Original Paper

Neurodegener Dis 2014;13:38–44
DOI: 10.1159/000352020

Received: November 27, 2012
Accepted after revision: May 12, 2013
Published online: September 6, 2013

Immune Activation in Amyloid-β-Related Angiitis Correlates with Decreased Parenchymal Amyloid-β Plaque Load

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Key Words
Angiitis · Amyloid-β · Neuropathology · Neuroinflammation · Angiopathy

Abstract
Background: Primary angiitis of the central nervous system (PACNS) is a rare but serious condition. A fraction of patients suffering from PACNS concurrently exhibit pronounced cerebral amyloid angiopathy (CAA) which is characterized by deposits of amyloid-β (Aβ) in and around the walls of small and medium-sized arteries of the brain. PACNS with CAA has been identified as a distinct disease entity, termed Aβ-related angiitis (ABRA). Evidence points to an immune reaction to vessel wall Aβ as the trigger of vasculitis. ***Objective:*** To investigate whether the inflammatory response to Aβ has (1) any effect on the status of immune activation in the brain parenchyma and (2) leads to clearance of Aβ from brain parenchyma. ***Methods:*** We studied immune activation and Aβ load by quantitative immunohistochemical analysis in brain parenchyma adjacent to affected vessels in 11 ABRA patients and 10 matched CAA controls. ***Results:*** ABRA patients showed significantly increased immune activation and decreased Aβ loads in the brain parenchyma adjacent to affected vessels. ***Conclusion:*** Our results are in line with the hypothesis of ABRA being the result of an excessive immune response to Aβ and show that this can lead to enhanced clearance of Aβ from the brain parenchyma by immune-mediated mechanisms.

Introduction

Primary angiitis of the central nervous system (PACNS) is defined by focal, inflammatory vascular lesions confined to the brain and spinal cord without systemic involvement. PACNS is rare, with an estimated incidence of 2–3 cases per million per year, a median age of 47 years, a progressive clinical course with frequent recurrences and high lethality if not adequately treated [1].

Cerebral amyloid angiopathy (CAA) is a common pathological finding in the elderly, characterized by deposition of amyloid-β (Aβ) within the cortical and meningeal arteries [2]. Its prevalence increases from 2–3% at the age of 70 to 15–30% at the age of 90 [2]. In Alzheimer's

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disease (AD), the prevalence of CAA is very high, possibly reaching 90% [2, 3]. Almost a third of intracranial lobar hemorrhages in the elderly are attributed to CAA-induced damage to vessel walls [2].

In certain instances, patients present with coincident Aβ deposition in vessel walls and angiitis [4, 5]. A variety of names such as granulomatous angiitis with CAA, inflammatory CAA or Aβ-related angiitis (ABRA) have been used to describe this clinicopathological entity [4–6]. Clinical and neuropathological investigations on ABRA have provided valuable insights into the pathogenesis of this disease [7–9]. Regarding its pathogenesis, there are at least three distinct possibilities: (1) Aβ deposition in vessel walls and angiitis concur by chance as stochastic events, (2) angiitis favors vessel wall deposition of Aβ [10] or (3) Aβ deposition in vessel walls leads to angiitis [5].

In a large successive case series of biopsy-proven PACNS, the co-occurrence of vascular Aβ and angiitis was recently studied [11–12]. Histological evidence of ABRA was detected in approximately 30% of PACNS with an average age of 46 years. Since the prevalence of CAA in this age group is 2–3% [2], chance association is highly unlikely. Thus, it seems that vascular Aβ deposits and angiitis are pathophysiologically interconnected. None of the studies on ABRA has provided convincing evidence that angiitis favors deposition of Aβ in cerebral vessels. Indeed, neuropathological studies showing that neuroinflammation is secondary to deposition of Aβ argue against the notion that inflammation promotes Aβ deposition [13, 14]. Thus, the cumulative amount of evidence implies that angiitis in ABRA is secondary to deposition of Aβ in vessels. Already, the initial report on ABRA has already pointed out that angiitis may be due to vascular Aβ [15]. Subsequent studies have shown that inflammation is specific to vessels with significant Aβ deposition [8, 16]. Furthermore, autoantibodies to Aβ can be observed in cerebrospinal fluid and are produced by expanded B cells derived from an ABRA patient [17, 18]. Finally, ABRA seems to occur spontaneously in a murine model with excessive production of vascular Aβ [19]. Interestingly, recent therapeutic trials in AD, with active immunization against Aβ as the mechanistic principle, have drawn attention to ABRA. In patients immunized against Aβ, occurrence of specific antibodies seems to go along with clearance of Aβ from brain parenchyma, immune activation and vascular inflammation [20–22], as well as potentially more prominent vessel wall deposition of Aβ [23]. Therefore, in order to explore whether enhanced clearance of parenchymal Aβ occurs in ABRA and to assess whether enhanced clearance of Aβ correlates with parenchymal immune activation, we carried out a case control study. For this, 11 patients with histologically proven ABRA and 10 patients with CAA were chosen. To exclude bias towards enhanced Aβ loads found in CAA with AD [2, 24], we recruited ABRA and CAA patients according to identical criteria with no reference to cognitive status. We could show that ABRA patients harbor significantly fewer plaques in cerebral parenchyma and show decreased Aβ loads compared to CAA controls. This reduction of plaques corresponds with a significant increase in activated macrophages and higher contents of microglia. This is in line with the hypothesis that ABRA results from Aβ-triggered immune activation.

Methods

Patients and Controls

Brain tissue specimen of 11 patients with ABRA (9 from diagnostic biopsies and 2 from autopsies) and 10 patients with CAA (only diagnostic biopsies) were collected in Northern Germany from 2002 to 2011. For biopsy samples, vascular events leading to diagnostic intervention were used as entry criteria to this study. The 2 autopsy patients died acutely due to ABRA-related events. Patients with clinical signs indicative of dementia or suspected AD were specifically excluded from this study. Selecting patients with vascular events in the absence of dementia may have introduced a bias towards severe forms of CAA, yet bias towards elevated levels of parenchymal Aβ, which strongly correlates with dementia, was avoided. The use of specimens and basic clinical information was in agreement with the regulations and ethical standards of the contributing hospitals and written consent by patients or relatives was obtained when appropriate.

Neuropathological Investigations and Immunohistochemistry

Paraffin-embedded tissue samples were cut into 3-μm thick-serial sections, mounted on glass slides and processed according to published protocols [25]. Besides hematoxylin-eosin, immunohistochemical stainings with the following primary antibodies were performed: Aβ (1:100; Mob410; DBS Emergo [26]), tau (1:1,500; Thermo), human leukocyte antigen-DR (HLA-DR; 1:100; M0775, Dako), CD68 (1:50; 2164; Immunotech). Primary antibodies were visualized using a standard diaminobenzidine streptavidin-biotin horseradish peroxidase method (for Aβ, tau and HLA-DR; Ventana/Roche) or an alkaline phosphatase method (for CD68; Ventana/Roche). Quantification of immunosignals was performed according to published methods [27] by experienced morphologists (S.B. and M.G.) blinded with respect to the experimental groups. Briefly, for quantification of diffuse and cored Aβ plaques, CD68-positive macrophages, HLA-DR-positive microglia and neurofibrillary tangles, we counted the presence of positive events (plaques, positive cells and tangles) in a representative fraction of the entire sample (at least 1 mm^2) using a Zeiss DMD 108 large image area microscope. For quantification of immunopositive ar-

Table 1. Demographic, imagenological and clinical features of 11 ABRA and 10 control (CAA) patients

	Age, years	Average	Sex	Main symptom	Neuroradiological findings	Site of biopsy[a]
ABRA	70	67.5±5.2	F	subacute confusion	multifocal leukoencephalopathy	n.a.
	67		M	grand-mal seizure	n.a.	n.a.
	69		M	aphasia, apraxia, dementia	temporal mass lesion	temporal
	73		F	hemiparesis, progressive dementia	occipital mass lesion	temporal
	61		M	headache, confusion	multiple vascular-like lesions	frontal
	63		M	n.a.	parietooccipital mass lesion	parietal
	78[a]		F	confusion	leukoencephalopathy	occipital
	60		F	n.a.	n.a.	n.a.
	66		M	n.a.	n.a.	n.a.
	68		F	grand-mal seizure	encephalitis-like pattern	n.a.
	68[a]		M	progressive confusion	n.a.	frontal
CAA	72	68.6±6.0	F	n.a.	intracranial hemorrhage	frontal
	74		F	n.a.	intracranial hemorrhage	frontal
	68		M	n.a.	intracranial hemorrhage	frontal
	63		F	n.a.	intracranial hemorrhage	frontal
	67		M	n.a.	subdural hygroma	n.a.
	76		M	n.a.	n.a.	frontoparietal
	67		F	acute aphasia	intracranial hemorrhage	frontal
	77		F	n.a.	n.a.	parietal
	56		F	n.a.	intracranial hemorrhage	n.a.
	68		M	n.a.	intracranial hemorrhage	temporal

n.a. = Not available. [a] Autopsy.

eas (Aβ, tau, CD68 and HLA-DR) fifteen randomly chosen 0.2-mm^2 regions of each sample were assessed using Zeiss AxioVision quantification software on images taken with a Zeiss Axiovert S100 microscope. Only parenchyma at least 200 μm distant from sites of angiocentric inflammation or vessels was included in the quantification.

Statistical Analysis

The data were analyzed using SPSS 17 statistical software (SPSS Inc., Chicago, Ill., USA). Analyses included Kolmogorov-Smirnov and Shapiro-Wilk tests for normal distribution assessment. For comparison of means, Student's t test was applied for two groups if they were normally distributed. Statistical significance of all analyses was determined at $p < 0.05$.

Results

Description of ABRA and CAA Cohorts

Details on the two patient groups are given in table 1. Within the ABRA patients (5 female, 6 male, average age 67.5 years, standard deviation, SD, 5.2 years) the principle presenting complaint was acute or subacute confusion. Neuroimaging and biopsy (or autopsy for the 2 individuals who died as a consequence of ABRA; for sites of biopsies see table 1) were performed to establish the diagnosis of cerebral angiitis. The CAA patients (6 female, 4 male, average age 68.6 years, SD 6.0 years) presented with intracerebral hemorrhage without distinct signs of dementia. Neuroimaging and eventually biopsy (for sites of biopsies see table 1) were performed to establish the diagnosis of CAA. The diagnosis of ABRA was made histologically according to current diagnostic criteria with prominent transmural lymphocytic and granulomatous infiltrates, mainly in smaller arteries (fig. 1) [5]. Deposits of Aβ could be seen in a substantial number of vessels. Notably, angiitis could only be observed in vessels with Aβ. In the CAA samples, well-defined Aβ deposits in the vessel walls were abundant. However, no signs of inflammation could be seen in Aβ-containing vessels (fig. 1).

ABRA Patients Have Less Aβ Plaques and Increased Immune Activation in Parenchyma Compared to CAA

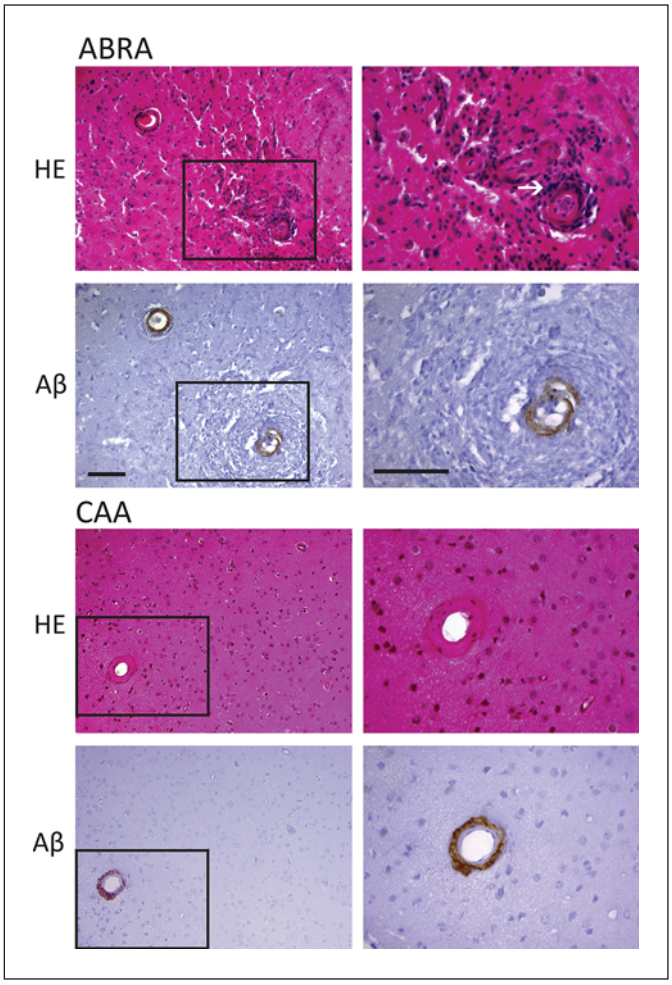
We were interested to see whether Aβ plaques or Aβ loads in ABRA patients differ from those found in CAA

controls. To this end, we counted Aβ plaques (diffuse and cored) and determined Aβ loads in brain parenchyma distant from sites of vascular inflammation. ABRA patients harbored significantly less Aβ plaques than CAA controls (16.2 ± 4.4 Aβ plaques/mm^2 for ABRA; 51.5 ± 11.4 Aβ plaques/mm^2 for CAA controls, p = 0.013), whereas for Aβ loads we detected a nonsignificant reduction in ABRA patients (1.2% ± 0.4/area for ABRA; 2.1% ± 1.1/area for CAA controls, p = 0.44; fig. 2). This means that reduction in plaque load is more drastic than reduction in diffusely deposited Aβ. In contrast, tau pathology was comparable in both cohorts by counting tau-positive neuropil threads or when quantifying brain parenchyma showing tau positivity (0.31% ± 0.11/area for ABRA; 0.20% ± 0.11/area for CAA controls, p = 0.51; 28 ± 11 tau-positive neuropil threads/mm^2 for ABRA; 11 ± 14 tau-positive neuropil threads/mm^2 for CAA controls, p = 0.68).

Since reduced Aβ burden has been shown to correlate with enhanced immune activation, and ABRA patients have been shown to mount an immune response to Aβ, we assessed immune activation in ABRA patients and CAA controls [13, 14, 17, 18]. We counted CD68-positive macrophages and HLA-DR-positive microglia in brain parenchyma distant from sites of vascular inflammation and determined percentages of brain parenchyma showing positivity for the above-mentioned markers. ABRA patients showed significantly more brain parenchyma with CD68-positive immunosignals and more CD68-positive macrophages than CAA controls (0.98% ± 0.24/area for ABRA; 0.24% ± 0.05/area for CAA controls, p = 0.015; 100 ± 60 CD68-positive macrophages/mm^2 for ABRA; 29 ± 23 CD68-positive macrophages/mm^2 for CAA controls, p = 0.31; fig. 2). Regarding HLA-DR-positive microglia there was no significant difference between groups (1.42% ± 0.37/area for ABRA; 1.01% ± 0.47/area for CAA controls, p = 0.50; 84 ± 24 HLA-DR-positive microglial cells/mm^2 for ABRA; 58 ± 11 HLA-DR-positive microglial cells/mm^2 for CAA controls, p = 0.33).

Macrophages Engulf Aβ in ABRA

In view of the assumption that the immune response in ABRA might be directed against Aβ, we performed immunohistochemical double-labeling using antibodies directed against CD68 and antibodies directed against Aβ in biopsies of ABRA and CAA patients. Only in ABRA patients could we observe CD68-positive macrophages in the immediate vicinity of Aβ plaques and vascular Aβ (fig. 3). We frequently found CD68-positive macrophages engulfing Aβ both in vessel walls (fig. 3a) and within the parenchyma at edges of Aβ plaques (fig. 3b).



Color version available online

Fig. 1. Representative HE and Aβ staining of ABRA (top 2 rows) and CAA (lower 2 rows) patient samples. Vessel walls show Aβ deposits in ABRA and CAA (rows 2 and 4). Inflammatory cells including the presence of giant cells (arrow) can only be seen in vessels of ABRA patients where Aβ deposits are prominent (rows 1 and 2). Right panels show magnification of indicated regions. Scale bar = 100 μm.

Discussion

Here we histologically analyzed the largest series of biopsies from ABRA patients to date [9]. ABRA is thought to be caused by an inflammatory response to Aβ deposited in vessel walls [5, 17, 18, 28]. The objective of this study was to investigate whether the inflammatory response to Aβ has any effect on the status of immune activation in the brain parenchyma at sites distant from vessels. Furthermore, we wanted to investigate whether this neuroinflammatory response would correlate with enhanced clearance of Aβ in ABRA. Our study clearly shows

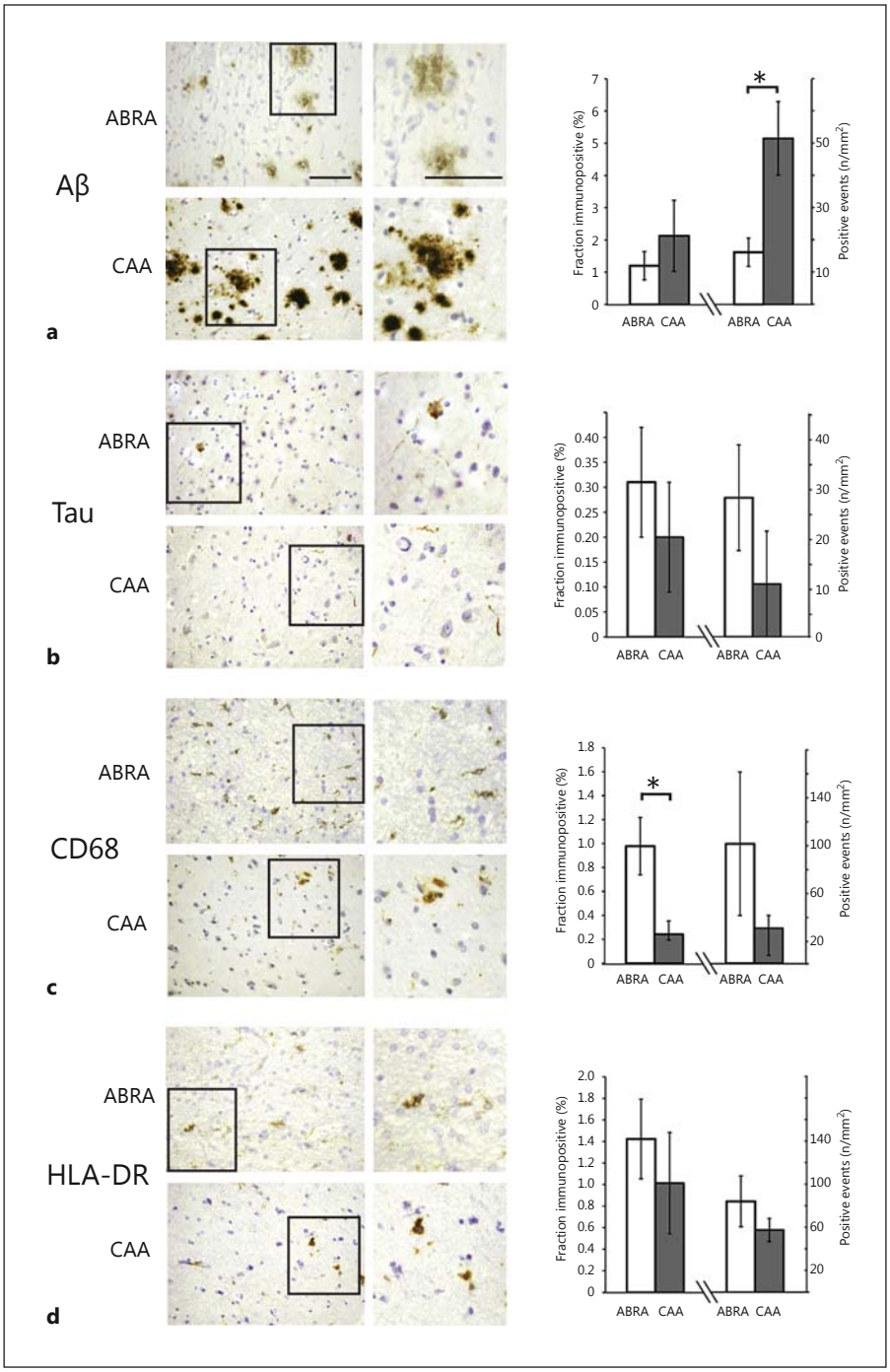


Fig. 2. Quantification of immunohistological staining for Aβ, tau, CD68 and HLA-DR. Representative histological images of immunohistological stainings for Aβ (**a**), tau (**b**), CD68 (**c**) and HLA-DR (**d**) are shown with magnification of indicated regions. Both positive events (plaques, positive cells and tangles) and percentages of immunopositive areas were quantified separately and are shown as group averages for percentages of immunopositive areas (left part of the graph) and positive events (right part of the graph). * $p < 0.05$, indicates significance (Student's t test). Scale bar = 100 μm.

that neuroinflammation in ABRA is not limited to affected vessel walls but also extends to the surrounding parenchyma with significantly more CD68-positive macrophages. This translates into a reduced Aβ plaque burden in these patients. We could also observe CD68-positive macrophages surrounding Aβ plaques and engulfing Aβ in ABRA but not in CAA controls, implying that the inflammatory response to Aβ triggered by vascular deposits of Aβ may lead to active clearance of parenchymal Aβ.

Why are these findings relevant? Over the last decade, ABRA has been recognized as a well-defined disease entity that represents a substantial subset of PACNS [4–6].

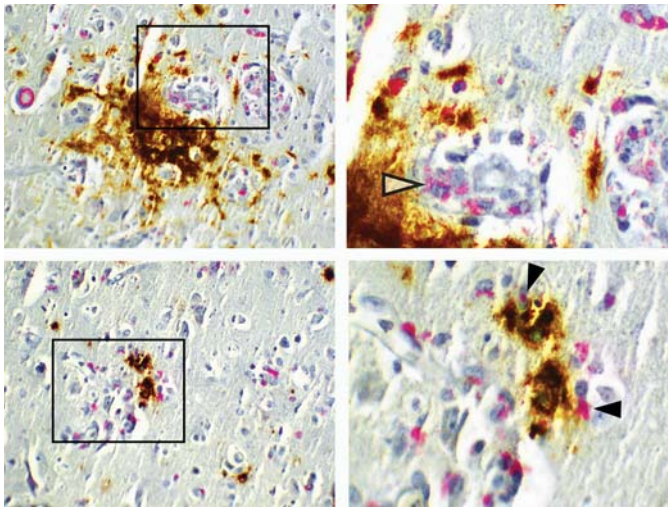


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Fig. 3. Immunohistochemical double-labeling for Aβ (brown; color refers to online version only) and CD68 (violet) of ABRA samples. CD68-positive activated macrophages engulfing Aβ-positive material can be found at the site of perivascular Aβ deposits (**a**, unfilled arrowhead) and in the immediate vicinity of plaques in the cortical parenchyma (**b**, solid arrowheads).

The pathophysiology of ABRA is not entirely understood, but the most likely etiology is excessive immune response to vascular Aβ [17, 18, 28]. Our data are in line with an immunological origin of ABRA and suggest that the immunological response to Aβ extends into the brain parenchyma with the potentially beneficial effects of an enhanced clearance of Aβ.

There is an ongoing discussion on the presence of Aβ and tau deposits in ABRA. While the majority of studies found intermediate levels of Aβ in ABRA, the presence of tau is reported less frequently [5, 29]. The fact that we found comparable, albeit low, amounts of tau in ABRA and CAA controls shows that tau deposits may be more frequent in ABRA than previously thought. We are confident that the observed decrease in parenchymal Aβ is not the result of a systemic error due to incorrect selection of patients. We specifically recruited patients to the CAA control group on the basis of vascular events leading to diagnostic intervention, with none of the patients presenting with obvious signs of AD-related dementia. The dissociation between comparable tau loads and significantly lowered Aβ loads in ABRA patients also speaks in favor of selective clearance of Aβ but not tau. Similar dissociation could be observed in patients with active immunization against Aβ [22, 30, 31]. Neuroradiological abnormalities have been reported in patients treated with monoclonal antibodies to Aβ [32]; these point to a correlation between neuroradiological findings indicative of vascular pathology and amyloid clearance. Our data show that ABRA patients with histologically proven inflammatory vascular pathology have enhanced amyloid clearance, further highlighting similarities between ABRA and Aβ antibody-based therapies [33]. Taken together, our findings support the ever-growing evidence that autoimmune dysregulation, characterized by an adaptive Aβ-directed immune response, plays a critical role in AD in general, but specifically in ABRA where vascular Aβ deposition may act as the triggering event [13, 18, 28].

Acknowledgments

This research was supported by grants from the DFG (GRK1459 and FG885 to M.G.), ERA-Net Neuron and the Landesexzellenzinitiative Hamburg (Neurodapt). Special thanks to the technical staff of the Institute of Neuropathology at the University Medical Center Hamburg-Eppendorf.

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