

Multiple Sclerosis and Alzheimer's Disease

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Objective: Chronic inflammation with microglia activation is thought to play a major role in the formation or clearance of Alzheimer's disease (AD) lesions, as well as in the induction of demyelination in multiple sclerosis (MS). In MS, the cortex is severely affected by chronic, long-lasting inflammation, microglia activation, and demyelination. To what extent chronic inflammation in the cortex of MS patients influences the development of AD lesions is so far unresolved.

Methods: The study was performed on autopsy tissue of 45 MS cases, 9 AD cases, and 15 control subjects. We analyzed lymphocyte and plasma cell infiltration in relation to microglia activation, to the presence of β -amyloid plaques and (AT8⁺) neurofibrillary tangles, and to myelin pathology.

Results: Profound microglia activation, determined by a broad spectrum of markers, was found in both MS and AD cortices, and the patterns of microglia activation were closely similar. Microglia activation in MS cortex, in contrast with that in AD and control cortex, correlated with lymphocyte and plasma-cell infiltrates in the meninges. MS cases older than 64 years experienced development of AD pathology in comparable incidence as seen in the course of normal aging. The density of β -amyloid plaques and neurofibrillary tangles did not differ between demyelinated and nondemyelinated cortical areas.

Conclusions: Our data suggest that microglia activation in the MS cortex alone has little or no influence on the development of cortical AD pathology.

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Microglia activation is believed to play a central role in the pathogenesis of lesions in the central nervous system of patients with Alzheimer's disease (AD) or multiple sclerosis (MS). In AD, abundant microglia activation is present in the affected cortex.¹ Moreover, microglia cells activated *in vitro* under AD-like conditions, such as by the addition of β -amyloid (A β) peptide to the culture medium, produce a variety of proinflammatory and toxic cytokines and mediators.^{2–4} Such toxic factors have been suggested to augment or promote amyloid deposition and neuronal degeneration.^{5–7} In contrast, active immunization or passive transfer of specific antibodies against the A β protein have been shown to reduce amyloid deposits in transgenic models of AD and in patients.⁸ Microglia cells are believed to play an important role in the clearing of A β deposits.^{9–11} This may occur in experimental models even in a bystander fashion without the involvement of specific adaptive immune responses against A β .¹²

Although MS has long been considered a demyelinating disease of the white matter, extensive cortical demyelination recently has been shown as a characteristic hallmark of the pathology of progressive MS.^{13,14} At this stage, profound inflammatory infiltrates com-

posed by T and B lymphocytes, sometimes even creating lymph-follicle-like structures, are present in the meninges.¹⁵ This inflammatory response in the meninges is associated with profound microglia activation in the cortex and the formation of widespread subpial bandlike demyelinated lesions.^{16,17} Microglia cells activated in the MS brain also produce proinflammatory cytokines and toxic factors, which contribute to demyelination and axonal lesions.¹⁸ MS thus represents an ideal human model disease to study the effect of long-lasting chronic cortical microglia activation on the formation or clearance of AD-type amyloid plaques or neurofibrillary tangles.

In this study, we addressed this question by analyzing a large sample of brains from MS cases who died in the progressive stage of the disease and comparing them with those from control subjects and AD cases.

Subjects and Methods

General Neuropathology

The study was performed on archival autopsy material from a total of 69 patients. It contained 45 MS cases, 9 cases with advanced AD, and 15 control subjects without neurological

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Table 1. Demographic and Clinical Data

Characteristics	Control Subjects	AD Cases	All MS Cases	MS Cases ≤ 65 yr	MS Cases > 65 yr
n	15	9	45	22	23
Female/male ratio	2	9	3.5	2.7	4.8
Median age, yr (range)	70 (30–97)	81 (60–92)	66 (28–85)	53.5 (28–64)	73 (66–85)

AD = Alzheimer's disease; MS = multiple sclerosis.

disease and brain lesions. Clinical histories of the patients were analyzed by retrospective chart analysis by an experienced neurologist (K.J.). Patient demographics are given in Table 1.

A detailed neuropathological survey was always performed, based on multiple brain tissue blocks, stained with hematoxylin and eosin, Luxol fast blue myelin stain, and Bielschowsky silver impregnation for axons and for A β deposition and neurofibrillary tangles. Histopathological evaluation was performed by a neuropathologist (K.J. or H.L.). MS cases demonstrated multiple focal demyelinated lesions in the gray and white matter. AD cases showed abundant amyloid plaques and neurofibrillary tangles (Fig 1), and pathology was staged according to Consortium to Establish a Registry for Alzheimer's Disease criteria¹⁹ and Braak stages.^{20,21} The control group did not show neuropathological alterations.

Regarding MS lesions, we determined the demyelinating activity of the lesions according to previously published criteria.^{14,22} Active lesions in the cortex showed a rim of high microglia density at the lesion margins, which were immunoreactive for major histocompatibility complex class II and CD68, and in part contained early myelin degradation products (see Fig 1). Inactive cortical plaques were demyelinated lesions without increased numbers of microglia at their borders and without myelin degradation products in macrophages or microglia. Normal-appearing gray matter did not contain demyelinated lesions.

Immunocytochemistry

Analysis of microglia activation in relation to demyelination and the development of AD lesions was restricted to cortical areas of the temporal lobe, including the temporal and the entorhinal cortex and hippocampus. The reason for this selection was that the temporal cortex is a predilection site for cortical demyelination in MS^{17,23} and for AD-related pathology.²⁰ Furthermore, the same region has been chosen previously for studying the incidence of AD lesions in a large cohort of aging humans.²⁴ Serial sections were performed and stained by immunocytochemistry with the markers (data are summarized in Supplementary Table 1). To evaluate microglia activation, we used different markers induced by different activation signals (adaptive vs innate immunity), associated with different microglia functions (antigen presentation, cytotoxic actions, or phagocytosis), or characteristic for defined proinflammatory or antiinflammatory microglia phenotypes (see also Discussion).

Immunocytochemistry was performed on formaldehyde-fixed and paraffin-embedded material with a biotin avidin

technique as described in detail previously.²⁵ Antigen retrieval was performed in a household steamer for 60 minutes with either EDTA buffer (pH 9.0) or citrate buffer (pH 5.0) when necessary (see Supplementary Table 1). Primary antibodies were applied overnight followed by incubation in biotinylated secondary antibodies (RPN 1001 or RPN 1004; Amersham, Buckinghamshire, United Kingdom) and avidin/peroxidase complex (Sigma, St. Louis, MO). Labeled antigen-antibody complexes were visualized with diaminobenzidine (Sigma).

For double staining, the first reaction was performed as described earlier; however, the reaction product was visualized with diaminobenzidine amplified with nickel sulfate. Then the second antibody was applied overnight and visualized with an alkaline phosphatase-labeled secondary antibody (Jackson ImmunoResearch Laboratories, Baltimore, MD) and fast red BB salt (Sigma) as a chromogen.

To control the specificity of the immunocytochemical reaction, we used antibodies of the same immunoglobulin class or polyclonal antibodies with irrelevant specificities. In addition, control sections were also stained in the absence of the primary antibody.

Quantitative Evaluation

The strategy of quantitative evaluation of the immunostained sections is depicted in Figures 2A–D. For quantification, each section was scanned and a camera lucida drawing was made, which depicted the global cortical area, the exact location and size of demyelinated cortical lesions, and the area of leptomeninges covering the cortex. The sections were then overlaid by a morphometric grid, and the number of grid points, located over meningeal areas, cortical areas, and lesions was determined. In the next step, the number of T cells, B cells, and plasma cells was determined within the entire area of meninges and cortex, and the density of macrophages and microglia was counted in six microscopic fields at a magnification of $\times 400$. A similar approach was used to evaluate the number of A β plaques and AT8⁺ neurofibrillary tangles. In parallel, we determined microglia activation within A β or neuritic plaques in comparison with the adjacent cortical tissue devoid of AD lesions. For this purpose, we used sections, which were double labeled for A β or AT8, together with the respective microglia marker (see Figs 1S, T). In the same sections, we determined the area of the cortex, which was covered by A β -plaques or AT8⁺ neuritic plaques. We then determined the density of microglia within and outside AD plaques. In sections from MS cases, we separately determined microglia

cell density within active or inactive demyelinated lesions, as well as in the normal-appearing gray matter. All values were normalized as cell counts per square millimeter of the respective tissue.

Determination of the Incidence of Alzheimer's Disease Lesions in the Global Multiple Sclerosis Population

To determine whether cortical injury in MS influences the development of AD lesions, we followed exactly the techniques described before in a study for determination of AD pathology in the normal aging population.²⁴ In brief, all sections were stained for A β and AT8, and plaques and neurofibrillary tangles were counted in the entire temporal cortex within the section. As in the previous study,²⁴ cases with more than two plaques or tangles per square millimeter were regarded as cases with AD pathology.

Determination of Plaque and Tangle Density in Demyelinated versus Nondemyelinated Cortical Areas in Multiple Sclerosis

This substudy was restricted to MS cases with evidence for AD pathology and where exactly matching demyelinated and nondemyelinated cortical areas could be identified ($n = 12$). Within the camera lucida drawings, the exact position and shape of the demyelinated lesion was determined. We then identified an area of normal-appearing gray matter in exactly the corresponding location within an adjacent gyrus in the same section. Then we determined the density of A β plaques and AT8⁺ neurofibrillary tangles in standardized microscopic fields of these appropriately selected areas. The values were expressed as plaques or tangles per square millimeter of demyelinated or nondemyelinated cortex.

Statistical Evaluation

Statistical analysis was performed using SPSS 11.5 statistical software system (SPSS, Chicago, IL). Nonparametric group tests (Kruskal–Wallis tests) were used to compare group differences (eg, different microglia activation marker expression within AD cases, MS cases, or control subjects). Consequently, to precisely determine existing significances among two particular groups, Mann–Whitney U tests were performed. p values were regarded as significant after Shaffer's correction for multiple testing. Linear regression analysis was used to identify interdependence of variables (eg, between meningeal inflammation and parenchymal microglia activation). The incidence of AD pathology in our MS sample was compared with Giannakopoulos and colleagues²⁴ sample, which is based on the analysis of 1,258 normal aged cases, by Fisher's exact test. We addressed the wide difference in the sample populations by calculating with absolute case numbers.

Results

General Neuropathology, Lymphocyte Infiltration, and Plasma Cell Infiltration

Control subjects showed some scattered lymphocytes within the meninges, but there were no changes reminiscent of inflammation. The cortical tissue did not show focal or diffuse abnormalities. In AD brains, lymphocyte infiltration in the meninges (see Fig 1) was

more pronounced compared with that in control subjects, and scattered T cells were seen within the cortical parenchyma and the perivascular space (Table 2). Within the cortex, numerous A β deposits were always present. Staining for AT8 showed numerous neurofibrillary tangles, neuropil threads, and a variable number of neuritic plaques (see Fig 1). All AD cases fulfilled the Consortium to Establish a Registry for Alzheimer's Disease criteria for AD. According to Braak classification, of the nine AD cases, two were stage IV, four were stage V, and three were stage VI.

MS cases showed demyelinated plaques in the cortex or the white matter, or both. Fourteen of 45 MS cases showed actively demyelinating lesions in the temporal cortex (termed active MS; see Fig 1). Eighteen of 45 cases showed only inactive lesions (inactive MS), whereas in 13 cases, no cortical demyelination was found in the temporal lobe. In sections from MS cases, the most intense infiltration with lymphocytes and plasma cells was found in the meninges (see Table 2 and Fig 1). As described previously,^{14,16} T-cell, B-cell, and plasma-cell infiltrates within the cortical parenchyma and around cortical vessels were sparse in MS cases.

Among the total cohort of 45 MS cases, 16 cases showed more than 2 A β plaques and/or neurofibrillary tangles per square millimeter (see Fig 2), all of them older than 64 years. In 8 of the 45 MS cases, plaque and tangle density exceeded the number required for neuropathological diagnosis of probable AD according to Consortium to Establish a Registry for Alzheimer's Disease criteria. Staging according to Braak showed 2 of 45 cases in stage I, 3 in stage II, 2 in stage III, 6 in stage IV, 1 in stage V, and 1 in stage VI. In one case, only A β plaques were present in the absence of neurofibrillary tangles.

Microglia Activation in the Cortex of Alzheimer's Disease and Multiple Sclerosis

Microglia activation was analyzed first in the global cortex regardless of the presence, size, and nature of lesions. In a second approach, we determined the expression of microglia activation markers separately in different lesion types or areas. In MS cases, they included actively demyelinating and inactive areas, as well as the normal-appearing gray matter. In AD, we analyzed the expression of the respective activation antigens within amyloid plaques in comparison with that in the global cortex.

Global microglia activation in the cortex was pronounced in sections of MS brain containing actively demyelinating cortical lesions (Act MS, Table 3). This was also reflected in the analysis of individual lesion areas, where all microglia markers were expressed to a significantly greater degree in actively demyelinating areas than in inactive areas or normal-appearing gray matter (Act MSL; see Table 3; see Fig 1).

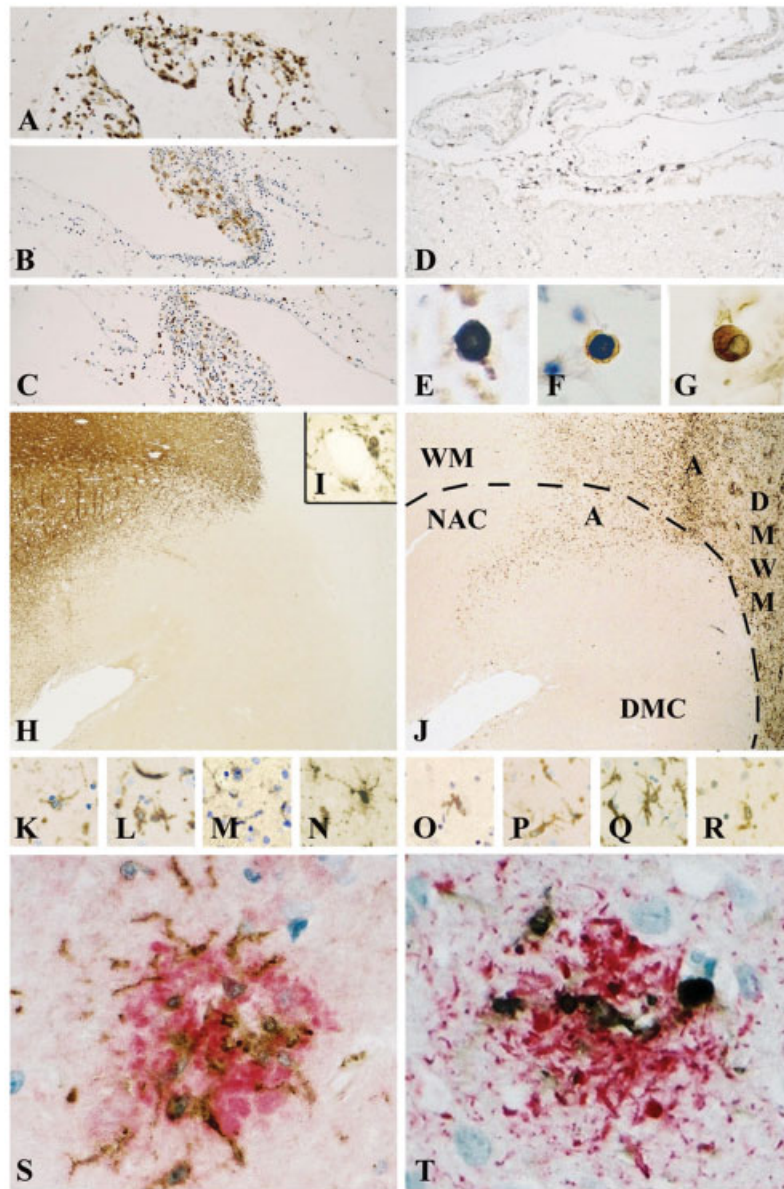


Fig 1. Brain inflammation and microglia activation in multiple sclerosis (MS) and Alzheimer's disease. (A–C) Meningeal inflammation in MS with actively demyelinating lesions in the temporal cortex; meninges are densely infiltrated by $CD3^+$ T cells (A), $CD20^+$ B cells, and immunoglobulin-containing plasma cells (C). (D–G) Meningeal inflammation in the temporal cortex of cases with Alzheimer's disease shows a moderate infiltration with $CD3^+$ T cells (D, E) and with single $CD20^+$ B cells (F) and immunoglobulin-containing plasma cells (G). (H–J) Temporal cortex of an MS case, containing an actively demyelinating lesion; serial sections, stained for proteolipid protein (PLP; H) and major histocompatibility complex (MHC) II (J). (J) Dotted line shows the border between cortex and subcortical white matter; there is a large, actively demyelinating lesion affecting both the cortex and the subcortical white matter; the area of demyelinating activity is marked by A; on the left side there is an area of normal-appearing cortex (NAC) and normal-appearing white matter (NAWM); the active zone is reflected by an increased density of MHC II-positive macrophages and microglia cells, which are densely packed in the active zone of the white matter lesion, but much less dense in the active area of the cortex; in the inactive portion of the lesions there is a moderate infiltration of MHC II+ cells in the white matter, but a low density of MHC II+ cells in the cortex; DMC and DMWM represent demyelinated cortex and demyelinated white matter; the inset (I) shows PLP-reactive myelin degradation products within macrophages and microglia cells in the active portion of the cortical lesion. (K–R) Expression of microglia activation antigens within an actively demyelinating MS lesion. (K) $\beta 2$ microglobulin. (L) MHC class I α chain. (M) Siglec 11. (N) CD68. (O) inducible nitric oxide synthase. (P) CD163. (Q) Allograft inflammatory factor 1 (AIF-1). (R) glut-5. (S, T) MHC class II-positive microglia cells in an $A\beta$ plaque (S) and a neuritic plaque (T) in Alzheimer's disease, (S) Double staining for $A\beta$ (red) and MHC class II (brown). (T) Double staining for AT8 (red) and MHC class II (brown). Original magnification $\times 100$ (A–D, K–M, O–T); $\times 1,000$ (E–G); $\times 10$ (H, J); $\times 300$ (I); $\times 200$ (N).

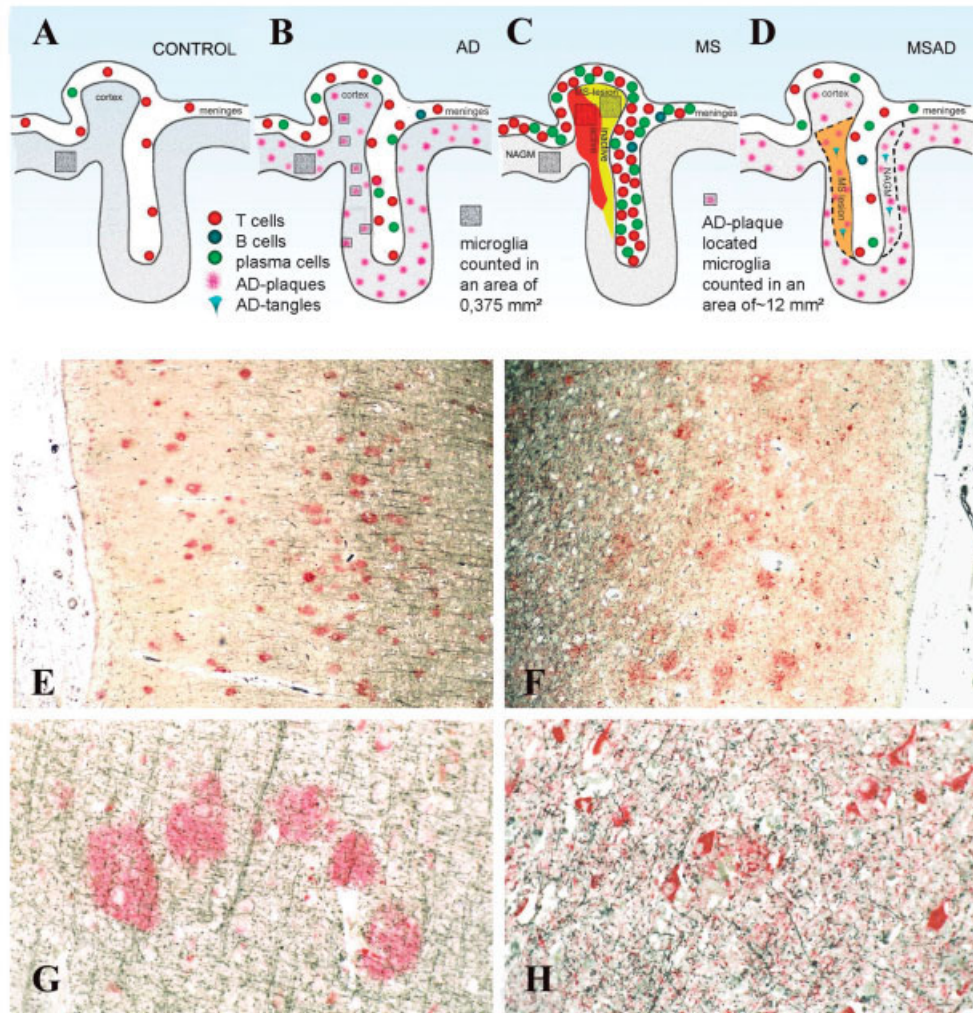


Fig 2. Schematic representation of quantitative methods and the relation between Alzheimer's disease (AD) and multiple sclerosis (MS) pathology. (A) Control brain; evaluation of inflammatory infiltrates in meninges and of the expression of microglia antigens in a standardized field of the cortex (gray box). (B) Quantitative evaluation of meningeal inflammation and cortical microglia activation in AD; meningeal inflammation was evaluated within the whole meninges; global microglia activation within the cortex was analyzed in standardized cortical areas (gray field); in addition, the density of microglia, expressing the different activation antigens, was determined separately within the $A\beta$ and $AT8^+$ neuritic plaques (small gray boxes overlying the red $A\beta$ plaques). (C) Quantitative evaluation of meningeal inflammation and cortical microglia activation in the temporal cortex of MS cases; meningeal inflammation was determined in the same way as in control subjects and AD cases; within the MS cortex, the density of microglia activation antigens was quantified in different areas separately, such as the actively demyelinating lesion areas (red), the inactive demyelinated areas (yellow), and the normal-appearing gray matter in standardized areas (gray boxes). (D) Determination of plaque and tangle densities in the cortex of MS patients with AD pathology: the demyelinated areas were identified by staining of the sections with proteolipid protein (PLP) (orange area); we then delineated an exactly corresponding area of nondemyelinated cortex in an adjacent gyrus within the same section. We then analyzed the density of $A\beta$ plaques and neurofibrillary tangles within these two corresponding areas. (E, F) Distribution of $A\beta$ plaques and neurofibrillary tangles (neuritic plaques) in partly demyelinated MS cortex; the sections were stained with PLP and show extensive subpial demyelination, involving the outer four layers of the cortex; $A\beta$ plaques and neurofibrillary tangles are present in comparable density in both the demyelinated and the normal cortical areas. (G, H) Double staining for PLP (black) and $A\beta$ (G) and $AT8$ (H), respectively (red); myelin sheaths are preserved within the $A\beta$ -plaques (G) and within the neuritic plaques (H). Original magnification $\times 10$ (E, F); $\times 100$ (G, H).

Microglia activation was also pronounced in the cortex of AD patients, and this was present to a degree that was similar to that in MS patients (see Table 3). Furthermore, the expression patterns of the different microglia activation antigens were strikingly similar between MS and AD patients (see Table 3). As de-

scribed previously,¹ microglia activation in AD patients was significantly greater within $A\beta$ plaques compared with plaque-free cortical areas (AD plaques; see Table 3 and Fig 1). Microglia activation in the AD cortex was not associated with demyelination (see Figs 2G, H).

Table 2. Median Quantitative Differences (Range) of Meningeal, Perivascular, and Parenchymal Infiltration of T Cells (CD3), B Cells (CD20), and Plasma Cells (Ig)

Patient Groups	Meningeal Inflammation			Perivascular Inflammation			Parenchymal Inflammation		
	CD3	CD20	Ig	CD3	CD20	Ig	CD3	CD20	Ig
Active MS (n = 14)	27.89 (10–138)	1.71 (0–21)	19.96 (2–141)	0.47 (0–3)	0.01 (0–0.4)	0.25 (0–4)	0.24 (0–1)	0.01 (0–0.2)	0.09 (0–2)
Inactive MS (n = 18)	5.77 ^a (2–16)	0.28 (0–6)	4.55^a (0–31)	0.11 ^a (0–1)	0.01 (0–0.1)	0.02 ^a (0–1)	0.02^a (0–1)	0 (0–0.1)	0 ^a (0–0.1)
AD (n = 9)	12.79^b (8–23)	0.19 (0–1)	5.99^b (2–12)	0.63 (0–2)	0 (0–0.02)	0.02 (0–0.5)	0.21 (0–3)	0 (0–0.1)	0.04 (0–0.1)
Control Subjects (n = 15)	8.00 (1–20)	0 (0–1)	0.89 (0–6)	0.31 (0–1)	0 (0)	0.03 (0–0.4)	0.17 (0–1)	0 (0–0.6)	0 (0–0.2)

Values in boldface are significant compared with control subjects. ^aSignificant difference between active and inactive multiple sclerosis (MS). ^bSignificant difference between Alzheimer's disease (AD) and active MS.

Microglia Activation Correlates with Meningeal Lymphocyte and Plasma-Cell Infiltration in Multiple Sclerosis Cases, But Not in Alzheimer's Disease Cases and Control Subjects

We have shown so far that in both diseases, AD and MS, there is profound microglia activation in the cortex

in comparison with control subjects, which is associated with increased meningeal T-cell, B-cell, and plasma-cell infiltration. The question thus arises whether in these different conditions microglia activation is driven by the inflammatory process. To answer this question, we analyzed the interdependence between microglia activation

Table 3. Microglia Activation (Density of Immunoreactive Microglia/mm² (Range)] in the Cortex of Multiple Sclerosis Cases, Alzheimer's Disease Cases, and Control Subjects

Marker	Act MS	Act MSL	Ia MS	Ia MSL	NAGM	AD	AD PI	Cont
MHC II	43.4 (6–133)	137.9 (10–228)	23.5 (1–63)	19.5 (0–84)	27.1 (0–133)	55.3 (5–104)	350.3 (91–522)	4.0 (0–50)
β2M	25.2 (3–53)	52.0 (10–145)	11.1 (3–37)	17.1 (0–51)	13.9 (3–61)	20.3 (0–43)	72.6 (0–377)	24.0 (3–56)
HC-10	21.6 (2–63)	43.3 (3–204)	14.8 (2–64)	16.0 (0–62)	16.5 (0–88)	25.0 (0–56)	213.2 (0–343)	14.7 (0–72)
Siglec	15.7 (5–52)	53.7 (4–198)	6.1 (0–33)	7.6 (0–77)	6.7 (0–43)	14.2 (0–48)	83.4 (0–516)	6.4 (0–32.0)
CD68	64.6 (1–123)	153.0 (54–354)	47.1 (28–70)	52.7 (0–141)	47.2 (0–116)	52.6 (27–103)	306.9 (86–478)	42.7 (35–61)
CD163	7.7 (0.1–43)	21.2 (3–102)	3.9 (0–15)	6.0 (0–42)	5.3 (0–41)	14.1 (5–46)	179.2 (6–429)	0 (0–13)
iNOS	15.0 (2–45)	40.8 (5–129)	9.0 (0–29)	6.0 (0–48)	10.7 (0–45)	10.0 (4–45)	108.9 (37–373)	5.3 (0–69)
HMGB	32.9 (8–70)	53.3 (8–132)	20.2 (8–66)	26.0 (3–74)	24.5 (8–85)	27.5 (11–63)	201.7 (53–303)	29.3 (5–67)
AIF-1	68.3 (32–181)	133.6 (40–326)	46.8 (10–127)	70.2 (6–162)	55.7 (10–181)	83.2 (17–203)	244.7 (81–639)	56.0 (8–80)
GLUT	38.3 (10–95)	71.7 (21–194)	28.0 (5–93)	36.6 (3–112)	32.7 (3–113)	24.6 (6–71)	158.2 (32–339)	42.7 (12–82)

Values in boldface are significantly different from control subjects; most pronounced microglia activation is seen in active multiple sclerosis (MS) lesions and within β-amyloid (Aβ) plaques in Alzheimer's disease (AD). The patterns of microglia activation are similar between MS and AD.

Act MS = global cortex in cases with active cortical lesions; Act MSL = actively demyelinating cortical lesions; Ia MS = global cortex of MS cases with inactive cortical lesions; Ia MSL = inactive cortical MS lesions; NAGM = normal-appearing gray matter in MS; AD = global cortex of cases with Alzheimer's disease; AD PI = Aβ plaques in the cortex of Alzheimer's disease cases; Cont = global cortex of control subjects; MHC = major histocompatibility complex; iNOS = inducible nitric oxide synthase; HMGB = high-mobility group box 1; AIF-1 = allograft inflammatory factor 1.

in the cortex and the density of T, B, and plasma cells in the meninges (Table 4). In the cortex of MS patients, we found a significant interdependence between microglial major histocompatibility complex, CD68, inducible nitric oxide synthase, and allograft inflammatory factor 1 expression and meningeal T-cell infiltration. This suggests that microglia activation in the cortex of MS patients is, at least in part, driven by the meningeal inflammatory response. A similar interdependence between meningeal inflammation and cortical microglia activation was not apparent in AD patients and control subjects (see Table 4).

Multiple Sclerosis Patients Develop Alzheimer's Disease Amyloid Plaques and Neurofibrillary Tangles in Similar Incidence Compared with an Age-Matched Control Population

Because MS patients and AD patients exhibit microglia activation in the cortex and similar activation patterns of microglia, we asked the question whether chronic microglia activation in MS influences the development of A β plaques or neurofibrillary tangles.

As in the normal aging cohort,²⁴ none of the MS cases, who died before an age of 64, showed neurofibrillary tangles or amyloid plaques in the temporal lobe, which according to the inclusion criteria exceeded 2/mm². In cases who died at later ages, the global incidence of cases with plaques and tangles in the temporal cortex was moderately greater as compared with the normal aging population. However, according to age group, there was no significant difference in global incidence of AD pathology (more than two plaques and/or tangles per mm²) in the temporal cortex between the MS population and the normal aging population (Fig 3).

We then quantified A β plaques and neurofibrillary tangles in demyelinated cortical plaques and compared their density with that in normal cortical areas, which were specifically selected to match the topographical orientation and the cortical layers. We found no significant difference in the density of A β plaques or neurofibrillary tangles between demyelinated and normal-appearing cortex in MS cases (Table 5).

Discussion

This study is the first to address the question whether chronic and long-lasting microglia activation in the human cortex influences the development of AD pathology. The key results of our study are that, in MS cases, who died before the age of 64 years, no AD pathology was found in the cerebral cortex, despite profound inflammation and a pattern of microglia activation, which was closely similar to that seen in the cortex of AD cases. In patients older than 64 years, AD pathology is seen, which in incidence and severity is similar to that present in the normal aging population. This

implies that, in aged MS patients, a cognitive decline may not only be related to MS-specific lesions but also to concomitant age-related development of AD pathology. Our study focused on the investigation of the temporal cortex, including the entorhinal cortex and the hippocampus, because cortical demyelination in MS and AD-type pathology is particularly pronounced in this area.^{15,20,23,26} This provides the unique opportunity to analyze directly whether these two types of pathologies influence each other in their development.

To study patterns of microglia activation, we used a panel of markers, which have been described to be induced by different activation signals and serving different functions in macrophages or microglia,²⁷ and which are expressed on microglia in normal or pathological conditions. Major histocompatibility complex antigens are instrumental for antigen presentation for T cells. They are readily induced by proinflammatory cytokines, and their expression is essential for the propagation of T-cell-mediated inflammation.²⁸ CD68 and CD163 are scavenger receptors that are involved in phagocytosis.^{29,30} High-mobility group box 1 is secreted in macrophages on activation by innate immunity. It stimulates adaptive immune responses and promotes recruitment and activation of inflammatory cells.³¹ Inducible nitric oxide synthase expression is triggered by proinflammatory cytokines and by innate immunity stimulation, and is a major source for nitric oxide radical production in inflammatory conditions.³² Siglec 11 is a member of the sialoadhesin family, which is prominently expressed in microglia.³³ Sialoadhesins are induced in macrophages by innate immunity stimulation.³⁴ They appear to play an inhibitory role in inflammatory conditions.³⁴ A similar antiinflammatory role is also ascribed to CD 163.³⁵ In addition, we used two markers that are prominently expressed in resting microglia. Allograft inflammatory factor is an actin binding protein. Its expression is enhanced in the course of macrophage and microglia activation under inflammatory conditions, and its expression correlates with tissue rejection.^{36,37} Finally, Glut-5 is a glucose/fructose transporter that is prominently expressed in brain microglia.³⁸ Thus, the panel of markers used in this study cover a wide range of different functional roles and activation mechanisms of microglia. Microglia activation in AD appears to be driven by innate immunity,^{39–41} and in MS by specific adaptive immune responses.¹⁸ This view is supported in our study by the significant interdependence between meningeal T-cell infiltration and microglia activation in the underlying MS cortex. In contrast, no such interdependence was found in AD cases and control subjects, despite profound microglia activation in the cortex of AD cases. Nevertheless, the expression patterns of activation antigens in microglia were strikingly similar in AD and MS cases. However, it must be emphasized that

Table 4. Interdependence of Meningeal Infiltration with T Cells with Cortical Microglia Activation in Multiple Sclerosis Cases (n = 32), Alzheimer's Disease Cases (n = 9), and Control Subjects (n = 15)

Patient Groups	MHC II	B2M	HC-10	Siglec	CD 68	CD 163	iNOS	HMGB1	AIF-1	GLUT-5
MS R (<i>p</i> value)	0.546 (0.001)	0.442 (0.011)	0.363 (0.041)	0.152 (0.407)	0.487 (0.005)	0.288 (0.110)	0.553 (0.001)	0.347 (0.052)	0.607 (0.001)	0.209 (0.251)
AD R (<i>p</i> value)	0.257 (0.504)	0.204 (0.599)	0.094 (0.809)	0.060 (0.879)	0.168 (0.665)	0.415 (0.267)	0.348 (0.358)	0.235 (0.543)	0.237 (0.538)	0.218 (0.574)
Control R (<i>p</i> value)	0.041 (0.884)	0.059 (0.834)	0.230 (0.410)	0.324 (0.239)	0.237 (0.394)	0.412 (0.127)	0.063 (0.824)	0.397 (0.142)	0.036 (0.899)	0.493 (0.062)

Linear regression analysis between meningeal CD3 cells and the expression of the respective microglia antigens in the underlying cortex. Values in bold show significant interdependence. MHC = major histocompatibility complex; iNOS = inducible nitric oxide synthase; HMGB = high-mobility group box 1; AIF-1 = allograft inflammatory factor 1; MS = multiple sclerosis; AD = Alzheimer's disease; R = correlation coefficient.

the spectrum of microglia activation markers used in our study, although it covers different activation mechanisms and functional states, is incomplete. Thus, more subtle or specific differences in microglia activation between AD and MS cases cannot be ruled out. It has, however, to be considered that a variety of different microglia-derived cytokines or molecules, which have been suggested to promote AD lesions, are also highly expressed in MS. They include, among others, interleukin-1,⁴² tumor necrosis factor- α ,⁴³ complement,⁴⁴ or inducible nitric oxide synthase.³²

Despite the similarities of microglia activation in MS and AD lesions, we did not find a difference in the development of AD pathology in aging MS patients in comparison with a normal human population. Because of the limited resource of autopsy samples from MS cases, this observation is based on 45 MS cases, which was compared with a previously published cohort of the normal aging population. One can thus argue that our study is underpowered to reach these conclusions. This question has to be addressed separately for the different aspects of the study, whether chronic inflammation and microglia activation in MS has an effect on either the development or the clearance of AD lesions and whether chronic tissue injury, which occurs in the

cortex of MS patients, may provoke amyloid deposition or neurofibrillary tangle formation.

Our results regarding the first question, that is, whether chronic microglia activation in MS promotes AD lesions, are based on a large sample of cases who died before 64 years of age. In these cases, A β plaques and neurofibrillary tangles were virtually absent, which is in accordance with the normal aging human population. Profound microglia activation is present in the cortex of most MS patients in the progressive stage of the disease,¹⁷ and this progressive phase of MS in general starts around the age of 40.⁴⁵ This implies that the cerebral cortex of MS patients is exposed to activated microglia cells for at least one or two decades without developing AD lesions.

Regarding the second question, that is, whether chronic inflammation and microglia activation in MS patients facilitates the clearance of AD lesions, it has to be considered that, at an age older than 65 AD, lesions develop in a subset of MS patients and the incidence of cases with AD lesions was moderately but not significantly greater compared with that in the normal aging cohort. Furthermore, in those cases who did develop AD lesions, the density of A β plaques and neurofibrillary tangles was not significantly different between de-

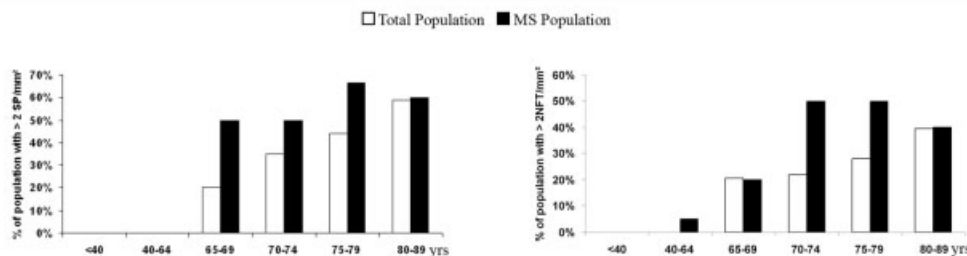


Fig 3. A β plaques and neurofibrillary tangles in the temporal cortex of multiple sclerosis (MS) cases in comparison with those in an aging human population of 1,258 cases that Giannakopoulos and colleagues²⁴ described in 1994. According to age group, there was no significant difference in global incidence of Alzheimer's disease pathology (more than two plaques and/or tangles per mm²) in the temporal cortex between the MS population and the normal aging population.

Table 5. Median β -Amyloid Plaque and Neurofibrillary Tangle Density (Range) in Demyelinated versus Myelinated Multiple Sclerosis Cortex

Cortex Type	A β Plaques (n = 12)	NFT (n = 8)
Demyelinated cortex	7.37 (0–62.77)	6.73 (0–53.99)
Myelinated cortex	10.92 (0–77.17)	9.33 (0–21.31)
<i>p</i>	0.755	0.959

A β = β -amyloid; NFT = neurofibrillary tangle.

myelinated and myelinated portions of the cortex. These data suggest that nonspecific activation of microglia, which has been described to decrease A β load in experimental animals sensitized with copolymer I or with adjuvant alone,¹² does not play a major role in A β clearance in humans, unless specific A β antibodies are present.¹⁰

The third question addresses the point whether chronic tissue injury in MS, induced by the demyelinating process, may promote AD pathology. This is particularly relevant for neurofibrillary tangles, which are seen occasionally in neurons in postencephalitic conditions.^{46,47} Like in AD, cortical MS lesions are associated with neuronal, axonal, or synaptic loss; its extent, however, is highly variable.^{14,48–50} To answer this question definitively, our study may indeed be underpowered, because in the limited number of MS cases included in our study, the incidence of cases with AD pathology in the temporal lobe was moderately but not significantly greater compared with that in the age-matched cohort. However, we did not observe a difference in the density of A β plaques and neurofibrillary tangles between demyelinated and nondemyelinated areas of the MS cortex. Because neurodegeneration is more pronounced within cortical plaques compared with that in normal-appearing cortical tissue in MS, such a difference would be expected when neurodegeneration in MS promotes AD pathology. Whether an age-related loss of neuroprotective functions of microglia contributes to AD pathogenesis^{40,51} in the normal cohort of aging humans or in our sample of MS patients is not addressed by our study and remains a plausible option.

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