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Meningoencephalitis associated with passive immunization of a transgenic murine model of Alzheimer's amyloidosis

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Abstract Immunization against the Aβ peptide reverses the pathologic and behavioral manifestations of Alzheimer's disease in murine models. Since active immunization is associated with an autoimmune meningoencephalitis in a subset of humans, passive transfer of anti-Aβ immunoglobulin is being pursued as a potentially safer alternative. We have identified cases of meningoencephalitis subsequent to peripheral and intracerebral passive immunization of Tg2576 mice. The vasocentric mononuclear infiltrate localized only to brain regions affected by Aβ amyloid deposits suggesting that the inflammatory reaction was Aβ specific. This report indicates that current passive immunization in humans should proceed with careful regard for autoimmune complications.

Keywords: Alzheimer; Autoimmunity; Inflammation; Aβ; Mononuclear; Vasocentric

1. Introduction

The cerebral accumulation of the hydrophobic peptide, Aβ, is an invariant feature of Alzheimer's disease (AD) [1]. Immune-based therapies directed against the Aβ peptide have been shown in transgenic murine models to ameliorate Aβ amyloid pathology and reverse cognitive behavioral deficits [2–6]. Based in part on the successful clearance of Aβ and the lack of toxicity in murine models, human clinical trials were performed using an active immunization paradigm. However, a major side effect of active immunization with $A\beta_{42}$ in humans is the development of an aseptic, vasocentric meningoencephalitis in a subset of patients [7,8]. As a result, passive immunization with anti-Aβ monoclonal antibodies is currently being pursued as a potentially safer alternative.

While the mechanism underlying the inflammatory reaction in humans has not been completely elucidated, the predominance of T cells in cerebral infiltrates suggested that a cell-mediated autoimmune response was responsible for the development of meningoencephalitis [7]. Furthermore, the development of meningoencephalitis did not correlate with ELISA titers for anti-Aβ antibodies, suggesting that humoral-mediated autoimmunity was not involved in the pathogenesis of autoimmunity upon active immunization with $A\beta_{42}$ [8].

Autoimmune reactions to active or passive anti-Aβ immunotherapy in murine models have not been reported. Here, we report the development of meningoencephalitis in the Tg2576 model of Alzheimer's amyloidosis upon passive immunization with an anti-Aβ monoclonal antibody.

2. Materials and methods

2.1. Peripheral immunization

Tg2576 mice overexpressing human APP harboring the Swedish mutation [9] were maintained on a C57B6/SJL F2 background by successive backcrossing to wild-type C57B6/SJL F1 females. All mice were generated and handled according to University of Pennsylvania Institutional Animal Care and Use Committee guidelines. Wild-type and transgenic littermates were administered an initial dose of 400 μg of protein G-purified NAB61, an anti-Aβ mouse monoclonal antibody generated in our laboratory using nitrated Aβ as immunogen, or non-specific mouse IgG (Sigma, St. Louis, MO) intraperitoneally on day 1, followed by maintenance doses of 200 μg on days 4, 10 and 16.

2.2. Intracerebral immunization

Six- to twelve-month old Tg2576 mice or Tg2576/PS-$1^{P246L/+}$ mice generated from crossing heterozygous Tg2576 mice with mice heterozygous for the P246L PS-1 familial AD mutation [10] were used. Fifteen mice received stereotaxic intracerebral injections of NAB61 ($n = 8$) or non-specific mouse IgG ($n = 7$) with a 33-gauge Hamilton syringe (Hamilton, Reno, NV) into the left hippocampus at one of the following two coordinates with respect to bregma: −2.7 mm posterior, +2.5 mm lateral, and −3.0 mm ventral; −2.3 mm posterior, +2.0 mm lateral, and −1.8 mm ventral. Mice received 2–4 μg of immunoglobulin and were sacrificed 3–7 days post-treatment.

2.3. Immunohistochemistry and histology

Following completion of the immunization protocols, mice were deeply anesthetized and transcardially perfused with heparinized PBS. Brains were removed and immersion fixed in 10% neutral buffered formalin. Samples were dehydrated through a series of graded ethanol solutions to xylene, and infiltrated with paraffin as described [11]. Six micrometer-thick sections were immunostained using standard avidin-biotin-peroxidase methods with 3-3′diaminobenzedene. Rat anti-mouse Mac-3 (BD Biosciences Pharmingen, San Diego, CA), rat anti-mouse CD45R/B220 (BD Biosciences Pharmingen) and rat anti-human CD3 (MCA1477; Serotec, Oxford, UK) were used as primary antibodies in addition to anti-Aβ antibodies to visualize Aβ deposits. To detect mouse immunoglobulin, the primary antibody was omitted. Species-specific HRP-conjugated anti-immunoglobulin

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Abbreviations: AD, Alzheimer's disease; CNS, central nervous system

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antibodies (Vector Laboratories, Burlingame, CA) were used as secondary antibodies. Routine histochemistry was also performed using hematoxylin and eosin, and thioflavin S.

3. Results

A novel, Aβ-specific monoclonal antibody, NAB61, was developed for a passive immunization trial. NAB61 recognizes mature senile plaques and amyloid angiopathy in affected AD and transgenic mouse brain sections (Fig. 1). No staining with NAB61 was seen in non-pathologic human and murine tissue. A short immunization schedule was followed in which a cohort of 18–19-month old APP transgenic mice [9] were injected intraperitoneally with 400 μg of NAB61 ($N = 21$) or non-specific murine IgG ($N = 17$), followed by booster immunizations of 200 μg on days 4, 10 and 16. As this was a pilot trial of this procedure, the mice reported here were sacrificed on day 20 for pathologic analysis.

Although, an autoimmune inflammatory response has not been described for transgenic murine models of intraparenchymal brain Aβ deposits, the complications encountered upon active immunization in humans prompted a thorough pathologic evaluation for complications secondary to passive immunization. Out of 21 transgenic mice immunized with NAB61, one case of meningoencephalitis was identified. Hematoxylin and eosin staining of sections taken throughout the central nervous system (CNS) demonstrated the presence of small, mononuclear inflammatory cells affecting the leptomeninges overlying regions which display Aβ pathology including the olfactory bulb, neocortex and hippocampus (Fig. 2, arrowheads). Consistent with an autoimmune inflammatory response, follicular aggregates with apparent germinal centers could be identified within the leptomeninges (Fig. 3). Furthermore, the absence of polymorphonuclear lymphocytes indicated that no acute inflammatory component was present. The inflammatory response extending along blood vessels also disrupted the blood–brain barrier, as evidenced by increased immunoreactivity for endogenous mouse immunoglobulin throughout the parenchyma of the brain subjacent to affected blood vessels (Fig. 4). Finally, the inflammatory response also involved the parenchyma, manifested as a vasocentric mononuclear infiltrate (Fig. 5). Regions without abundant Aβ pathology such as the striatum, cerebellum, brainstem and spinal cord were generally not affected (Fig. 2). Furthermore, no inflammatory response has been identified in transgenic mice treated with non-specific IgG ($N = 17$), or non-transgenic mice treated with NAB61 ($N = 10$), suggesting that the meningoencephalitis was specific to NAB61 treatment in animals containing Aβ amyloid pathology.

To further define the inflammatory response, immunohistochemistry for Mac3, CD3 and CD45R was performed to identify macrophages, T cells and B cells, respectively (Fig. 3). In contrast with the described case from the human immunization

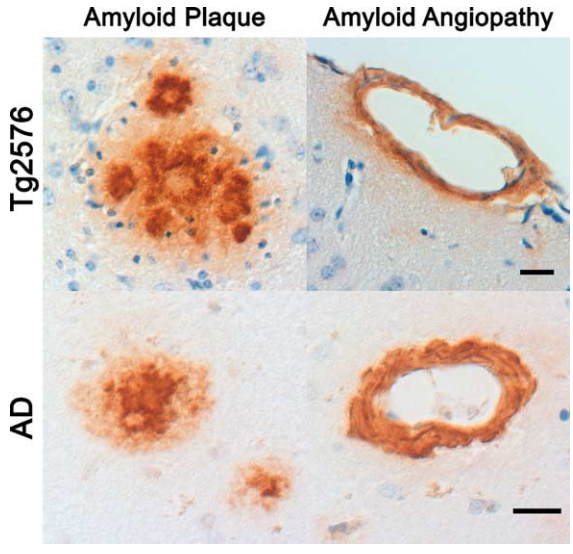

Fig. 1. NAB61 recognizes senile plaques and amyloid angiopathy. NAB61 is an Aβ-specific mouse monoclonal antibody which recognizes pathologic Aβ deposits by immunohistochemistry. Brain sections of Tg2576 brain (top) and AD brain (bottom) were stained with NAB61 demonstrating specific staining of amyloid plaques and amyloid angiopathy. No NAB61 immunostaining was present in sections from unaffected human brain, non-transgenic mouse brain, and young Tg2576 mouse brains prior to onset of Aβ amyloid plaques (data not shown). Scale bars, 20 μm.

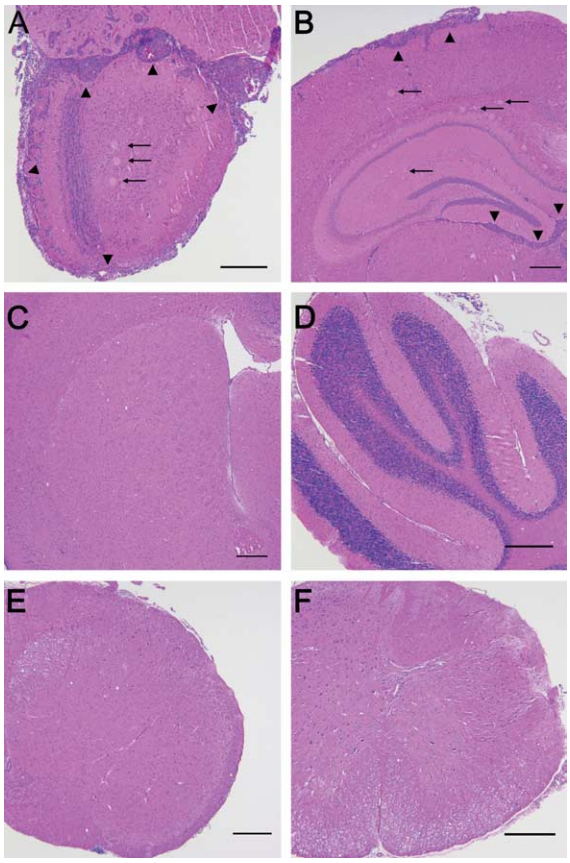

Fig. 2. Region-specific meninoencephalitis. Sections from various brain regions were stained with hematoxylin and eosin. Mononuclear infiltrates were observed in regions affected by Aβ amyloid pathology including the (A) olfactory bulbs, (B) cortex and hippocampus. Other regions, such as the (C) striatum, (D) cerebellum, (E) brainstem and (F) spinal cord were generally free of inflammatory cells. No polymorphonuclear lymphocytes were observed. Arrowheads point to the extensive leptomeningeal involvement. Arrows point to amyloid plaques. Scale bars, 300 μm.

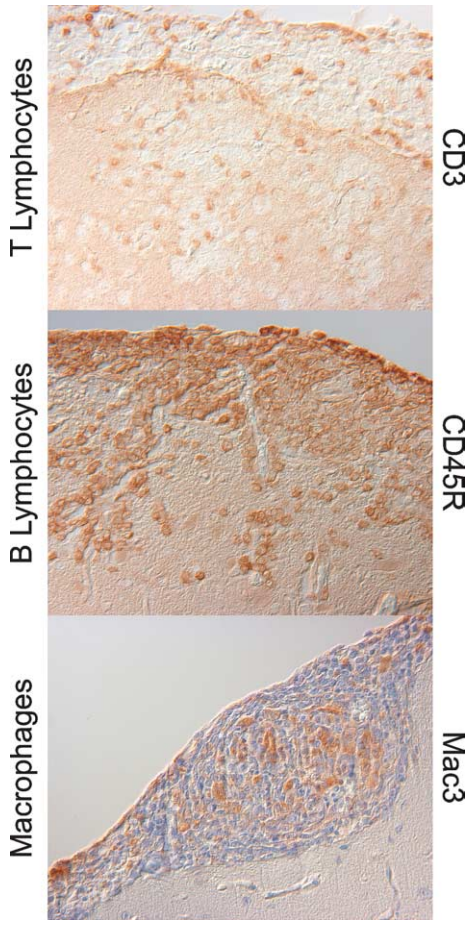

Fig. 3. Mononuclear lymphocytic chronic inflammation. Immunohistochemical detection of inflammatory infiltrates was conducted using antibodies that recognize CD3, CD45R and Mac3 which demonstrated the presence of T cells, B cells and macrophages, respectively. The bottom panel shows an example of the follicular aggregates of lymphocytes forming apparent germinal centers within the leptomeninges.

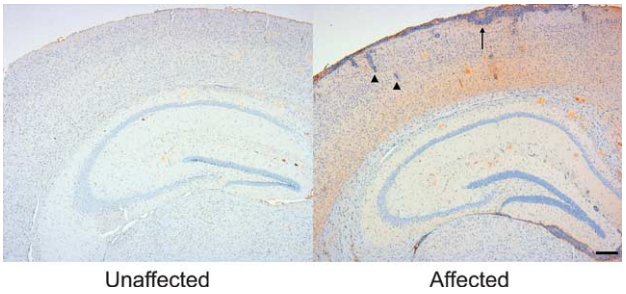

Fig. 4. Disruption of the blood–brain barrier. Direct detection of immunoglobulin using HRP-conjugated anti-mouse IgG resulted in diffuse DAB staining within the brain parenchyma of the mouse affected by meningoencephalitis (right). Unaffected mice showed little to no parenchymal staining for immunoglobulin (left). Nuclei are stained blue with a hematoxylin counterstain. Arrowheads point to inflammatory infiltrates which track with vascular structures. The arrow points to a follicular aggregate of lymphocytes. Scale bars, 200 μm.

trial [7], this inflammatory response appeared to be predominantly comprised of B cells, although macrophages and T cells were also present.

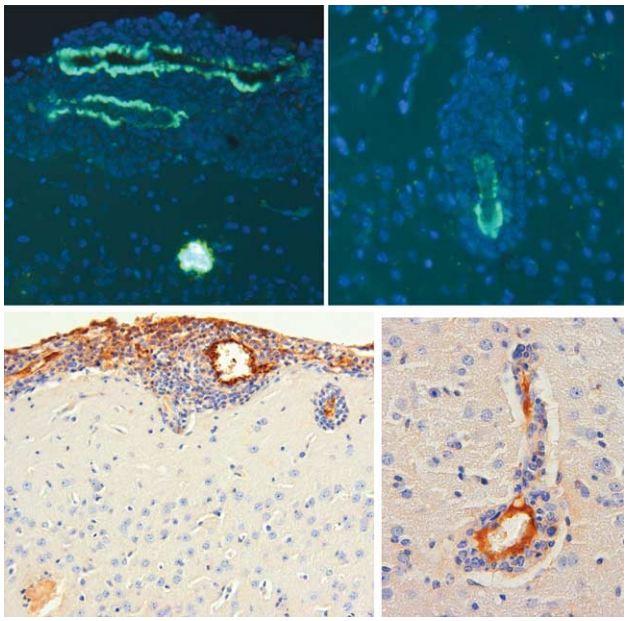

Fig. 5. Vasocentric mononuclear infiltration. Sections were stained with Thioflavin S for Aβ amyloid (green) and counterstained with DAPI for nuclei (blue). Mononuclear infiltrates were associated with amyloid-laden blood vessels in both the meninges and within the cortical parenchyma (top panels). Direct detection of immunoglobulin using HRP-conjugated anti-mouse IgG demonstrated the presence of immunoglobulin deposits (brown) in affected meningeal and cortical vasculature (bottom panels, with a hematoxylin counterstain for nuclei).

Since NAB61 was administered via the peripheral circulatory system, we analyzed the meningeal and intraparenchymal vasculature of affected brain regions. Vasocentric lymphocytic infiltration was often centered around blood vessels containing amyloid angiopathy (Fig. 5, top panels), as determined by Thioflavin S staining for amyloid. Furthermore, affected blood vessels contained immunoglobulin deposits as determined by immunohistochemistry (Fig. 5, bottom panels). These findings suggest that immunoglobulin deposition within amyloid laden blood vessels may serve as a nidus for an autoimmune inflammatory response.

In addition to the systemic passive immunization trials, intracerebral injections of NAB61 were performed in transgenic mice. Out of 8 mice which received intrahippocampal injections of NAB61 (2–4 μg), two had significant infiltrates in close proximity to the injection track. Similar to the case identified after peripheral immunization, hematoxylin and eosin staining demonstrated perivascular mononuclear infiltrates which were immunoreactive for macrophage, T lymphocyte and B lymphocyte markers (Fig. 6). Mac3-positive macrophages were also found along the length of the cortical needle track. Infiltrates were not observed in the contralateral hemisphere or sections of cortex distant to the injection track, and polymorphonuclear inflammatory cells were not present. In contrast, no inflammation could be identified by hematoxylin and eosin staining of mice injected with non-specific IgG ($n = 7$). However, immunohistochemical staining of IgG-injected mice did show macrophages along the injection track consistent with the typical reaction to a stab wound. Lymphocytes were generally absent, with the exception of two mice

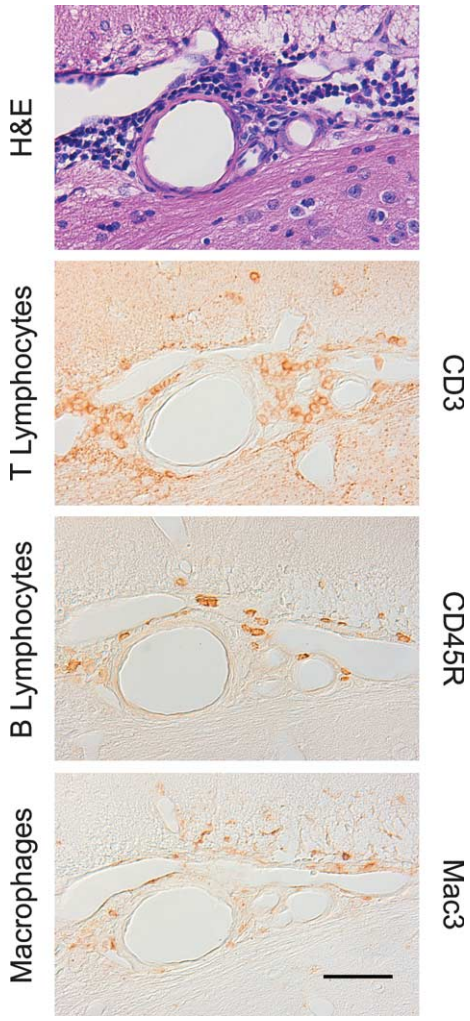

Fig. 6. Inflammation with intracerebral NAB61 injection. Tg2576 and Tg2576/PS-1$^{P246L/+}$ mice showed leptomeningeal, vasocentric, mononuclear infiltrates as determined by hematoxylin and eosin (H&E) staining after intrahippocampal injection of NAB61. Additional immunostaining for CD3, CD45R and Mac3 demonstrated the presence of T cells, B cells and macrophages, respectively. Scale bars, 50 μm.

which had very sparse, scattered lymphocytes without perivascular cuffing, perhaps representing a low-level non-specific response to the trauma of intracerebral injection with immunoglobulin. However, significant lymphocytic infiltration could only be identified in mice passively immunized with NAB61.

4. Discussion

The development of meningoencephalitis in 6% of individuals immunized with $A\beta_{42}$ halted a phase II human AD clinical trial. This adverse event calls into question the safety of active immunization for the treatment of AD [7,8]. Anti-Aβ immunotherapy-induced meningoencephalitis appears to be due to the aberrant activation of a deleterious T cell response that can compromise blood vessel integrity. Furthermore, it does not correlate with the presence of anti-Aβ antibodies as determined by ELISA titers [8]. Therefore, administration of anti-Aβ antibodies could be a safer alternative to active Aβ immunization for the treatment of AD.

This is the first report of meningoencephalitis in APP transgenic mice that model brain parenchyma deposits of Aβ upon active or passive immunization. The Tg2576 transgenic mice used in this study were maintained on a mixed C57B6 × SJL background and, therefore, display considerable haplotype heterogeneity which might account for the variable and infrequent occurrence of the adverse consequence of passive immunization described here. Further investigation is necessary to better understand the mechanisms underlying the variable penetrance of autoimmunity in both mice and humans.

Multiple mechanisms may be responsible for inflammatory changes after active or passive Aβ immunization. The pathologic findings of passive immunization described in this report are consistent with an inflammatory mechanism triggered by antibody binding to Aβ amyloid angiopathy. Such binding may enhance antigen presentation of Aβ resulting in activation of reactive T cells. Alternatively, immunoglobulin deposition may lead to local disruption of the blood–brain barrier whereby antigens normally sequestered in the CNS are presented to the immune system, triggering a more generalized autoimmune response. In support of the latter hypothesis, the incidence of intracerebral hemorrhage is increased in APP transgenic mice with prominent Aβ vascular amyloid deposits that resemble congophilic angiopathy upon passive immunization with anti-Aβ antibodies [12]. Furthermore, we found that direct inoculation of the CNS with NAB61 resulted in perivascular inflammation. In either situation, humoral and cellular-based immunity cannot be clearly segregated into isolated systems. Indeed, autoantibodies have been demonstrated to exacerbate the development of type 1 diabetes which is typically considered to be a T cell mediated autoimmune disease [13], suggesting that the humoral and cellular components of autoimmunity can be interdependent.

Regardless of the mechanism, any immunologic approach to therapy should be undertaken with careful regard towards unwanted complications such as those reported here. An autoimmune response to active immunization with $A\beta_{42}$ resulted in adverse events in clinical trial subjects despite the fact that similar adverse effects had not been described for active immunization in murine models. Given the current attempts at passive immunization for the treatment of AD in humans, the presence of aseptic autoimmune meningoencephalitis in a murine model of Alzheimer's Aβ amyloidosis warrants careful monitoring for similar complications in humans.

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