Aβ-Immunotherapy for Alzheimer’s Disease Using Mannan–Amyloid-Beta Peptide Immunoconjugates

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ABSTRACT

In Alzheimer’s disease (AD) the accumulation of pathological forms of the beta-amyloid (Aβ) peptide are believed to be causal factors in the neurodegeneration that results in the loss of cognitive function in patients. Anti-Aβ antibodies have been shown to reduce Aβ levels in transgenic mouse models of AD and in AN-1792 clinical trial on AD patients; however, the clinical trial was halted when some patients developed meningoencephalitis. Theories on the cause of the adverse events include proinflammatory “primed patients,” a Th1-inducing adjuvant, and Aβ autoreactive T cells. New immunotherapy approaches are being developed to eliminate these putative risk factors. Mannan, which is recognized by pattern recognition receptors of the innate immune system, can be utilized as a molecular adjuvant to promote a Th2-mediated immune response to conjugated B cell epitopes. The N-terminus of Aβ was conjugated to mannan, and used to immunize mice with low concentrations of immunoconjugate, without a conventional adjuvant. Mannan induced a significant and highly polarized toward Th2 phenotype anti-Aβ antibody response not only in BALB/c, but also in B6SJL F1 mice. New preclinical trials in AD mouse models may help to develop novel immunogen–adjuvant configurations with the potential to avoid the adverse immune response that occurred in the first clinical trial.

INTRODUCTION

ALZHEIMER’S DISEASE (AD) is a most common form of dementia in the elderly and is characterized by a progressive loss of memory and general cognitive decline. The neuropathological features of the disease include neurofibrillary tangles (NFT), deposition of amyloid-beta (Aβ) in senile plaques, and neuronal loss in affected brain regions (Price and Sisodia, 1994). The amyloid cascade hypothesis that proposed a central role of Aβ deposition in the brain in the onset and progression of AD (Hardy and Higgins, 1992; Hardy and Selkoe, 2002), remains to be a rationale for therapeutic strategies (Golde, 2005). Thus, reduction of the level of Aβ in the brain may diminish learning and memory deficits observed in AD patients. Recently, several groups have demonstrated that active immunization of amyloid precursor protein (APP) transgenic (Tg) mice with fibrillar Aβ, as well as passive transfer of anti-Aβ antibodies, significantly reduced Aβ plaque deposition, neuritic dystrophy, and astrogliosis in the brains of these mice (Schenk et al., 1999; Bard et al., 2000; Morgan et al., 2000; Wilcock et al., 2004a). Improvements in learning and memory were also observed after either active or passive immunization of APP/Tg mice (Janus et al., 2000; Morgan et al., 2000; Dodart et al., 2002; Sigurdsson et al., 2004; Wilcock et al., 2004a; 2004b).

Based on these results, the AN-1792 vaccine clinical trial was initiated with AD patients, but was halted because a subset of participants developed meningoencephalitis. Although the results of the first vaccination of elderly AD patients with Aβ₁₋₁₄₂ peptide raised concerns about the safety of AN-1792 vaccine, follow-up studies suggest that anti-Aβ antibodies were capable of reducing AD pathology and, at least in some patients, diminishing the progressive cognitive decline associated with the disease (Hock et al., 2003; Nicoll et al., 2003; Ferrer et al.,

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Mannan has been investigated extensively as a molecular adjuvant due to its ability to enhance both B- and T-cell immune responses (Okawa et al., 1992; Apostolopoulos et al., 1995, 2000; Karanikas et al., 1997; Vaughan et al., 2000; Stambas et al., 2002a, 2002b, 2005). The adjuvant function is dependent on the ability to target the immunogen to antigen-presenting cells expressing receptors specific to mannosylated sugars. Mannose-binding receptors (MBRs) are expressed on dendritic cells, some endothelial cells, and macrophages (Engering et al., 1995, 2000; Cribbs and Agadjanyan, 2005). Previously, it was demonstrated that a Th2-type of humor response in APP/Tg mice was therapeutically effective (Weiner et al., 2000). Thus, an adjuvant that can direct the immune response towards a Th2 phenotype may be critical for the design of a safe and effective immunotherapy for AD patients.

In this paper we report the development of a novel AD vaccine consisting of the N-terminus of Aβ28, conjugated to mannan. Previously, it was demonstrated that B-cell antigenic determinants using a competition ELISA plate may mask some of the peptide's epitopes, we determined B-cell antigenic determinants using a competition ELISA. To identify the B-cell antigenic determinant/s within Aβ42, we used four overlapping 15-mer peptides (Aβ1-15, Aβ16-20, Aβ11-25, and Aβ16-30) for epitope mapping. Since adsorption of a peptide to an ELISA plate may mask some of the peptide’s epitopes, we detected B-cell antigenic determinants using a competition ELISA as previously described (Cribbs et al., 2003b).

**Detection of anti-Aβ42 antibodies**

Eight to 9 days after each immunization, sera from mice were collected, and anti-Aβ42 antibodies, as well as their isotypes, were determined by ELISA (Cribbs et al., 2003b; Ghochikyan et al., 2003; Petrushina et al., 2003; Agadjanyan et al., 2005). To identify the B-cell antigenic determinant/s within Aβ42, we used four overlapping 15-mer peptides (Aβ1-15, Aβ16-20, Aβ11-25, and Aβ16-30) for epitope mapping. Since adsorption of a peptide to an ELISA plate may mask some of the peptide’s epitopes, we detected B-cell antigenic determinants using a competition ELISA as previously described (Cribbs et al., 2003b).

**Detection of anti-Aβ T-cell proliferation and production of cytokines by immune splenocytes**

Nine days after the last boost, BALB/c mice were sacrificed, and anti-Aβ T-cell responses were analyzed using splenocyte
cultures from individual mice. We used HL-1 serum-free synthetic medium (Cambrex, Baltimore, MD) for our T-cell stimulation assays, because it significantly decreases nonspecific activation of splenocytes, allowing measurement of T-cell activation (proliferation, cytokine production, and Th1 and Th2 subsets) more accurately. To detect proliferation of splenocytes, we restimulated individual culture of cells with A/H9252 40 peptide and measured 3[H]thymidine uptake, as described previously (Cribbs et al., 2003b). Data are presented as the Stimulation Index (SI), and were calculated for each mouse.

We used the ELISPOT technique to detect production of IFNγ-Th1) or IL4 (Th2) lymphokines, as well as TNFα (proinflammatory) cytokine in restimulated splenocytes from experimental mice. Experiments were conducted as recommended by the manufacturer (PharMingen, San Diego, CA) and as we described previously (Cribbs et al., 2003b). The colored spots were counted, and the results were examined for differences between stimulated and nonstimulated conditions for each experiment using one-way ANOVA and Tukey’s posttest, Graph Pad Prism 3.03.

Detection of Aβ plaques in human brain tissues

Sera from immunized mice were screened for the ability to bind to Aβ plaques on tissue sections from an AD case as we described previously (Ghochikyan et al., 2003; Agadjanyan et al., 2005). Briefly, pooled sera (dilution 1:500) were added to the serial 50-μm brain sections of formalin-fixed frontal cortical tissue from patients with neuropathological and behavioral patterns typical of severe AD. Sections were pretreated with 90% formic acid, and exogenous peroxidase was quenched. A negative control, monoclonal antihuman Aβ antibody 6E10 (Signet Laboratories, Dedham, MA) was used. Binding of antibodies to the brain sections was detected by Vectastain Elite ABC Mouse anti-IgG/avidin–HRP system (Vector Labs, Burlingame, CA) with DAB, according to the manufacturer’s recommendations. A digital camera (Olympus, Japan) was used to collect images of the plaques at 20× image magnification.

RESULTS

Immunogenicity of Aβ28 peptide

Prior to testing the Aβ28–mannan conjugates, we first evaluated the immunogenicity of the peptide alone in different strains of mice. Previously, we showed that BALB/c mice recognized the B- and T-cell antigenic determinants of Aβ42 within the first 28 aa of this peptide (Cribbs et al., 2003b). In fact, BALB/c mice of H-2d immune haplotype generated high titers of anti-Aβ antibodies (Petrushina et al., 2003; Gevorkian et al., 2004), and the highest level of anti-Aβ T cell responses after immunizations with fibrillar Aβ42 peptides (Cribbs et al., 2003b). We directly analyzed the immunogenicity of fibrillar Aβ28 peptide in this strain of mice and compared it with that in mice of H-2b (C57BL/6), H-2b (SJL), and H-2b/c (B6SJL F1),
were moderate. However, a difference in the level of anti-Aβ bodies. Next, we tested the potency of mannan as an adjuvant with fibrillar Aβ28 at all, indicating that this immune haplotype did not recognize a T-cell epitope within this immunogen. B6SJL F1 animals induced the highest titer of anti-Aβ antibodies, whereas the levels of anti-Aβ antibody in SJL and BALB/c mice were moderate. However, a difference in the level of anti-Aβ antibody response between these groups is not significant (p > 0.05). Immunization of APP/Tg 2576 mice of H-2<sup>b</sup>s, H-2<sup>d</sup> immune haplotypes, as well as APP/Tg 2576 animals, which have H-2<sup>b</sup>d background induced the lowest level of anti-Aβ<sub>42</sub> antibody response. These results were consistent with our previous findings that C57BL/6 do not respond to Aβ<sub>28</sub>, and that for APP/Tg 2576 mice human Aβ<sub>28</sub> represents a self-antigen. However, collectively these data confirmed that wild type mice of H-2<sup>b</sup>, H-2<sup>b</sup>/4<sup>+</sup>, H-2<sup>d</sup> immune haplotypes, as well as APP/Tg 2576 mice recognized Aβ<sub>28</sub> immunogen and produced anti-Aβ antibodies. Next, we tested the potency of mannan as an adjuvant in BALB/c wild-type mice.

**Conjugation with mannan significantly enhances immunogenicity of the Aβ<sub>28</sub> peptide**

To determine the ability of mannan to enhance the immunogenicity of Aβ<sub>28</sub> peptide, we vaccinated BALB/c mice with mannan–Aβ<sub>28</sub> conjugate or fibrillar Aβ<sub>28</sub>. The groups of experimental mice were injected with 2.5, 5, or 10 µg of Aβ<sub>28</sub> conjugated with mannan, whereas control mice were immunized with 10 µg Aβ<sub>28</sub>. Of note, we did not use any additional adjuvant, and prepared both immunogens in PBS. The sera collected from experimental and control mice were analyzed for the presence of anti-Aβ<sub>42</sub> antibodies after one, two, and three biweekly boosts (Fig. 2). Three immunizations of mice with 10 µg of fibrillar Aβ<sub>28</sub> were not sufficient to generate a detectable anti-Aβ<sub>42</sub> antibody response. However, after a third boost this group of mice generated a low level of antibody response that was equal to that induced in animals immunized four times with 2.5 µg of mannan–Aβ<sub>28</sub> conjugate. In contrast, mice immunized with 5 and 10 µg of mannan–Aβ<sub>28</sub> induced low titers of anti-Aβ antibodies after the first boost. The second and third boosts with these doses of mannan–Aβ<sub>28</sub> significantly enhanced anti-Aβ antibody production (≥4–8 times). Of note, although 5 µg peptide induced higher response than 10 µg, the difference was not statistically significant. These mice were boosted one more time after a 2-month rest period. Anti-Aβ<sub>42</sub> antibody responses were analyzed before and 9 days after the fourth boost (Fig. 3). After 2 months of rest, the level of anti-Aβ<sub>42</sub> antibodies decreased slightly (32.5 ± 6% on average). As expected, a single boost enhanced a production of anti-Aβ<sub>42</sub> antibodies with the largest increase detected in groups of mice immunized with 5 and 10 µg (data not shown) mannan–Aβ<sub>28</sub>. We concluded that mannan was an effective molecular adjuvant that induced long-lasting anti-Aβ antibody responses in mice immunized with 5 and 10 µg of mannosylated Aβ<sub>28</sub> peptide, although variability of humoral immune responses in individual mice was significant.

**Mannosylated Aβ<sub>28</sub> induced a Th2 polarized immune response**

Antibody isotyping has been used as an indirect measure of the contribution of Th1 (IgG2a) and Th2 (IgG1) cytokines to the humoral response (Finkelman et al., 1990). In addition, it was demonstrated that the subclass of anti-Aβ<sub>42</sub> antibodies might correlate with their therapeutic potential (Solomon et al., 1996; Bard et al., 2000; Dodart et al., 2002; McLaurin et al., 2002). Thus, we measured production of IgG1, IgG2a, IgG2b, and IgM anti-Aβ antibodies in the sera of immunized BALB/c mice. Both fibrillar Aβ<sub>28</sub> and mannan–Aβ<sub>28</sub> induced primarily IgG1 antibodies after immunization and three boosts (Fig. 4A). The ratio of IgG1 to IgG2a antibody in mice immunized with Aβ<sub>28</sub> was 6. However, sera from mice immunized with mannan conjugated to Aβ<sub>28</sub> significantly enhanced the highly polarized Th2 type immune response. The IgG1/IgG2a ratios in the sera of these animals increased to 15. Since Balb/c mice are

![FIG. 2. Mannan enhanced immunogenicity of Aβ<sub>28</sub> peptide. BALB/c mice were immunized and boosted one, two, or three times with the indicated dose of mannan–Aβ<sub>28</sub> or fibrillar Aβ<sub>28</sub>. Pooled sera were used for detection of binding to Aβ<sub>42</sub>-coated wells. Representative ELISA data from three experiments are presented.](image-url)
Th2-prone, in order to demonstrate the real contribution of mannan in Th2 polarization of immune response we immunized B6SJL F1 mice with mannan–Aβ28 and measured production of IgG1 and IgG2a/b antibodies (Fig. 4B). The ratio of IgG1 to IgG2a/b antibody in immunized B6SJL F1 mice was 11 (Fig. 4C). Thus, mannan conjugation enhanced Th2-polarized anti-Aβ antibody responses, as has been observed after conjugation of mannan with other peptide immunogens (Okawa et al., 1992; Apostolopoulos et al., 1995, 1996; Vaughan et al., 1999; Apostolopoulos and McKenzie, 2001).

To directly demonstrate a role of the mannan conjugation in Th1– and Th2-type immune responses, we analyzed the cellular immune responses in individual vaccinated mice. The splenocytes from immune mice were restimulated with Aβ42, and the T-cell proliferation was analyzed (Fig. 5A). Both fAβ28 and mannan–Aβ28 induced robust T-cell proliferation with stimulation index of 8.2 and 7.7, respectively (Fig. 5A), although antibody responses in mice immunized with fAβ28 were significantly weaker than in mice administered with mannan–Aβ28 (Figs. 2 and 3). Next, we analyzed a production of Th1 (IFN-γ) and Th2 (IL-4) lymphokines by immune splenocytes isolated from mice immunized and boosted four times with 5 μg of mannan–Aβ28 and compared it with data obtained after vaccination with fibrillar Aβ28. In addition, we detected a production of the pro-inflammatory cytokine TNFα that is expressed by activated macrophages, monocytes, neutrophils, lymphocytes, and natural killer cells and has been suggested to play a pivotal role in regulation of the synthesis of other pro-inflammatory cytokines (Arend and Dayer, 1995). Our data demonstrated that only a small number of splenocytes from mice immunized with the mannan-conjugated immunogen, but not fibrillar Aβ28, produced this pro-inflammatory cytokine after in vitro restimulation with Aβ28 (Fig. 5B). On the contrary, the same immune splenocytes generated the highest number of cells producing IL4, and immunization of mice with fibrillar Aβ28 was less effective than vaccination of mice with Aβ28 conjugated with mannan.

**B-cell epitope specificity of anti-Aβ28 antibodies**

To demonstrate the specificity of antibodies and to identify the B-cell antigenic determinant/s within the Aβ28 immunogen, we screened the antisera with a series of short overlapping peptides encompassing entire Aβ28 peptide using a competition ELISA assay (Cribbs et al., 2003b). Preincubation of antisera with 2.5 μM of the full-length Aβ42 peptide resulted in strong

![FIG. 3. Long-lasting anti-Aβ antibody responses in mice immunized with 5 μg of Aβ28 conjugated with mannan (the data with similar profile were obtained with 2.5 and 10 μg mannan–Aβ28). After vaccination and three booster injections mice were rested for 2 months, and the immune response was recalled with the appropriate antigen. Individual sera from animals were diluted 1:250 and tested for detection of anti-Aβ28 antibodies in ELISA. The experiment was repeated with similar results.](image)

![FIG. 4. (A) BALB/c mice immunized with mannan–Aβ28 (5 μg) or fibrillar Aβ28 formulated in PBS induced Th2-polarized anti-Aβ42 antibodies of IgG1 isotype (similar results were obtained with 2.5 and 10 μg mannan–Aβ28). Sera for this assay were collected before the rest period (after the third boost) and diluted 1:250 prior to the detection of isotypes. These results were generated with individual mice. (B) Immunization of B6SJL F1 mice with mannan–Aβ28 also induced anti-Aβ42 antibodies of IgG1 isotype. (C) IgG1/IgG2a (ab) ratio for BALB/c mice immunized with mannan–Aβ28 or fibrillar Aβ28 and B6SJL F1 mice immunized with mannan–Aβ28.](image)
inhibition of antibody binding to Aβ1-15 on the plate (Fig. 6). At 2.5 μM, the Aβ1–15 peptide was equally effective at blocking the binding of antibodies from mice immunized with fibrillar Aβ28 or the mannan–Aβ28 immunogen. Notably, Aβ6–20, Aβ11–25 or Aβ16–30 peptides were ineffective (Fig. 6). Thus, vaccination with both fibrillar Aβ28 and mannosylated Aβ28 activated B cells specific to the B-cell epitope in the Aβ1–15 peptide.

To further analyze the potential therapeutic efficacy of anti-Aβ antibodies generated in response to the Aβ28–mannan conjugate vaccine, we also determined binding to amyloid plaques in human brain tissue. We used pooled sera from mice immunized with mannan–Aβ28 and observed that this antiserum bound to amyloid plaques on the brain sections of cortical tissues from a severe AD case (Fig. 7). Preimmune sera from these mice and irrelevant immune sera did not bind to the amyloid plaques (data not shown). These data suggest that anti-Aβ antibodies raised after immunizations with Aβ28–mannan conjugate were potentially functional, as it was demonstrated previously with antibodies specific to fibrillar Aβ42 (Schenk et al., 1999; Bard et al., 2000; Janus et al., 2000; Morgan et al., 2000) or Aβ1–15 peptide fused with foreign T-cell epitope (Agadjanyan et al., 2005).

DISCUSSION

Anti-Aβ-immunotherapy is a novel strategy to induce antigen-specific humoral immune responses therapeutic for AD. Although the failure of the first anti-Aβ-immunotherapy clinical trial was disappointing, the follow-up studies indicate that Th2-prone immune response may be more beneficial and safer than a Th1 response. Mannan has previously been shown to be a potent molecular adjuvant enhancing both B- and T-cell immune responses, as well as antigen uptake and presentation (Okawa et al., 1992; Apostolopoulos et al., 1995, 2000; Engering et al., 1997a, 1997b; Karanikas et al., 1997; Gröger et al., 2000; Linehan et al., 2000; Vaughan et al., 2000; Stambas et al., 2002a, 2002b, 2005). This molecular adjuvant not only enhanced antibody responses specific to appropriate peptide attached to it (Okawa et al., 1992; Apostolopoulos et al., 1995, 2000), but under certain conditions also induced Th2-polarized immunity (Okawa et al., 1992; Apostolopoulos et al., 1995, 1996; Vaughan et al., 1999; Apostolopoulos and McKenzie, 2001). Taking advantage of this property of mannan, we designed a novel AD vaccine that will induce Th2-prone immune responses directed to the Aβ peptide. The data presented here further support the previous observations and demonstrate that mannan conjugates can enhance Th2-polarized immune responses to the Aβ28 peptide immunogen.

Previously, we have demonstrated that Aβ28 peptide possessed both B- and T-cell antigenic determinants of Aβ12 in BALB/c mice (Cribbs et al., 2003a). In this study, we confirm
this observation by demonstrating production of anti-Aβ42 antibodies in wild-type mice of H-2<sup>b</sup>, H-2<sup>k</sup>, and H-2<sup>b</sup>/k immune haplotypes immunized with 100 μg of Aβ28 peptide formulated in alum, which is a Th2-type adjuvant (Fig. 1). Of note, previously several groups, including us (Das et al., 2003; Petrushina et al., 2003; Seabrook et al., 2004; Kutzler et al., 2005), demonstrated that C57BL/6 mice respond to fibrillar Aβ42 poorly. The results generated here indicate that H-2<sup>b</sup> immune haplotype does not respond to Aβ28 peptide immunization, suggesting that these mice may not recognize a T-cell epitope within this peptide. While further investigation of these data is required in order to demonstrate an exact mechanism for the lack of response in H-2<sup>b</sup> mice, it does emphasize the significant hurdle facing development of a small epitope vaccine in humans, which have multiple MHC haplotypes. We chose the Aβ28 peptide as a prototype immunogen to test the effectiveness of mannan as a molecular adjuvant to break tolerance against a self-peptide. We hypothesized that mannan should not only enhance antigen uptake and presentation, but may also induce better crosslinking of B-cell receptors, which amplifies the signal to Aβ specific B cells (Reth and Wienands, 1997; Wagle et al., 2000). The data presented here showed that very low doses of mannan–Aβ28 induced activation of B cells and generation of anti-Aβ28 antibodies after only one boost (Fig. 2). Even 2.5 μg of mannan–Aβ28 was active after two additional boosts with mannan–Aβ28. In fact, the level of the humoral immune response in this group was similar to that generated in BALB/c mice immunized with 10 μg of fibrillar Aβ28 (Fig. 2). To check the longevity of the immune responses, mice from all groups were allowed to rest for 2 months. Importantly, mice boosted with mannan–Aβ28 responded to a single recall injection with the same antigen, suggesting that significant immunological memory was present in these mice (Fig. 3). The specificity of these antibodies was confirmed by a competition ELISA in which antiserum from immune mice were preadsorbed by small overlapping linear peptides Aβ1-15, Aβ6-20, Aβ11-25, or Aβ16-30. Consistent with previous studies with fibrillar Aβ42 vaccine (Cribbs et al., 2003b), antiserum raised in mice immunized with mannan–Aβ28 was specific only to Aβ1-15 (Fig. 6) and bound to amyloid plaques in cortical tissue from an AD patient (Fig. 7).

Next, we tested the Th1 and Th2 phenotype of humoral immune responses by analyzing isotypes of anti-Aβ antibodies generated in vaccinated mice. Antibody isotyping has been used as an indirect measure of the contribution of Th1 (IgG2a) and Th2 (IgG1) cytokines to the humoral response (Finkelman et al., 1990); thus, we measured anti-Aβ42 IgG2a and IgG1 antibodies in the sera of immune mice. Data indicated that both mannan–Aβ28 and fibrillar Aβ28 in PBS induced highly polarized Th2-type humoral immune responses (IgG1/IgG2a ratios were equal to 15 and 6, respectively) (Fig. 4). To confirm that mannan–Aβ28 induces Th2-type immune responses not only in typically Th2-prone Balb/c mice, we measured the production of anti-Aβ42 IgG2a<sub>b</sub> and IgG1 antibodies in B6SJL F1 mice immunized with mannan–Aβ28 and showed that IgG1/IgG2a<sub>b</sub> ratio is 11. Analysis of T-cell responses supported these results. More specifically, immune splenocytes from BALB/c mice immunized with mannan–Aβ28 after restimulation with Aβ40 peptide induced robust T-cell proliferation and generated substantial amounts of CD4<sup>+</sup>Th2 cells, as well as a higher percent of cells producing IL4 (Th2) than IFNγ (Th1) cytokines (Fig. 5). Thus, mannan–Aβ28 induced predominantly anti-inflammatory Th2 type immune responses in BALB/c mice immunized with mannan–Aβ28 peptide. Th1 cytokines (IL12, IL18, and IFNγ) have been implicated in many autoimmune disorders, whereas Th2 type responses (IL-4, IL-10, and TGFβ) in some cases have been shown to attenuate cell-mediated immunity and inhibit autoimmune disease (Smeltz and Swanborg, 1998; Aharoni et al., 2000; O’Shea et al., 2001; Swanborg, 2001; Weiner and Selkoe, 2002). Therefore, the bias of the anti-Aβ immune responses towards a Th2 phenotype may be potentially beneficial for AD patients. As stated above, we are currently investigating neuropathological changes in APP/Tg 2576 mice vaccinated with mannan–Aβ28, and preliminary data suggest that these animals generated robust anti-Aβ antibody production that can clear/inhibit AD-like pathology in the brains of these animals (Petrushina et al., 2006). The development of second-generation vaccine candidates, which promote a Th2-mediated immune response and the removal of the self T-cell epitope of the Aβ from the immunogen, may help to develop novel immunogen–adjuvant configurations which reduce the risk of adverse events that occurred during the first clinical trial in AD patients.

FIG. 7. Mannan–Aβ28 induced potentially therapeutic anti-Aβ<sub>1-15</sub> antibodies, which are capable of binding to amyloid plaques in human brain tissue (A). Coincubation with Aβ28 peptide blocked this binding (B). 6E10 antibodies were used as a positive control (C). Picture represents binding of 1:500 diluted pooled sera to a 50-μm brain section of formalin-fixed cortical tissue from an elderly individual with neuropathological and behavioral patterns typical to severe AD. Original magnification, ×20.
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