamethasone was the most effective drug with NCX-2216 having a partial inhibition followed by an even weaker inhibition caused by minocycline.

Total Aβ immunohistochemistry in mice administered the control antibody directed against human immunodeficiency virus (HIV) protein gp120 was similar to that described previously in the APP transgenic mouse (Gordon et al., 2002; Hsiao et al., 1996). The ratio of Aβ in the right/left sides was also the same as that observed previously in unmanipulated APP transgenic mice (Wilcock et al., 2003). The Aβ immunohistochemistry showed a few large, intensely stained deposits, which are normally also stained by Congo red or thioflavine-S, indicating fibrillar compact amyloid deposits. There were also a large number of smaller, less intensely stained deposits analogous to diffuse amyloid deposits observed in human AD brain tissue. In the hippocampus, Aβ deposition was localized primarily to the molecular layers of the dentate gyrus and Ammon’s horn adjacent to the hippocampal fissure, as well as a large concentration in the subiculum (Fig. 1J).

Anti-Aβ antibody administration into frontal cortex and hippocampus resulted in a reduction of total Aβ immunohistochemistry 72 h following injection (Fig. 1F). This reduction was approximately 80% in frontal cortex and 65% in hippocampus compared

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to APP transgenic mice administered HIV antibody (Fig. 2B). The anti-inflammatory agents dexamethasone, NCX-2216 and minocycline had no effect on the removal of this largely diffuse Aβ deposits. The only antibody which activated microglia 72 h following intracranial injection into frontal cortex and hippocampus was the intact anti-Aβ antibody 2286 and a control monoclonal antibody 2286 and a control monoclonal antibody directed against the Drosophila protein amnesiac were analyzed via SDS-polyacrylamide gel electrophoresis (PAGE). The gel showed very pure product, with a single band at approximately 105 kDa, the molecular weight for F(ab')2 fragments. The intact IgG molecule produced one intense band at approximately 150 kDa, the correct molecular weight for IgG molecules and a less intense band at approximately 110 kDa. Following confirmation of purity via SDS-PAGE, we then performed a Bradford assay to assess the concentration of F(ab')2 fragments injected intracranially was 1.2 μg/μl, while the complete IgG concentration was 1 μg/μl, resulting in an excess of anti-Aβ Fv domains in the (ab')2 solutions.

The only antibody which activated microglia 72 h following intracranial injection into frontal cortex and hippocampus was the intact anti-Aβ 2286 antibody. The frontal cortex shows a greater degree of activation than the hippocampus, however, in both regions, the activation is significantly greater than that in the groups receiving control anti-amnesiac protein IgG, F(ab')2, or anti-Aβ F(ab')2 (Figs. 3A, C, D and 4A; P < 0.01 or greater in all comparisons). The pattern of activation in the hippocampus following the anti-Aβ antibody 2286 injection resembled that shown in Fig. 1A when using the anti-Aβ antibody 44-352. There is a very intense area of activation in the granule cell layer of the dentate gyrus, with a much more diffuse activation filling the remainder of the dentate gyrus (Fig. 3A). Interestingly, the anti-Aβ F(ab')2 fragments produced no microglial activation in either the frontal cortex and hippocampus (Figs. 3B and 4A).

Aβ immunohistochemistry in the two anti-amnesiac protein control groups shows the typical staining pattern observed in APP transgenic mice at 19.5 months (Figs. 3G and H). This pattern was qualitatively the same as observed at 16 months (Fig. 1J), although quantitatively greater as the mice were 3.5 months older. Both the anti-Aβ antibody and the anti-Aβ F(ab')2 groups significantly reduced total Aβ immunohistochemistry to a similar extent 72 h following injection into frontal cortex and hippocampus. In the frontal cortex, there was a reduction of approximately 60% (Fig. 4B). In the hippocampus, the reduction was approximately 65% (Figs. 3E, F and 4B).

Thioflavine-S staining detects only compact fibrillar amyloid deposits. The mice receiving intracranial injections of either
control anti-amnesiac protein IgG or control F(ab')2 resembled the typical staining observed in the APP transgenic mouse at this age. In the hippocampus, the majority of thioflavine-S-positive plaques were in the outer molecular layer of Ammon’s horn and the dentate gyrus near the hippocampal fissure (Figs. 3K and L). Anti-Aβ antibody IgG significantly reduced thioflavine-S-positive compact plaque by approximately 90% in the frontal cortex and hippocampus (Fig. 4C). There were no, or very few, remaining thioflavine-S-positive deposits in the hippocampus (Fig. 3I). In contrast, the anti-Aβ F(ab')2 fragments did not remove compact amyloid plaques as effectively as the whole IgG molecule. In the frontal cortex, there was no significant reduction in thioflavine-S staining when compared to either control antibody group (Fig. 4C). In the hippocampus, there was a significant difference between the anti-Aβ F(ab')2 group and the control groups (P < 0.05), however, this reduction was also significantly less than the reduction observed with the whole IgG molecule (Figs. 3J and 4C; P < 0.02 or greater).

Discussion

The data presented here support the argument that activation of microglia in APP transgenic mice facilitates the removal of compact amyloid plaques. The first experiment, using several anti-inflammatory agents to regulate the microglial response, showed that the extent of fibrillar amyloid removal roughly corresponds to the extent of microglial activation 3 days after intracranially applied anti-Aβ antibody. The second study identified that anti-Aβ F(ab')2 fragments were less capable of activating microglia (presumably because they lacked the Fc domain) and were significantly less effective than the corresponding whole IgG in removing fibrillar Aβ, despite the presence of excess anti-Aβ Fv in the F(ab')2 injections.

Our earlier work in this system demonstrated that intracranial administration of anti-Aβ antibodies into APP Tg2576 mice resulted in diffusion of the antibody throughout most of the hippocampus by 4 h, however, no specific localization to amyloid plaques was noted. This caused a rapid removal of diffuse Aβ deposits between 4 h (when no reduction is detected) and 24 h (when removal of diffuse deposits appeared complete; Wilcock et al., 2003). This removal was not associated with any apparent activation of microglia using markers such as MHC-II or CD45 at 24 h, nor was there any reduction of the fibrillar amyloid deposits measured with thioflavine-S staining. However, by 72 h following the injection, there was a dramatic reduction of thioflavine-S-positive compact amyloid deposits associated with a florid microglial activation as detected by CD45 immunohistochemistry. One week following the anti-Aβ antibody injection, the injection site remained devoid of most forms of amyloid, the microglial reaction had terminated and the injected antibody had been fully cleared from the area.

An issue of concern with these studies is whether the Aβ epitope utilized for immunohistochemistry is masked by the injected antibody. This issue was addressed in our previous work where it was shown that although there is a broad distribution of the injected antibody 4 h following injection, no reduction in Aβ immunohistochemistry is apparent (Wilcock et al., 2003). Also, if the reduction observed is simply an artifact of masking the Aβ epitope, a reduction in thioflavine-S staining would not be observed since this is a conformation-dependent stain and not epitope dependent. Further, the stoichiometry of injected antibody (13 pmol) to Aβ in deposits (estimated at 250 pmol in 0.5 mg, Chapman et al., 1999) is likely too low to interfere substantially with the histochemical reaction. Finally, 7 days after injection, the injected antibody is no longer detectable, yet the Aβ immunostaining remains absent.

The present report further investigated the relationship between activation of microglia and the clearance of the fibrillar amyloid plaques associated with anti-Aβ antibody injections. We used several distinct pharmacological agents in an attempt to inhibit the microglia activation observed 72 h following intracranial injection of anti-Aβ antibodies. Dexamethasone is a glucocorticosteroid that inhibits the cyclooxygenase and lipoxygenase inflammatory pathways as well as inducing a general state of immunosuppression. It has been shown that microglia respond differently to mineralocorticoid and glucocorticoid receptor stimulation. Mineralocorticoid receptor activation stimulates the microglia while glucocorticoid receptor activation inhibits microglia (Tanaka et al., 1997). All pharmacological glucocorticosteroids possess some degree of mineralocorticoid action also. For the present study, we selected dexamethasone as it has the maximum glucocorticoid receptor activity with the minimum mineralocorticoid receptor activity detectable among all available pharmacological glucocorticosteroids (Schimmer and Parker, 2001). It was found that dexamethasone was the most efficacious compound for the inhibition of microglial activation among those used in this study. Dexamethasone administered immediately following intracranial anti-Aβ antibody administration completely inhibited the microglial activation caused by the injection. Associated with this profound arrest of microglial activation was a complete inhibition of the antibody’s ability to remove compact amyloid deposits detected with thioflavine-S staining, strongly suggesting a role for microglial involvement in the removal of compact amyloid deposits.

The novel nonsteroidal anti-inflammatory (NSAID) NCX-2216, which is a flurbiprofen molecule conjugated to an antioxidant and a nitric oxide releasing group, was moderately effective at inhibiting microglial activation. Interestingly, this compound has previously been shown to cause the activation of microglia and removal of amyloid from the brains of otherwise untreated doubly transgenic APP+PS1 mice (Jantzen et al., 2002). NCX-2216 has also been shown to inhibit the microglial activation caused by intracranial infusion of lipopolysaccharide (LPS), a pro-inflammatory agent in young rats, but to increase microglial activation in old rats (Hauss-Wegrzyniak et al., 1999). In the present study, NCX-2216 partially reduced the activation of microglia caused by antibody injection. Associated with this partial inhibition of microglial activation is also a partial impairment of the anti-Aβ antibody’s capacity to remove the compact amyloid plaques. The discrepancy between the effects observed in the current study and the effects previously observed by Jantzen et al. (2002) may be explained by the fact that NCX-2216 essentially is three drugs. In a situation where there is intense, local microglial activation, the anti-inflammatory properties of the drug appear to dominate. In a situation where there is diffuse microglial activation, it appears that maybe the nitric oxide release dominates to enhance microglial activation and aid in the clearance of Aβ. Although not quantified, the microglial reaction immediately adjacent to amyloid deposits appeared more intense in NCX-2216-treated mice in regions distant from the injection (e.g. contralateral anterior cortex). A similar bidirectional effect of this drug was found by Hauss-Wegrzyniak et al. (1999) who found reduced microglial activation.
in young rats, but enhanced activation in old rats treated with the NCX-2216 relative, nitrofurflurbiprofen.

Minocycline is a tetracycline derivative which has been shown to have a novel action independent of its antibiotic property. This agent has been shown to inhibit microglial activation following excitotoxicity (Tikka et al., 2001), ischemia (Yrjonheikki et al., 1998) and 6-hydroxydopamine lesions (He et al., 2001). In the present study, we demonstrate that minocycline is capable of a modest inhibition of microglial activation following antibody injection. Associated with this is no difference in compact plaque removal in either the frontal cortex or hippocampus.

To further investigate whether the activation of microglia, which appears to be specific to the anti-Aβ antibody, is due to Fc receptor activation, we administered F(ab′)2 fragments intracranially into APP transgenic mice as well as the whole IgG, control IgG and F(ab′)2 fragments from the control IgG. We found that of the four experimental groups, only the animals receiving anti-Aβ IgG showed significant activation of microglia. The fact that anti-Aβ F(ab′)2 fragments were unable to activate microglia strongly suggests that Fc receptor activation is required for the significant activation of microglia following intracranial administration of anti-Aβ antibodies. Associated with this inability to activate microglia was a significantly impaired capacity to remove fibrillar amyloid deposits. Still, at least in hippocampus, there was some residual capacity for fibrillar amyloid removal using the F(ab′)2 fraction. However, with respect to diffuse Aβ clearance, the F(ab′)2 are just as effective as the corresponding intact IgG. These results suggest that there may be an equilibrium between the fibrillar deposits and the diffuse deposits, and the F(ab′)2 antibody fragments can whittle away at the fibrillar plaques without requiring Fc receptor-mediated phagocytosis. This is also the likely explanation why Bacskai et al. (2002) found topically applied F(ab′)2 fragments equally effective to intact antibodies in the clearance of thioflavine-S-labeled material. It is plausible that had we injected a greater amount of F(ab′)2 fragments, or extended the post-injection interval, we also might have found complete clearance of fibrillar amyloid with the F(ab′)2 material. However, although it may be capable of clearing fibrillar amyloid, the results presented here demonstrate F(ab′)2 fragments were much less efficient than the intact IgG molecule in mediating clearance associated with microglial activation. It is plausible that this also explains the recent observations from Bard et al. (2003) that the ability of different monoclonal anti-Aβ antibodies to clear brain amyloid when administered systemically was correlated better with their capacity to bind Fc receptors than with their affinity for Aβ. The amounts of antibody entering the brain are roughly 0.1% of the injected amount per hour (Banks et al., 2002). Thus, a 500-μg injection of a monoclonal antibody should result in 0.5 μg entering the CNS within 60 min, somewhat less than the amounts injected directly in our system (2 μg). Therefore, with systemically injected antibodies, the facilitation of amyloid removal by Fc receptor-mediated phagocytosis is likely to be even greater than that observed here with intracranially administered antibodies.

Certainly, it will be useful to measure Aβ content by means other than histochemistry. Increasingly complex methods of fractionating homogenates are being used to identify Aβ pools linked most closely to neuropathology and cognitive disruption (Golde et al., 2000; Lue et al., 1999), but consensus regarding the specific fractions corresponding to the most toxic form of amyloid has not yet been achieved (Kayed et al., 2003; Walsh et al., 2002). Additionally, the relatively small portions of the brain affected by the intracranial injections and difficulty dissecting these regions consistently limits the ability to use solution methods to evaluate Aβ loads in our studies. As consensus emerges regarding the relationships among soluble, oligomeric and fibrillar forms of Aβ in the solution domain and the histochemical domain, and larger portions of the brain become involved with either intraventricular or systemic antibody injections, we will investigate the effects of anti-Aβ antibodies on these different Aβ pools in both domains.

It has been shown previously that vaccination using Aβ1-42 results in activation of microglia, which is associated with a reduction in Aβ accumulation in PDAPP transgenic mice (Schenk et al., 1999) and APP+PS1 transgenic mice (Wilcock et al., 2001). It has also been shown that following direct application of anti-Aβ antibodies to the brains of PDAPP mice, there is an activation of microglia and a reduction in amyloid deposits (Bacskai et al., 2001). In vitro studies using F(ab′)2 fragments demonstrated that they were unable to activate microglia despite retaining full ability to bind to Aβ (Bard et al., 2000). These fragments also failed to remove fibrillar Aβ in an ex vivo assay. Human postmortem microglia have been shown to phagocytose opsonized Aβ, which is inhibited by excess nonspecific IgG, suggesting this phagocytosis is Fc receptor mediated (Lue and Walker, 2002). All of these data suggest that one likely mechanism of antibody action in removing amyloid deposits from transgenic mouse brains is via binding to microglial Fc receptors and triggering activation of the microglia, possibly including phagocytosis of the opsonized amyloid. This by no means precludes other possible mechanisms, such as catalytic dissolution of amyloid fibrils (Solomon et al., 1997), or sequestration of Aβ in the periphery, effectively drawing Aβ out of the brain (DeMattos et al., 2001).

Recently, the first pathology report from a patient receiving the Aβ1-42 vaccination (AN1792) was published (Nicoll et al., 2003). This report showed that the patient had considerably fewer amyloid deposits than would have been predicted from other AD cases. Interestingly, it is reported that in those regions devoid of amyloid plaques, the remaining Aβ immunoreactivity was associated with activated microglia. This patient did develop meningocerephalitis and other symptoms of CNS inflammation, as did several others in the trial. The treatment chosen for the CNS inflammation in this case was dexamethasone.

Assuming that the mechanisms of Aβ vaccination in clearing amyloid is similar to that demonstrated in the present work, it might be anticipated that dexamethasone would counter the amyloid removing effects of the vaccine. Although the case described by Nicoll et al. above did show evidence of removal, the patient had received five inoculations before developing adverse reactions and being administered dexamethasone. It was also not indicated what the antibody titer was in the patient, nor how long the dexamethasone treatment had continued. The data presented here suggest that administration of glucocorticoids to vaccinated patients may counteract any benefit the vaccine has with respect to amyloid clearance and possibly cognitive function. It is also conceivable that removal of only the soluble and diffuse Aβ may provide cognitive improvement and inhibition of microglial activation by anti-inflammatory drugs or administration of F(ab′)2 fragments may avoid some of the inflammatory adverse effects observed in the human clinical trial (Orgogozo et al., 2003). The inhibition of microglial-mediated amyloid clearance may also have been a factor in the failure of the prednisone clinical trial for AD (Aisen et al., 2000).
The adverse reactions in the human vaccine trial demonstrates a need to more fully investigate the mechanisms involved in beneficial and detrimental effects of immunotherapy. Encouraging data recently published by Hock et al. (2003) showed that the a subset of patients administered the Aβ vaccine remained cognitively stable for 1 year after treatment while the control patients declined at a normal rate, some vaccinated patients actually improved. While the immunotherapeutic approach may hold promise for the treatment of AD, it would appear very important to better understand both the mechanisms of vaccine action, and how the tools to effectively modulate the immune reaction interact with these mechanisms. The data presented here make some headway toward determining what effects modulation of the immunotherapeutic approach mechanisms would have pathologically. Future studies will extend these investigations to a more clinically relevant, systemically administered, passive immunization regimen to determine the importance of the mechanisms discussed in the current study.

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References


