

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 (A β) 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 A β -related angiitis (ABRA). Evidence points to an immune reaction to vessel wall A β as the trigger of vasculitis. **Objective:** To investigate whether the inflammatory response to A β has (1) any effect on the status of immune activation in the brain parenchyma and (2) leads to clearance of A β from brain parenchyma. **Methods:** We studied immune activation and A β 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 de-

creased A β 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 A β and show that this can lead to enhanced clearance of A β from the brain parenchyma by immune-mediated mechanisms.

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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 (A β) 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

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 A β deposition in vessel walls and angiitis [4, 5]. A variety of names such as granulomatous angiitis with CAA, inflammatory CAA or A β -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) A β deposition in vessel walls and angiitis concur by chance as stochastic events, (2) angiitis favors vessel wall deposition of A β [10] or (3) A β deposition in vessel walls leads to angiitis [5].

In a large successive case series of biopsy-proven PACNS, the co-occurrence of vascular A β 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 A β deposits and angiitis are pathophysiologically interconnected. None of the studies on ABRA has provided convincing evidence that angiitis favors deposition of A β in cerebral vessels. Indeed, neuropathological studies showing that neuroinflammation is secondary to deposition of A β argue against the notion that inflammation promotes A β deposition [13, 14]. Thus, the cumulative amount of evidence implies that angiitis in ABRA is secondary to deposition of A β in vessels. Already, the initial report on ABRA has already pointed out that angiitis may be due to vascular A β [15]. Subsequent studies have shown that inflammation is specific to vessels with significant A β deposition [8, 16]. Furthermore, autoantibodies to A β 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 A β [19]. Interestingly, recent therapeutic trials in AD, with active immunization against A β as the mechanistic principle, have drawn attention to ABRA. In patients immunized against A β , occurrence of specific antibodies seems to go along with clearance of A β from brain parenchyma, immune activation and vascular inflammation [20–22], as well as potentially more prominent vessel wall deposition of A β [23]. Therefore, in order to explore whether enhanced clearance of parenchymal A β occurs in ABRA

and to assess whether enhanced clearance of A β 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 A β 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 A β 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 A β -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 A β , 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- μ m 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: A β (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 A β , 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 A β 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 mm²) 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 (A β , tau, CD68 and HLA-DR) fifteen randomly chosen 0.2-mm² 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 μ m 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 individu-

als 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 A β could be seen in a substantial number of vessels. Notably, angiitis could only be observed in vessels with A β . In the CAA samples, well-defined A β deposits in the vessel walls were abundant. However, no signs of inflammation could be seen in A β -containing vessels (fig. 1).

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

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

controls. To this end, we counted A β plaques (diffuse and cored) and determined A β loads in brain parenchyma distant from sites of vascular inflammation. ABRA patients harbored significantly less A β plaques than CAA controls (16.2 ± 4.4 A β plaques/mm² for ABRA; 51.5 ± 11.4 A β plaques/mm² for CAA controls, $p = 0.013$), whereas for A β loads we detected a nonsignificant reduction in ABRA patients ($1.2\% \pm 0.4/\text{area}$ for ABRA; $2.1\% \pm 1.1/\text{area}$ for CAA controls, $p = 0.44$; fig. 2). This means that reduction in plaque load is more drastic than reduction in diffusely deposited A β . In contrast, tau pathology was comparable in both cohorts by counting tau-positive neuropil threads or when quantifying brain parenchyma showing tau positivity ($0.31\% \pm 0.11/\text{area}$ for ABRA; $0.20\% \pm 0.11/\text{area}$ for CAA controls, $p = 0.51$; 28 ± 11 tau-positive neuropil threads/mm² for ABRA; 11 ± 14 tau-positive neuropil threads/mm² for CAA controls, $p = 0.68$).

Since reduced A β burden has been shown to correlate with enhanced immune activation, and ABRA patients have been shown to mount an immune response to A β , 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 ($0.98\% \pm 0.24/\text{area}$ for ABRA; $0.24\% \pm 0.05/\text{area}$ for CAA controls, $p = 0.015$; 100 ± 60 CD68-positive macrophages/mm² for ABRA; 29 ± 23 CD68-positive macrophages/mm² for CAA controls, $p = 0.31$; fig. 2). Regarding HLA-DR-positive microglia there was no significant difference between groups ($1.42\% \pm 0.37/\text{area}$ for ABRA; $1.01\% \pm 0.47/\text{area}$ for CAA controls, $p = 0.50$; 84 ± 24 HLA-DR-positive microglial cells/mm² for ABRA; 58 ± 11 HLA-DR-positive microglial cells/mm² for CAA controls, $p = 0.33$).

Macrophages Engulf A β in ABRA

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

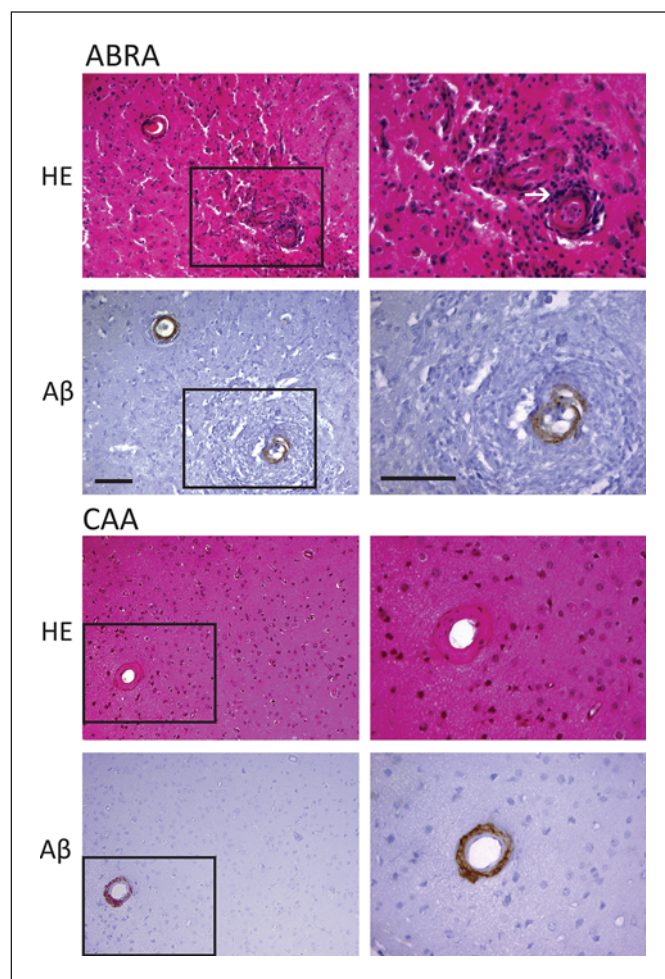


Fig. 1. Representative HE and A β staining of ABRA (top 2 rows) and CAA (lower 2 rows) patient samples. Vessel walls show A β deposits in ABRA and CAA (rows 2 and 4). Inflammatory cells including the presence of giant cells (arrow) can only be seen in vessels of ABRA patients where A β deposits are prominent (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 A β deposited in vessel walls [5, 17, 18, 28]. The objective of this study was to investigate whether the inflammatory response to A β 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 A β in ABRA. Our study clearly shows

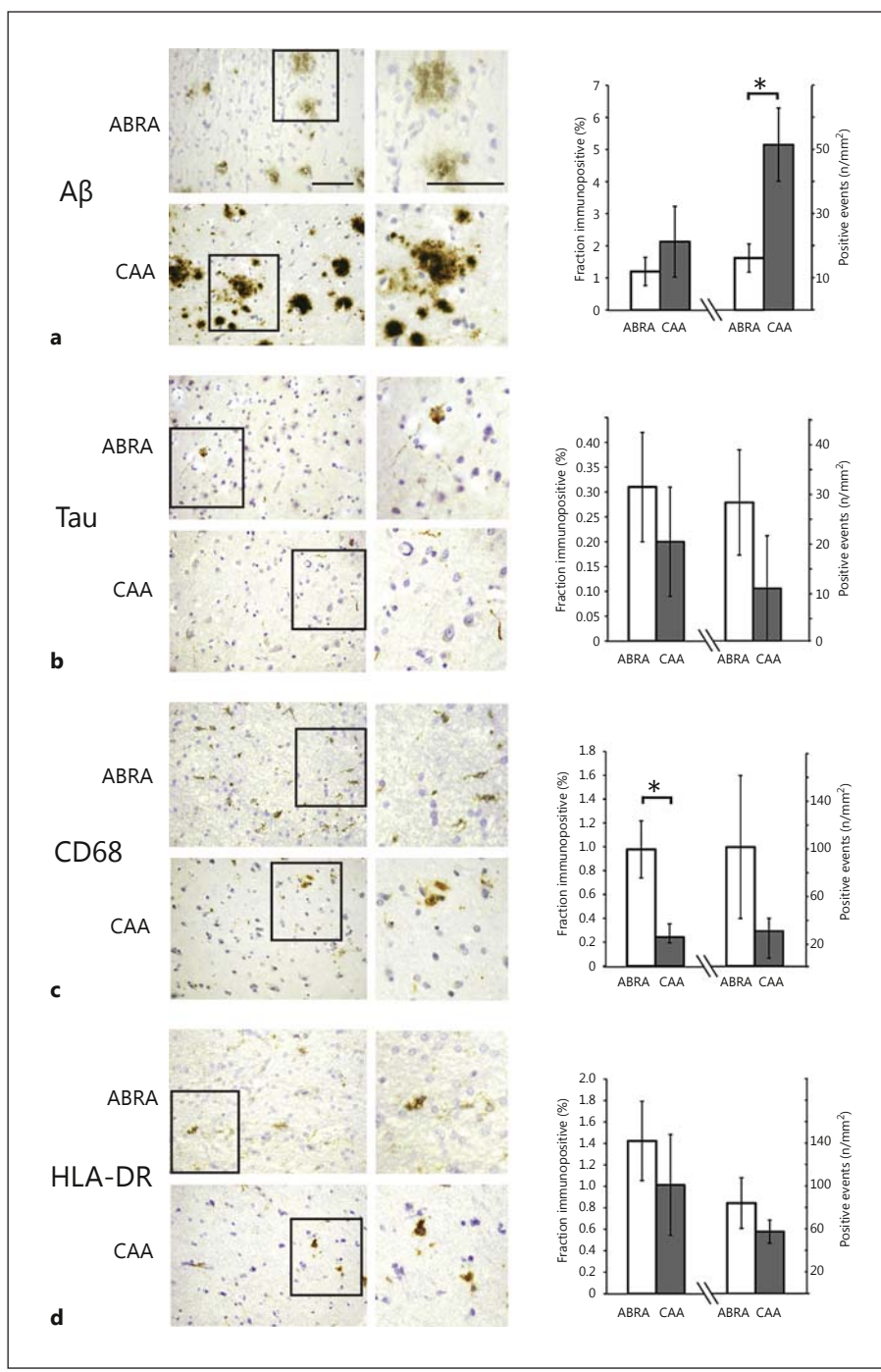
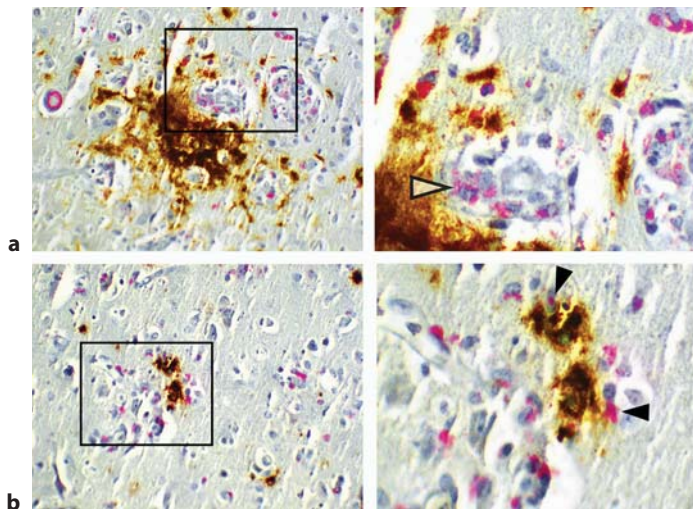


Fig. 2. Quantification of immunohistological staining for A β , tau, CD68 and HLA-DR. Representative histological images of immunohistological stainings for A β (**a**), tau (**b**), CD68 (**c**) and HLA-DR (**d**) are shown with magnification of indicated regions. Both positive events (plaques, positive 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 μ m.

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 A β plaque burden in these patients. We could also observe CD68-positive macrophages surrounding A β plaques and engulfing A β

in ABRA but not in CAA controls, implying that the inflammatory response to A β triggered by vascular deposits of A β may lead to active clearance of parenchymal A β .

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].



Color version available online

Fig. 3. Immunohistochemical double-labeling for A β (brown; color refers to online version only) and CD68 (violet) of ABRA samples. CD68-positive activated macrophages engulfing A β -positive material can be found at the site of perivascular A β 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 A β [17, 18, 28]. Our data are in line with an immunological origin of ABRA and suggest that the immunological response to A β extends into the brain parenchyma with the potentially beneficial effects of an enhanced clearance of A β .

There is an ongoing discussion on the presence of A β and tau deposits in ABRA. While the majority of studies found intermediate levels of A β 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 A β 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 A β loads in ABRA patients also speaks in favor of selective clearance of A β but not tau. Similar dissociation could be observed in patients with active immunization against A β [22, 30, 31]. Neuroradiological abnormalities have been reported in patients treated with monoclonal antibodies to A β [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 A β antibody-based therapies [33]. Taken together, our findings support the ever-growing evidence that autoimmune dysregulation, characterized by an adaptive A β -directed immune response, plays a critical role in AD in general, but specifically in ABRA where vascular A β deposition may act as the triggering event [13, 18, 28].

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