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*J Immunol* 2010; 185:6338-6347; Prepublished online 13 October 2010; doi: 10.4049/jimmunol.1001765

http://www.jimmunol.org/content/185/10/6338

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**Supplementary Material**

http://www.jimmunol.org/content/suppl/2010/10/14/jimmunol.1001765.DC1

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Active Immunization with Amyloid-β 1–42 Impairs Memory Performance through TLR2/4-Dependent Activation of the Innate Immune System

Patrick Vollmar,* Jennifer S. Kullmann,† Barbara Thilo,‡ Malte C. Clausen,* Veit Rothhammer,* Hortenzia Jacobi,* Johann Sellner,* Stefan Nessler,*1 Thomas Korn,*2 and Bernhard Hemmer*2

Active immunization with amyloid-β (Aβ) peptide 1–42 reverses amyloid plaque deposition in the CNS of patients with Alzheimer’s disease and in amyloid precursor protein transgenic mice. However, this treatment may also cause severe, life-threatening meningoencephalitis. Physiological responses to immunization with Aβ1-42 are poorly understood. In this study, we characterized cognitive and immunological consequences of Aβ1-42/CFA immunization in C57BL/6 mice. In contrast to mice immunized with myelin oligodendrocyte glycoprotein (MOG)35–55/CFA or CFA alone, Aβ1-42/CFA immunization resulted in impaired exploratory activity, habituation learning, and spatial-learning abilities in the open field. As morphological substrate of this neurocognitive phenotype, we identified a disseminated, nonfocal immune cell infiltrate in the CNS of Aβ1-42/CFA-immunized animals. In contrast to MOG35–55/CFA and PBS/CFA controls, the majority of infiltrating cells in Aβ1-42/CFA-immunized mice were CD11b+CD14+ and CD45 high, indicating their blood-borne monocyte/macrophage origin. Immunization with Aβ1-42/CFA was significantly more potent than immunization with MOG35–55/CFA or CFA alone in activating macrophages in the secondary lymphoid compartment and peripheral tissues. Studies with TLR2/4-deficient mice revealed that the TLR2/4 pathway mediated the Aβ1-42-dependent proinflammatory cytokine release from cells of the innate immune system. In line with this, TLR2/4 knockout mice were protected from cognitive impairment upon immunization with Aβ1-42/CFA. Thus, this study identifies adjuvant effects of Aβ1-42, which result in a clinically relevant neurocognitive phenotype highlighting potential risks of Aβ immunotherapy.


Abbreviations used in this paper: Aβ, amyloid β; AD, Alzheimer’s disease; APP, amyloid precursor protein; BL, baseline; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; KO, knockout; MOG, myelin oligodendrocyte glycoprotein; PAMP, pathogen-associated molecular pattern; p.i., post-immunization; PTX, pertussis toxin.

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The online version of this article contains supplemental material.
aimed at characterizing: 1) the cognitive profile and the histopathological manifestation of wild-type mice challenged with AB1–42 immunization; 2) distinguishing the inflammatory response in AB1–42-immunized animals from classical experimental auto-immune encephalomyelitis (EAE); and 3) unraveling the immunological mechanisms behind the inflammatory processes in AB1–42-immunized mice in the systemic compartment and within the CNS.

**Materials and Methods**

**Mice**

Female C57BL/6 mice were obtained from Charles River Laboratories (Sulzfeld, Germany) and were used in experimental paradigms at the age of 6–8 wk. TLR2/4-deficient mice on the C57BL/6 background were provided by C. Kirschning (Institute of Medical Microbiology, Technische Universität Munich, Munich, Germany). All procedures were conducted in compliance with the local guidelines for animal experimentation.

**Immunization procedures**

Animals were immunized s.c. with 100 μg/animal human AB1–42 peptide (American Peptide Company, Sunnyvale, CA; EZBiolab, Carmel, CA) emulsified in CFA containing 5 mg/ml Mycobacterium tuberculosis (strain H37Ra, DIFCO Laboratories, Detroit, MI). EAE induction was performed by s.c. injection of 100 μg/animal of myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (Jerini, Berlin, Germany) emulsified in CFA. Control animals received CFA with PBS. On days 0 and 2, all animals were injected with 500 ng/animal pertussis toxin (PTX; Sigma-Aldrich, Munich, Germany) i.p.

**Behavioral tests**

Open field. For evaluation of habituation and visuospatial learning, the open field test was conducted as previously described (13, 14). Briefly, the open field was a square arena (30 × 30 × 40 cm) with clear Plexiglas walls and a grid square floor composed of nine equal quadrants. At the beginning of the test, mice were placed in the center of the open field and left to freely explore. The total number of quadrant borders the mice crossed and the number of rearings were counted by a blinded observer during a 10-min observation period. Baseline values were assessed prior to immunization. According to O’Keefe and Nadel’s cognitive map theory (15), exploration of a novel environment is used by the animal to construct a cognitive map, and activity wanes once such a map is established. Therefore, habituation to an open field is a measure of memory, and the faster a cognitive map is established, the sooner exploration activity will decrease. To assess a habituation learning measure (habituation learning index), the difference of crossed segments in the first and last 150 s of each 10-min observation period was determined (16). The open field test was repeated every 3 d.

Clinical signs of EAE were ranked with an established score from 0–5: 0 (normal); 1 (tail limpness), 2 (paraparesis with clumsy gait); 3 (hind limb paralysis); 4 (hind limb and forelimb paralysis); and 5 (death). All ratings were done by observers blinded to the treatment.

**Visuospatial learning task.** Visuospatial learning performance was tested in the open field paradigm with slight modifications from published protocols (17). For ethical reasons, the water maze paradigm was not applied, as some of the animals in the MOG/1–42/CFA-immunized control group developed severe parases.

For 3 consecutive learning d, mice were placed into the open field in which two identical objects (bottles) in terms of height, color, shape, and surface texture were located. Spatial configuration did not change for three training sessions. On day 4, the bottle in the corner was moved to the opposite corner, leaving the configuration and distance of the objects undisturbed. The total exploration time for each object was determined during a 10-min observation period. Object exploration was defined as physical contact with the bottle by mouth, vibrissae, and forepaws. Compassing or sitting inactively next to the objects was not regarded as object exploration. For statistical evaluation, the initial exploration time for each stimulus in the first session was calculated, and the relative change in exploration time of the replaced stimulus in the fourth session was determined.

**Macrophage depletion**

For systemic depletion of macrophages, mice were given i.p. injections of clodronate liposomes according to established protocols (18). Briefly, mice received an initial dose of 100 mg/kg clodronate liposomes (kindly provided by R. Schwendener, Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland) followed by subsequent injections of 50 mg/kg every fourth day. Control mice were injected with empty liposomes. Immunization with PBS/CFA or AB1–42/CFA plus PTX was performed 3 d after the initial clodronate injection.

**Serum Ab production**

Serum Abs against human and murine AB1–42 peptides were determined by ELISA according to established protocols (19). On day 28 p.i. with PBS/CFA or human AB1–42/CFA, anti-AB1–42 Abs in the sera of the animals were captured by solid-phase human or murine AB1–42 followed by detection of mouse IgG with HRP-labeled goat anti-mouse IgG (AbD Serotec, Raleigh, NC).

**Cell separation**

Cells immunoreactive for CD11b and CD11c were isolated from naïve mouse spleen tissue by magnetic cell sorting with MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. Purity of cells (>90%) was confirmed by FACS analysis.

**Peritoneal macrophages**

Primary macrophages were isolated from the peritoneal cavity of mice 12 d p.i. according to previously published protocols (20). For assessing cytokine production, these cells were cultured (2 × 10^6 cells/ml) in media (DMEM medium containing 10% FCS, nonessential amino acids, HEPES, t-glutamine, and antibiotics) for 48 h at 37°C in a humidified incubator at 5% CO2. For gene expression studies, mRNA was isolated directly after harvesting the cells from the peritoneal cavity.

**Isolation of mononuclear cells from the CNS**

Mice were perfused with cold PBS through the left cardiac ventricle on day 10 p.i. The brain was dissected, and the spinal cord was flushed out by hydrostatic pressure. CNS tissue was cut into pieces and digested with 2.5 mg/ml Collagenase D (Roche Diagnostics, Indianapolis, IN) and 1 mg/ml DNase I (Sigma-Aldrich) in DMEM medium at 37°C for 40 min. Single-cell suspensions were prepared using a 70-μm cell strainer followed by percoll gradient centrifugation (70%–37%). Mononuclear cells were removed from the interphase, washed, and resuspended in culture medium.

**Surface staining and flow cytometry**

Mononuclear cells were stained for CD11b, CD14, and CD45 (BD Biosciences, San Jose, CA) according to the manufacturer’s instructions. Analyses were performed on a Dako CyAn flow cytometer system (DakoCytomation, Glostrup, Denmark). Flow cytometric data were analyzed with FlowJo (Tree Star, Ashland, OR).

**AB peptide and cell stimulation**

Lyophilized human AB1–42 peptide (obtained from American Peptide Company or EZBiolab) was reconstituted with PBS at a concentration of 2 mg/ml. Dissolved peptide was stored at 4°C for up to 48 h. Where indicated, murine AB1–42 (American Peptide Company) was used. In stimulation experiments, CD11b+ and CD11b+CD11c+ cells (2 × 10^6 cells/ml) were stimulated with different concentrations of AB1–42 peptide (0.1–50 μg/ml) or 100 ng/ml LPS (Sigma-Aldrich) for 48 h at 37°C in culture medium in a humidified incubator at 5% CO2.

**Cytokines**

Cytokine levels were determined in sera and culture supernatants. Cell culture supernatants were collected after indicated incubation periods and stored at −80°C until analysis. Levels of IL-1β, IL-6, IFN-γ, and TNF were measured by commercial ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**RNA isolation and real-time PCR**

Isolation of RNA (RNeasy, Qiagen, Hilden, Germany) from whole brain tissue and immune cells, its quantification, and the RT reactions (High-capacity RT Kit, Applied Biosystems, Foster City, CA) were performed according to established protocols. Expression of mRNA of target genes and the endogenous control gene GAPDH was assessed by real-time PCR (with TaqMan Gene Expression Assay products on StepOne Plus PCR System, Applied Biosystems) according to the manufacturer’s recommendations. Expression levels for each gene of interest were calculated by normalizing the quantified mRNA amount to GAPDH. Relative gene expression was determined and used to test significance between different groups. The following gene expression assays (Applied Biosystems) were used: IL-1β (Mm00434228_m1),...
IL-6 (Mm00446190_m1), CD14 (Mm00438094_g1), glial fibrillary acidic protein (GFAP; Mm01253033_m1), S100A8 (Mm00496696_g1), and TNF (Mm00432558_m1).

**Histology**

Mice were anesthetized with isoflurane and perfused with ice-cold PBS and 4% paraformaldehyde. Brains were dissected and embedded in paraffin. Immunohistochemistry was performed with a rat Ab against mouse Mac-3 (1:200; clone M3/84, BD Biosciences) as described previously (21, 22). Briefly, tissues were pretreated by microwaving in 10 mM citrate buffer (pH 6) for two cycles of 5 min each. Immunolabeling was detected by the avidin-peroxidase method and visualized with diaminobenzidine by incubation for 5 min. Control sections were incubated in the absence of primary Ab or with nonimmune sera. Slides were counterstained with hematoxylin and coverslipped.

**Data analysis**

For statistical comparisons, a one-way multiple-range ANOVA test for multiple comparisons was employed. Unpaired t tests were used for comparison of two groups. Values of $p < 0.05$ were considered significant. Graphs were generated using GraphPad Prism software (GraphPad, San Diego, CA).

**Results**

**Immunization with $A\beta_{1–42}$ is associated with alterations of behavioral and cognitive performances**

Because APP and its cleavage products, the $A\beta$ peptides, are present in the normal CNS, we wished to investigate in more detail how immunotherapeutic approaches designed to remove $A\beta$ deposits interfere with regular functions of the CNS. Active immunization with $A\beta_{1–42}$/CFA significantly altered the psychomotor and cognitive phenotype of mice in comparison with various control groups. Observations in the open field revealed pronounced deficits in three cognitive parameters: first, open field testing of $A\beta_{1–42}$/CFA-immunized mice showed a significant reduction of locomotion (Fig. 1A) as compared with MOG/CFA- or PBS/CFA-immunized animals. Changes in locomotion were detected as early as on day 10 p.i., and reduced locomotion in $A\beta_{1–42}$-immunized mice persisted over the entire observation period until day 28. Reduced rearing behavior was detected already on day 4 and persisted until day 18 (Fig. 1B). Second, we observed a significant decrease in habituation learning ability. Whereas control animals showed habituation to a persisting environment by reduction of exploration over time, $A\beta_{1–42}$/CFA-immunized mice had a significantly lower habituation learning index (Fig. 1C) from day 3 p.i. Even compared with MOG$_{35–55}$/CFA-immunized mice (EAE scores are shown in Fig. 1D), we found significant differences in $A\beta_{1–42}$-immunized mice on days 10, 17, and 28 p.i. Experiments in aged mice (12 mo old) revealed similar deficits in explorative behavior after $A\beta_{1–42}$/CFA immunization (Supplemental Fig. 1). Third, we found that mice immunized with $A\beta_{1–42}$/CFA developed profound deficits in visuospatial learning both in the acute (observation between days 9 and 14 p.i.) and chronic (observation between days 23 and 28 p.i.) phases of disease (Fig. 2A, 2B). As compared with controls, $A\beta_{1–42}$/CFA-immunized animals spent significantly less time to explore a novel stimulus in a known environment (reduced memory gain) both in the acute and chronic phases of disease. Together, these behavioral data indicate a profound and persistent decline in motivational and cognitive performance in $A\beta_{1–42}$/CFA-immunized animals.

$A\beta_{1–42}$ immunization results in macrophage infiltration and reactive astroglisis in the CNS

In contrast to immunization with MOG$_{35–55}$/CFA or CFA only, immunization with $A\beta_{1–42}$ emulsified in CFA induced profound and persistent behavioral changes in wild-type animals. To investigate the potential immunological substrate of this behavioral phenotype, we performed immunohistochemical studies of CNS tissue specimens 18 d p.i. Immunohistochemistry revealed perivascular and subpial infiltrates of mononuclear cells in the brain and brainstem of $A\beta_{1–42}$/CFA-immunized mice (Fig. 3B), but not...
in PBS/CFA controls (Fig. 3A). These infiltrates in the hippocampal region mainly consisted of macrophages as shown by MAC-3 staining with few CD3+ T cells. Infiltrates in Aβ1-42/CFA-immunized mice (Fig. 3D) were disseminated and nonfocal, whereas MOG35-55/CFA controls showed focal meningeal and perivascular inflammatory infiltrates (Fig. 3C). We wondered whether the cellular infiltrate in MOG35-55/CFA- versus Aβ1-42/CFA-immunized mice was also quantitatively different. To quantify various immune cell populations in the CNS of immunized mice, we isolated mononuclear cells from the CNS and performed flow cytometric analysis. Because behavioral differences between the groups of MOG35-55/CFA-versus Aβ1-42/CFA-immunized animals were evident as early as 10 d p.i., whereas animals in the MOG35-55/CFA group did not yet show signs of paralytic disease at this time point, we chose to perform quantitative analyses of CNS infiltrates on day 10 p.i. In PBS/CFA-immunized control animals, most of the CNS-derived CD11b+ cells were CD45low, indicating their microglial origin (Fig. 4A, 4D, 4G). Whereas in MOG35-55/CFA-immunized mice, T cells (CD11b-CD45high) were already starting to accumulate in the CNS on day 10 p.i., the majority of CD11b+ cells were still CD45low, again suggesting their microglial origin (Fig. 4B, 4E, 4G). In contrast, the majority of CD11b+ cells isolated from the CNS of Aβ1-42/CFA-immunized mice were CD45high, indicating that these CD11b+ cells were macrophages that had invaded the CNS as early as on day 10 p.i. (Fig. 4C, 4F, 4G). Moreover, the fraction of CD14+ cells within the population of CD11b+CD45high macrophages in the CNS was significantly higher in Aβ1-42/CFA-immunized mice than in either control group (Fig. 4H, 4I). Together, these data indicate that Aβ1-42/CFA immunization leads to early and massive recruitment of blood-borne macrophages into the CNS.

Consistent with the immunohistochemical and flow cytometric analyses, the expression of macrophage-associated genes, such as S100A8 and CD14 (Fig. 5A, 5B), was upregulated in whole brain tissue of Aβ1-42/CFA-immunized animals compared with PBS/CFA and MOG35-55/CFA controls. Quantitative RT-PCR from whole brain tissue isolated from Aβ1-42/CFA-immunized mice demonstrated a 10-fold higher expression of S100A8 as compared PBS/CFA-immunized animals and a 3-fold higher expression as compared with MOG35-55/CFA-immunized mice (Fig. 5A). CD14 expression was ~2-fold increased as compared with either control group (Fig. 5B). When comparing the CNS parenchyma between
the groups at late stages of the disease (4 wk p.i.), we found prominent signs of astrogliosis in the Aβ1–42/CFA-immunized mice as determined by a disproportionate upregulation of GFAP mRNA expression in Aβ1–42/CFA-immunized animals (Fig. 5C).

Immunization with Aβ1–42 induces systemic release of proinflammatory cytokines by activated macrophages

Cognitive changes in Aβ1–42/CFA-immunized mice developed in the absence of focal neurologic symptoms. We wondered whether systemic and CNS specific release of inflammatory cytokines induced by immunization with Aβ1–42/CFA was responsible for the neurocognitive phenotype. To test this hypothesis, we determined the level of TNF in the sera of mice immunized with Aβ1–42/CFA. On day 15 p.i., serum TNF was significantly increased in Aβ1–42/CFA-immunized mice as compared with control groups (Fig. 6A).

To identify possible cellular sources of TNF, we measured both the expression and the production of TNF and IL-6 in peritoneal macrophages of the various experimental groups. Consistent with the elevated serum concentration of TNF, peritoneal macrophages isolated from Aβ1–42/CFA-immunized mice showed a higher expression of TNF, IL-6, CD14, and S100A8 on a per-cell basis and higher secretion of TNF and IL-6 into the culture supernatant than macrophages that were isolated from MOG/CFA- or CFA-only-immunized mice (Fig. 6B, 6C). Taken together, these data suggest that immunization with Aβ1–42/CFA induces an exaggerated systemic inflammatory response by activating cells of the innate immune system.

To assess the functional relevance of systemic activation of macrophages and their recruitment to the CNS for the neurocognitive phenotype of Aβ1–42/CFA-immunized mice, we performed macrophage-depletion experiments. Clodronate liposomes were used to deplete macrophages in the secondary lymphoid tissues prior to immunization with Aβ1–42/CFA or PBS/CFA. The depletion of macrophages by clodronate liposomes prevented Aβ1–42-induced effects on psychomotor behavior (Fig. 7). In contrast to control mice receiving empty liposomes, macrophage-depleted mice receiving Aβ1–42/CFA showed a significant improvement in the performance of the Barnes maze test compared to control mice receiving empty liposomes (Fig. 7A).

FIGURE 4. Infiltrating cells in Aβ1–42/CFA-immunized animals are primarily of blood-borne macrophage origin. Mononuclear cells were isolated from the CNS of PBS/CFA, MOG35–55/CFA, and Aβ1–42/CFA-immunized animals on day 10 p.i. and analyzed by flow cytometry. Representative cytograms (A–C) and histograms (D–F) are shown to illustrate the fractions of CD11b+CD45low cells (microglia) and CD11b+CD45high cells (macrophages) in PBS/CFA, MOG35–55/CFA, and Aβ1–42/CFA-immunized animals, respectively. G. Ratio of macrophages versus microglial cells in the CNS of the three experimental groups. One symbol represents one animal. Note that in contrast to PBS/CFA and MOG35–55/CFA-immunized controls, the majority of cells isolated from the CNS of Aβ1–42/CFA-immunized mice on day 10 p.i. were CD11b+CD14+CD45high cells (H, I), indicating their macrophage origin. *p < 0.05 as determined by Mann-Whitney U test.
depleted animals did not show impaired locomotion (Fig. 7A), rearing (Fig. 7B), and habituation performance (Fig. 7C) as a result of challenge with Aβ1–42/CFA, suggesting that peripheral macrophages are crucial effector cells in inducing the clinical phenotype of Aβ1–42/CFA-challenged animals. Accordingly, resident microglial cells (CD11b+CD45low) and not blood-borne macrophages (CD11b+CD45high) constituted the majority of CD11b+ cells in the CNS of macrophage-depleted Aβ1–42/CFA-immunized mice (Supplemental Fig. 3). Thus, clodronate administration was able to abrogate both the behavioral and the immunopathological phenotype in Aβ1–42/CFA-immunized mice. The stimulatory effects of Aβ1–42 in macrophages and dendritic cells are TLR2/4 dependent

Because we observed a profound activation of the innate immune system p.i. with Aβ1–42, we investigated the stimulatory properties of Aβ peptide in vitro and tested the relevance of specific TLR systems that have been implicated with immunostimulatory effects of Aβ peptide in previous studies. It has been reported that the activation of microglial cells by Aβ peptide requires both TLR2 and TLR4 pathways to activate intracellular signaling (23).

In this study, stimulatory effects of Aβ1–42 on CD11b+ macrophages and CD11b+CD11c+ dendritic cells isolated from naive wild-type and TLR2/4-deficient mice were evaluated in vitro. Aβ1–42 induced large amounts of IL-6 and TNF in macrophages (Fig. 8A, 8B) and IFN-γ in dendritic cells from wild-type mice in a dose-dependent manner (Fig. 8C). Similar effects were observed poststimulation with murine Aβ1–42 peptide (Supplemental Fig. 2). In contrast, this effect was not detected in macrophages and dendritic cells derived from TLR2/4-deficient mice, suggesting that either TLR2 or TLR4 or the combined activation of these TLRs mediate the stimulatory effect of Aβ1–42.

To corroborate whether activation of the TLR2/4 pathway by Aβ1–42 was relevant in vivo, we immunized TLR2/4 knockout (KO) animals with Aβ1–42/CFA. We consistently observed a differential disease-promoting effect of Aβ1–42/CFA versus PBS/CFA treatment in wild-type animals. Indeed, we determined...
a significant decrease in locomotion and rearing in wild-type C57BL/6 mice immunized with Aβ1–42 as compared with CFA only (Fig. 9). In contrast, we did not find any additional neurocognitive phenotype (surplus effect) upon immunization with Aβ1–42/CFA challenge. Statistical comparisons are based on the surplus effect of Aβ1–42/CFA immunization as compared with PBS/CFA immunization within the respective treatment group. Note that the surplus effect of Aβ1–42/CFA immunization was abrogated in the clodronate group. **p < 0.01. BL, baseline.

Discussion

In this study, we showed that active immunization with Aβ1–42/CFA induced sustained cognitive and behavioral impairment in wild-type C57BL/6 mice. We identified a disseminated, nonfocal infiltrate of CD11b+/CD14+/CD45high cells in the CNS of Aβ1–42/CFA-immunized mice. We propose that this infiltrate of activated macrophages represented the immunopathogenetic correlate of the neurocognitive phenotype in Aβ1–42/CFA-immunized mice because challenge with Aβ1–42/CFA failed to induce neurocognitive impairment in animals that had been depleted of macrophages. Furthermore, immunization with Aβ1–42/CFA induced a systemic inflammatory response including the systemic release of cytokines. Peritoneal macrophages from Aβ1–42/CFA-immunized animals were characterized by an increased activation state as compared with MOG35–55/CFA-immunized mice, suggesting the capacity of Aβ peptide to activate the innate immune system in a manner reminiscent of pathogen-associated molecular patterns (PAMPs). Using TLR2/4-deficient mice, we showed that the TLR2/4 pathway mediated the Aβ1–42-induced proinflammatory cytokine release from cells of the innate immune system. Accordingly, TLR2/4 KO mice were protected from cognitive impairment upon immunization with Aβ1–42/CFA. We concluded that vaccination with Aβ1–42/CFA lead to the activation of innate immune cells in the systemic and CNS compartments in a TLR-dependent manner. Thus, this study identified adjuvant effects of Aβ1–42, which resulted in a clinically relevant and sustained neurocognitive phenotype.

Induction of EAE with myelin Ags emulsified in CFA is a widely used model to study autoimmune diseases of the CNS, such as multiple sclerosis. We used classical MOG35–55/CFA-induced EAE as a control condition for our vaccination protocol with Aβ1–42/CFA. MOG 35–55-induced EAE has been extensively investigated (24–27). In MOG35–55/CFA-immunized animals, focal perivascular and parenchymal infiltrates of T cells and macrophages primarily in the spinal cord lead to demyelination and axonal damage resulting in paralytic disease. However, less is known about the neurocognitive status in MOG35–55/CFA-immunized animals. A depression-like syndrome is reported, but cognitive alterations are rarely seen (28). In contrast, only a few days p.i. with Aβ1–42/CFA, mice exhibited a significant decrease in their psychomotor
performance as well as deficient habitual learning abilities and impaired performance in complex visuospatial tasks in the absence of apparent focal neurologic deficits. Although neurocognitive phenotypes have been extensively characterized in previous reports and in our present study, memory performance in mice can only be captured in the context of locomotive behavior tasks that reflect both motivational and cognitive components. Dissection of these components is not possible in the absence of verbal communication. Thus, we cannot exclude that the extent of exploration in our setup was also influenced by the lack of motivation due to systemic release of proinflammatory cytokines.

The clinical syndrome exhibited by Aβ1-42/CFA-immunized mice was reminiscent of the apathic condition that was the result of a cytokine release syndrome. In fact, deficits in visuospatial tasks are reported in mice injected with LPS. After LPS treatment, mice show impaired performance in tests of cognition that require effective integration of new information to complete a spatial task (29). A further study in mice provides evidence for hippocampus-dependent learning and memory impairment after LPS injection (30). Systemic administration of LPS is reported to induce TLR4-dependent secretion of proinflammatory cytokines such as IL-1β, IL-6, and TNF in the CNS (31–34). Furthermore, activation of TLR4 by LPS increases IFN-γ levels in mice and stimulates IDO in peripheral tissues and the brain (35). IDO activation results in decreased tryptophan levels and increased production of kynurenine promoting depression-like behavior in mice (36). LPS-induced sickness behavior is characterized both by systemic inflammation (35) and activation of local microglial cells (37) in the absence of cellular infiltrates in the CNS. In contrast to detrimental effects of proinflammatory cytokines on cognitive functioning, other cytokines may have beneficial effects on cognitive processes in the normal brain (38, 39). For example, IL-4 in meningeal T cells is involved in maintaining cognitive abilities in spatial learning and memory tasks (40). In the absence of T cell-derived IL-4, mononuclear cells in the meningeal compartment become activated and contribute to impaired learning abilities. In line with this concept, we showed that extensive production of proinflammatory cytokines like TNF and IL-6 by Aβ1-42-activated macrophages resulted in reduced cognitive performance.

A study by Furlan et al. (19) reports an Aβ1-42-specific CD4 T cell response and mild neurologic signs p.i. of C57BL/6 mice with Aβ1-42/CFA, but did not test for behavioral deficits. Mice develop an inflammatory disease of the CNS characterized by the presence of perivenular inflammatory foci containing macrophages, T and B cells, and Igs both in the brain and spinal cord. In our immunohistochemical analyses of Aβ1-42/CFA-immunized mice, we consistently observed disseminated macrophage infiltrates without focal accumulation of immune cells. S100A8 and CD14 were more prominently expressed in the CNS of Aβ1-42/CFA-immunized mice than in control animals. Calprotectin (S100A8) induces cytokine-like effects in the local environment.
and is expressed in activated macrophages, endothelial cells, and epithelial cells (41). CD14 is reported to function as a coreceptor for Aβ1–42 (42, 43). Both Ab blocking of CD14 and knockdown of the CD14 gene reduce the Aβ peptide-induced release of inflammatory cytokines and NO in microglial cells and peritoneal macrophages (44). We hypothesize that the upregulation of CD14 is absolutely dependent on TLR2 and TLR4. Thus, we propose that TLR2 and TLR4 are dispensable for inducing a paralytic syndrome of EAE is similar to wild-type animals (49). TLR4 and TLR9 KO mice do not improve cognitive performance in cognitively impaired APP + PS1 mice. Behav. Neurosci. 117: 478–484.

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Disclosures

The authors have no financial conflicts of interest.


