CD4⁺ T Cells Predominate in Cerebrospinal Fluid and Leptomeningeal and Parenchymal Infiltrates in Cerebral Amyloid β–Related Angiitis

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Background: In amyloid β (Aβ)–related angiitis (ABRA) of the central nervous system (CNS), cerebral amyloid angiopathy occurs in association with primary vasculitis of small- and medium-sized leptomeningeal and cortical arteries. It has been suggested that ABRA is triggered by vascular deposition of Aβ followed by an Aβ-directed (auto)immune response.

Objective: To provide a detailed description of the cellular composition of the inflammatory infiltrates in the cerebrospinal fluid (CSF) and CNS and their response to immunotherapy in a typical case of ABRA.


Setting: Neurologic referral center.

Patient: 67-year-old white woman.

Main Outcome Measures: Neurologic examination, magnetic resonance imaging, lumbar puncture, flow cytometry, leptomeningeal biopsy, and histopathologic analysis.

Results: In a typical case of ABRA, we demonstrate for the first time the presence of a vast majority of partially activated CD4⁺ T cells in CSF and leptomeningeal and parenchymal (peri)vascular infiltrates, which were frequently found in close proximity to major histocompatibility complex (MHC) class II–expressing microglia, epithelioid macrophages, and multinucleated giant cells containing intracellular deposits of Aβ.

Conclusion: Our findings support the notion of adaptive Aβ-directed autoimmunity as the underlying pathogenic mechanism in ABRA.


In amyloid β (Aβ)–related angiitis (ABRA) of the central nervous system (CNS), cerebral amyloid angiopathy (CAA) occurs in association with vasculitis of small- and medium-sized leptomeningeal and cortical arteries. It has been suggested that ABRA is triggered by vascular deposition of Aβ followed by an Aβ-directed (auto)immune response, which on the one hand may clear Aβ from the parenchyma but on the other hand causes vasculitis and increased deposition of Aβ within small leptomeningeal and penetrating cerebral arteries. Elderly men and women are equally affected by ABRA in the sixth and seventh decades of life. Patients present with altered mental status, headaches, seizures, and persistent or even only transient multifocal neurologic deficits. Magnetic resonance imaging (MRI) findings are variable and include diffuse white matter lesions, mass lesions, focal edema, hemorrhages, infarction, and atrophy. Histopathologic analysis reveals leptomeningeal and parenchymal amyloid angiopathy and chronic inflammation within the leptomeninges and within and around the walls of amyloid-laden vessels. Thickening and splitting of the vessel walls, fibrinoid necrosis, acute thrombosis and subsequent recanalization, and microbleeding are frequent. The perivascular and intramural inflammatory infiltrates are known to consist mainly of CD68⁺ (epithelioid-shaped) macrophages and multinucleated giant cells. CD3⁺ T lymphocytes are also present, but only at low numbers of both CD4⁺ and CD8⁺ T cells.

Cerebrospinal fluid findings in ABRA include nonspecific changes such as mixed pleocytosis, with a lymphocytic predominance and a mean cell count of 36/µL (range, about 10/µL-700/µL), and elevated protein levels, with a median level of 1.0 g/L (range, about 0.5-5 g/L). Oligoclonal bands are usually absent in the...
Response to treatment with steroids alone or in combination with cyclophosphamide is considerably variable and ranges from accomplishment of an asymptomatic status to a fatal outcome.2

Herein, we provide for the first time to our knowledge a detailed description of the cellular composition of the inflammatory infiltrates in the CSF and CNS and their response to immunotherapy in a typical case of ABRA, demonstrating a so far unknown predominance of CD4+ T cells.

METHODS

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging was performed on a 3-T scanner. Also performed were diffusion-weighted imaging with calculation of apparent diffusion coefficient map, axial and coronal T1-weighted spin-echo before and after application of gadolinium, coronal fluid-attenuated inversion recovery (FLAIR), axial fast T2-weighted field-echo, and axial turbo T2-weighted spin-echo sequences.

HISTOPATHOLOGIC ANALYSIS

Sections were deparaffinized, rehydrated, and washed in distilled water. For antigen retrieval, slides were microwaved and pretreated. They were blocked in 20% goat serum and then incubated with the following antibodies: monoclonal mouse antihuman β-amyloid (clone 6F/3D [1:100]; DakoCytomation Denmark A/S), monoclonal mouse antihuman CD3 (clone F7.2.38 [1:25]; DakoCytomation Denmark A/S), monoclonal mouse antihuman CD8 (clone 8/144B [1:100]; DakoCytomation Denmark A/S), monoclonal mouse antihuman HLA-DP, DQ, DR (major histocompatibility complex (MHC) class II clone CR3/43 [1:100]; DakoCytomation Denmark A/S). After washes, the sections were incubated with a biotinylated goat antirabbit secondary antibody following incubation with the Vectastain ABC kit (Vector Laboratories Inc); then the signal was developed using a DAB substrate kit (Vector Laboratories Inc). After washes, the sections were incubated with a biotinylated goat antirabbit secondary antibody following incubation with the Vectastain ABC kit (Vector Laboratories Inc); then the signal was developed using a DAB substrate kit (Vector Laboratories Inc). Sections were counterstained with hematoxylin-eosin.

Staining for CD4 (monoclonal mouse antihuman CD4, Novocastra clone 4B12 [1:20]; Novocastra Laboratories Ltd) was performed using the alkaline phosphatase anti-alkaline phosphatase clone 4B12 [1:20]; Novocastra Laboratories Ltd) was per-
CD4⁺ T cells predominate in the cerebrospinal fluid in amyloid β–related angiitis. A-C, Magnetic resonance imaging reveals extensive leptomeningeal infiltration of the parietal and temporal lobes on gadolinium-enhanced fluid-attenuated inversion recovery sequences (A, white arrows) and T1-weighted sequences (B, white arrows); T₂⁺-weighted sequences show hemosiderin in a sulcal pattern (C, white arrows). D, Gross aspect of the meningeal and cortical surface on leptomeningeal biopsy; note the xanthochromic cerebrospinal fluid (CSF) within the subarachnoid space. E-J, Flow cytometry of the peripheral blood (E, G, and I) and CSF (F, H, and J) shows lymphocytic pleocytosis (F), predominantly by CD4⁺ T cells (H), a substantial fraction of which are positive for the activation marker CD69 (J). These changes were not detected in the peripheral blood (E, G, and I), K and L, Cerebrospinal fluid cell count, CSF protein level, serum to CSF albumin ratio (K), CD4/CD8 ratio, and fraction of CD69⁺ CD4⁺ T cells (L) regressed on combined therapy with intravenous and oral steroids and cyclophosphamide, as revealed by serial analysis for the first 2 months after admission (H). FSC indicates forward scatter; gran, granulocytes; lymph, lymphocytes; mono, monocytes.

Results of a computed tomography scan of the chest and abdomen excluded a malignant tumor and showed no evidence of systemic sarcoidosis or vasculitis.

Biopsy specimens of the leptomeninges and superficial cerebral cortex showed pronounced perivascular and intramural inflammatory infiltration of the leptomeningeal and cortical arteries (Figure 1D and Figure 2A-D). Vessels showed thickening and splitting of their walls, fibrinoid necrosis, acute thrombosis and subsequent recanalization, and microbleeding, as revealed by the presence of hemosiderin-laden macrophages. Vessel walls stained positive with congo red, corroborating the presence of congophilic cerebral angiopathy. Immunohistochemical analysis revealed Aβ deposition within the vessel walls. Furthermore, abundant primitive cortical amyloid plaques became evident, which contained many activated microglia (Figure 2E). Inflammatory infiltrates consisted of CD3⁺ T cells (about 40%) (Figure 2I) and CD68⁺ macrophages (about 55%) (Figure 2G), many of the CD68⁺ macrophages exhibiting an epithelioid or multinucleated giant cell appearance and containing intracellular deposits of Aβ (Figure 2F and G). Corresponding to the results from flow cytometry of the CSF, CD4⁺ T cells were the predominant lymphocyte subtype within the (peri)vascular inflammatory infiltrates, accounting for about 80% (Figures 2H-L), and were found in close proximity to MHC class II–expressing microglia, epithelioid macrophages, and multinucleated giant cells (Figures 2M-P). Notably, there was no evidence of the presence of bacteria (Gram staining), fungi (periodic acid–Schiff staining) or mycobacteria (Ziehl-Neelsen staining).

Based on the presence of cerebral amyloid angiopathy together with vasculitis of leptomeningeal and penetrating cortical vessels, the histopathologic diagnosis of ABRA was established. Consecutive genetic testing for apolipoprotein (APO) E, as a risk factor for CAA-associated perivascular inflammation, revealed an APOE ε2/ε3 genotype in our case.

The patient received intravenous high-dose dexamethasone pulse therapy, 3 × 4-mg/d, for 3 days, immediately followed by methylprednisolone, 1 g/d, for 5 days. Afterwards, an oral maintenance dose of prednisolone, 80 mg/d, was administered for 6 weeks, followed by a...
tapering phase of 60, 40, 20, 10, and 5 mg/d for 2 weeks each. In parallel, immunosuppressive treatment with cyclophosphamide was administered at recurrent intravenous pulses of 750 mg/m² of body surface area every 6 weeks for a total of 6 months.

Follow-up studies revealed a stable clinical status and regression of leptomeningeal and cerebral inflammatory lesions on MRI. Serial CSF examination revealed normalization of cell counts (4/µL) and regression of protein levels within 60 days (protein, 0.8 g/L; CSF to serum albumin ratio, 8.5–10⁻³) (Figure 1K). Over the same period, the CD4/CD8 ratio decreased from 12.1 to 7.7 in the CSF (Figure 1L) and from 5.9 to 3.1 in the peripheral blood (data not shown). The fraction of activated CD69⁺ CD4⁺ T cells decreased from 18.1% to 1.2% in the CSF (Figure 1L) and from 1.1 to 0.8 in the peripheral blood (data not shown).

**Comment**

We provide herein, for the first time to our knowledge, a detailed description of the cellular composition of the inflammatory infiltrates in the CSF and in and around the leptomeningeal and penetrating cortical arteries in a typical case of ABRA as well as the response to a treatment regimen usually applied for primary angiitis of the CNS without CAA.

The biopsy specimens demonstrated about 50% CD68⁺ macrophages with intense expression of MHC class II,
many of which exhibited an epithelioid cell or multinucleated giant cell appearance and contained intracellular deposits of Aβ, suggesting that they ingested Aβ and might be capable (together with dendritic cells, plasma cells, and microglia) of processing and presenting Aβ as an antigen. CD3+ T cells made up about 40%, the vast majority of which were CD4+ T cells (about 80%) present in close proximity to MHC class II–expressing epithelioid macrophages and multinucleated giant cells. Moreover, the CSF contained a substantial faction of activated CD69+ CD4+ T cells even remote from the site of inflammation.

Hence, one might assume that CD4+ T cells are activated in an antigen-dependent manner by Aβ-presenting APCs. Such an interaction between CD4+ T cells and Aβ-presenting APCs would on the one hand enhance phagocytic capacity of macrophages and microglia and explain the partial clearance of Aβ from the CNS parenchyma found in ABRA. On the other hand, given a respective genetic susceptibility, this interaction between CD4+ T cells and Aβ-presenting APCs might trigger autoimmune destructive vessel inflammation, which in turn would impair the blood-brain barrier and promote enhanced drainage of Aβ from the circulation into the CNS parenchyma and thus sustained vessel inflammation.2

The presence of the APOE ε4/ε4 genotype in about 75% of patients with CAA-associated perivascular inflammation has been suggested as a susceptibility factor,4,5 which may exert a specific but yet unknown effect on the individual immune response to vascular Aβ deposition. However, the absence of this genotype in our case (APOE ε2/ε3) shows that this effect is not absolutely dispositive.

Taken together, our results support the notion of adaptive Aβ-directed autoimmunity as the underlying pathogenic mechanism in ABRA.