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ORIGINAL ARTICLE

B-Cell Enrichment and Epstein-Barr Virus Infection in Inflammatory Cortical Lesions in Secondary Progressive Multiple Sclerosis

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Abstract

Gray matter lesions are thought to play a key role in the progression of disability and cognitive impairment in multiple sclerosis (MS) patients, but whether gray matter damage is caused by inflammation or secondary to axon loss in the white matter, or both, is not clear. In an analysis of postmortem brain samples from 44 cases of secondary progressive MS, 26 cases were characterized by meningeal inflammation with ectopic B-cell follicles and prominent gray matter pathology; subpial cortical lesions containing dense perivascular lymphocytic infiltrates were present in 11 of these cases. Because intracortical immune infiltrates were enriched in B-lineage cells and because we have shown previously that B cells accumulating in the MS brain support an active Epstein-Barr virus (EBV) infection, we investigated evidence of EBV in the infiltrated cortical lesions. Cells expressing EBV-encoded small RNA and plasma cells expressing EBV early lytic proteins (BZLF1, BFRF1) were present in all and most of the intracortical perivascular cuffs examined, respectively. Immunohistochemistry for CD8-positive cells, granzyme B, perforin, and CD107a indicated cytotoxic activity toward EBV-infected plasma cells that was consistently observed in infiltrated cortical lesions, suggesting active immune surveillance. These findings indicate that both meningeal and intraparenchymal inflammation may contribute to cortical damage during MS progression, and that intracortical inflammation may be sustained by an EBV-driven immunopathologic response, similar to findings in white matter lesions and meninges.

Key Words: B cells, Cortical lesions, Cytotoxicity, Epstein-Barr virus, Inflammation, Multiple sclerosis.

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INTRODUCTION

It is increasingly recognized that CNS pathology in multiple sclerosis (MS) is not restricted to the white matter (WM) and that abnormalities in the cortical and deep gray matter (GM) are a common and early feature of MS (1–3). Importantly, disease progression and cognitive impairment correlate better with cortical lesion load and GM atrophy than with WM damage (2–4), indicating that GM abnormalities play an important role in MS evolution. Despite this evidence, a full understanding of mechanisms underlying GM pathology in MS lags behind (4, 5).

Studies in postmortem brain tissue from patients with long-standing progressive MS have identified 3 types of cortical lesions (mostly subpial, but also intracortical and leukocortical) (6, 7) and have shown substantial intralesional and extralesional neuronal and glial cell damage and loss (8–11). The observation that cortical lesions lack the typical lymphocytic infiltrates, complement activation, and myelin-laden macrophages of WM lesions has led to the suggestion that GM damage is not caused primarily by inflammation but develops as a consequence of remote myelin and axon loss in the WM (6, 12, 13). However, we (10, 11, 14) and others (15, 16) have shown that cortical demyelination, neurodegeneration, and atrophy in MS positively correlate with the presence of diffuse inflammatory infiltrates and lymphoid follicle-like structures (ectopic B-cell follicles) in the leptomeninges; this implies that immune activation in this compartment contributes to cortical pathology in MS. We have also demonstrated that, in secondary progressive MS (SPMS), florid meningeal inflammation is accompanied by damage to the glia limitans and that there is a gradient of neuronal loss that is greater in the superficial cortical layers nearest the pial surface than in the inner cortical layers (11). These findings strongly suggest that inflammatory and cytotoxic factors diffusing from the infiltrated meninges may play a major role in the development of subpial cortical lesions. In support of the inflammatory origin of cortical lesions, a recent study of a large number of brain biopsies from patients with the recent diagnosis of MS showed that actively demyelinating cortical lesions are frequent in early MS, that they associate with meningeal inflammation, and, in contrast with observations in postmortem tissue, that they show prominent immune infiltrates and myelin phagocytosis (17). These pathologic features could be related to the more active inflammatory process at MS onset

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(18), suggesting that the contribution of meningeal and intraparenchymal inflammation to cortical damage may differ as disease progresses.

During extensive screening of postmortem MS brain samples, we observed cortical lesions containing dense perivascular lymphocytic infiltrates in approximately 40% of the SPMS cases with more prominent CNS inflammation (10, 11, 14, 19). This unexpected finding led us to investigate the cellular composition of intracortical immune infiltrates at late MS stages and to observe that these contained abundant B-lineage cells. Because a persistent infection in the CNS with the B-lymphotropic herpesvirus Epstein-Barr virus (EBV) is currently considered as a possible mechanism contributing to the pathogenesis of MS (20–22), we investigated the relationship between B-cell/plasma cell accumulation and EBV infection in MS cortical lesions.

MATERIALS AND METHODS

Tissues and Neuropathologic Evaluation

Brain tissue was obtained from the UK MS Tissue Bank at Imperial College via a UK prospective donor scheme with full ethical approval (08/MRE09/31). Neuropathologic confirmation of the diagnosis of MS was carried out according to the ICDNS criteria (www.ICDNS.org) (23). The presence of inflammatory cell infiltrates in the cerebral cortex was evaluated in 181 brain tissue blocks from 44 SPMS cases, 26 with meningeal B-cell follicle (F+) and 18 without meningeal B-cell follicles (F-); the cases have been extensively characterized for lesional and inflammatory features (10, 11, 14). The 17 frozen tissue blocks containing the infiltrated cortical lesions analyzed in this study were from the cerebral hemispheres of 11 F+ SPMS cases. Cortical and WM lesions were classified according to published criteria (6, 10).

Immunohistochemistry and Immunofluorescence

Air-dried 10-μm-thick cryosections cut from 4% paraformaldehyde–fixed or snap-frozen tissue blocks were stained with hematoxylin and eosin (H&E) and with antibodies specific for myelin-oligodendrocyte glycoprotein, laminin, CD20, human immunoglobulin A (IgA), IgG, IgM, CD3, CD8, MHC class II, CD107a, perforin, and EBV early lytic antigen BFRF1, following previously described procedures (10, 11, 19, 20, 24). For BZLF1 immunostaining, paraformaldehyde-fixed sections were postfixed in acetone and then treated with citrate buffer (pH 6) in a microwave, permeabilized with 0.5% Triton X-100 for 10 minutes, incubated with a mouse monoclonal antibody specific for BZLF1 protein (BZ-1; kind gift of Prof J. Middledorp, Vrije Universiteit University Medical Center, Amsterdam, The Netherlands), and diluted in PBS containing 0.1% Triton X-100 overnight at 4°C. Anti–granzyme B mouse monoclonal antibody (clone GrB7, 1:50 dilution; Dako, Glostrup, Denmark) was used for double immunofluorescence staining with anti-CD8 rabbit polyclonal antibody (1:50 dilution; Pierce, Thermo Fisher Scientific, Inc., Rockford, IL). Anti–granzyme B goat polyclonal antibody (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) was used for triple immunofluorescence staining with anti-CD8

TABLE 1. Demographic, Clinical, and Neuropathologic Characteristics of the SPMS Cases Analyzed

	Female/Male	Age of Onset, years	Age at Death, years	Disease Duration	Age at Wheelchair (EDSS = 7)	Percentage of Brain Tissue Blocks With Active WM Lesions	Percentage of Brain Tissue Blocks With Chronic Active WM Lesions	Percentage of Brain Tissue Blocks With Active GM Lesions	Percentage of Brain Tissue Blocks With Chronic Active GM Lesions
F- SPMS cases (n = 18)	6/12	31.3 ± 6.6*	58.8 ± 12.3	27.6 ±13.4	46.6 ± 10.0	11.6 ± 14.9	56.2 ± 21.0	2.8 ± 8.1	27.8 ± 24.1
F+ SPMS cases (n = 26)	19/7	24.1 ± 5.6†	44.3 ± 7.1‡	19.6 ± 7.9	34.4 ± 7.6§	37.7 ± 25.0‡	65.7 ± 26.0	34.7 ± 21.8‡	88.6 ± 14.9‡
F+ SPMS cases with infiltrated cortical lesions (n = 11)	9/2	23.7 ± 7.3 †	42.5 ± 5.6‡	17.5 ± 8.6	34.8 ± 8.7†	51.3 ± 28.2‡	79.6 ± 21.7‖	32.5 ± 19.2‡	93.7 ± 11.1‡

*Data are expressed as mean ± SD; p values were calculated using nonparametric Mann-Whitney U test.
†$p < 0.01$ F+ SPMS cases and F+ SPMS cases with infiltrated cortical lesions versus F- SPMS cases.
‡$p < 0.0001$ F+ SPMS cases and F+ SPMS cases with infiltrated cortical lesions versus F- SPMS cases.
§$p < 0.001$ F+ SPMS cases and F+ SPMS cases with infiltrated cortical lesions versus F- SPMS cases.
‖$p < 0.05$ F+ SPMS cases with infiltrated cortical lesions versus F- SPMS cases.
EDSS, Expanded Disability Status Scale; F+, cases with meningeal B-cell follicles; F-, cases without meningeal B-cell follicles.

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mouse monoclonal (clone C8/144B, Dako) and anti-BFRF1 rabbit polyclonal antibodies. For immunohistochemistry, sections were incubated with the appropriate biotinylated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA), followed by avidin–biotin peroxidase complex (ABC; Vector Laboratories Burlingame, CA), and the immune reactions were visualized with diaminobenzidine (Sigma, St. Louis, MO). After counterstaining with hematoxylin, sections were sealed with Canadian Balsam and viewed and photographed with an Axiophot Zeiss microscope equipped with an Axiocam digital camera using the Axiovision 4 AC software. For indirect immunofluorescence, sections were incubated with Alexa Fluor 488–conjugated goat or donkey anti-mouse IgG (Invitrogen, Eugene, OR), AMCA-conjugated donkey anti-goat IgG (Jackson ImmunoResearch Laboratories), or Cy3-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) alone or in the appropriate combinations. Negative control staining was performed using Ig-isotype controls and/or preimmune sera. Sections were sealed in ProLong Gold antifade reagent with 4′,6′-diamidino-2-phenylindole ([DAPI] Invitrogen). Images were acquired and analyzed with a Leica DM-4000B Microsystems microscope or with a laser scanning confocal microscope (LSM 5 Pascal, Carl Zeiss).

Total cell nuclei (hematoxylin-positive or DAPI-positive nuclei) and CD20-positive, CD8-positive, and Ig-positive cells present in individual intracortical perivascular cuffs were counted by 2 investigators (Roberta Magliozzi and Barbara Serafini). Thirty-three brain sections from 15 brain tissue blocks from 9 F+ SPMS cases were evaluated. Perivascular CD8-positive, CD20-positive, and Ig-positive cells were counted in 11, 17, and 8 brain sections, respectively. Data are expressed as percentages of immunostained cells in the total perivascular cell population; mean values ± SD are shown.

In Situ Hybridization for EBV-Encoded Small RNA

In situ hybridization for EBV-encoded small RNA (EBER) was performed using the Epstein-Barr virus peptide neucleic acid (PNA) probe/fluorescein and PNA ISH Detection Kit (Dako) following the manufacturer's instructions and as previously described (20). An irrelevant probe and a probe for GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (Dako) were used as internal negative and positive controls, respectively. Brain sections from 1 MS case were also hybridized with radiolabeled EBER probes. Briefly, sections were exposed to αP^{33}-labeled probes synthesized from plasmids containing cDNAs for EBER1 and EBER2 (kindly provided by Prof G. Niedobitek, Sana Klinikum Lichtenberg/Unfallkrankenhaus Berlin, Berlin, Germany) (25) and then to the autoradiography emulsion, following previously published procedures (26). After counterstaining with DAPI, images of stained sections were digitalized using a darkfield-equipped microscope.

RESULTS

Frequency, Localization, and Cellular Composition of Perivascular Immune Infiltrates in Cortical Lesions From SPMS Cases

Investigation of immune infiltrates in the cerebral cortex of MS cases was performed on brain tissue from 44 SPMS

TABLE 2. Demographic, Clinical, Autopsy, and Neuropathologic Characteristics of SPMS Cases With Meningeal B-Cell Follicles and Infiltrated Cortical Lesions

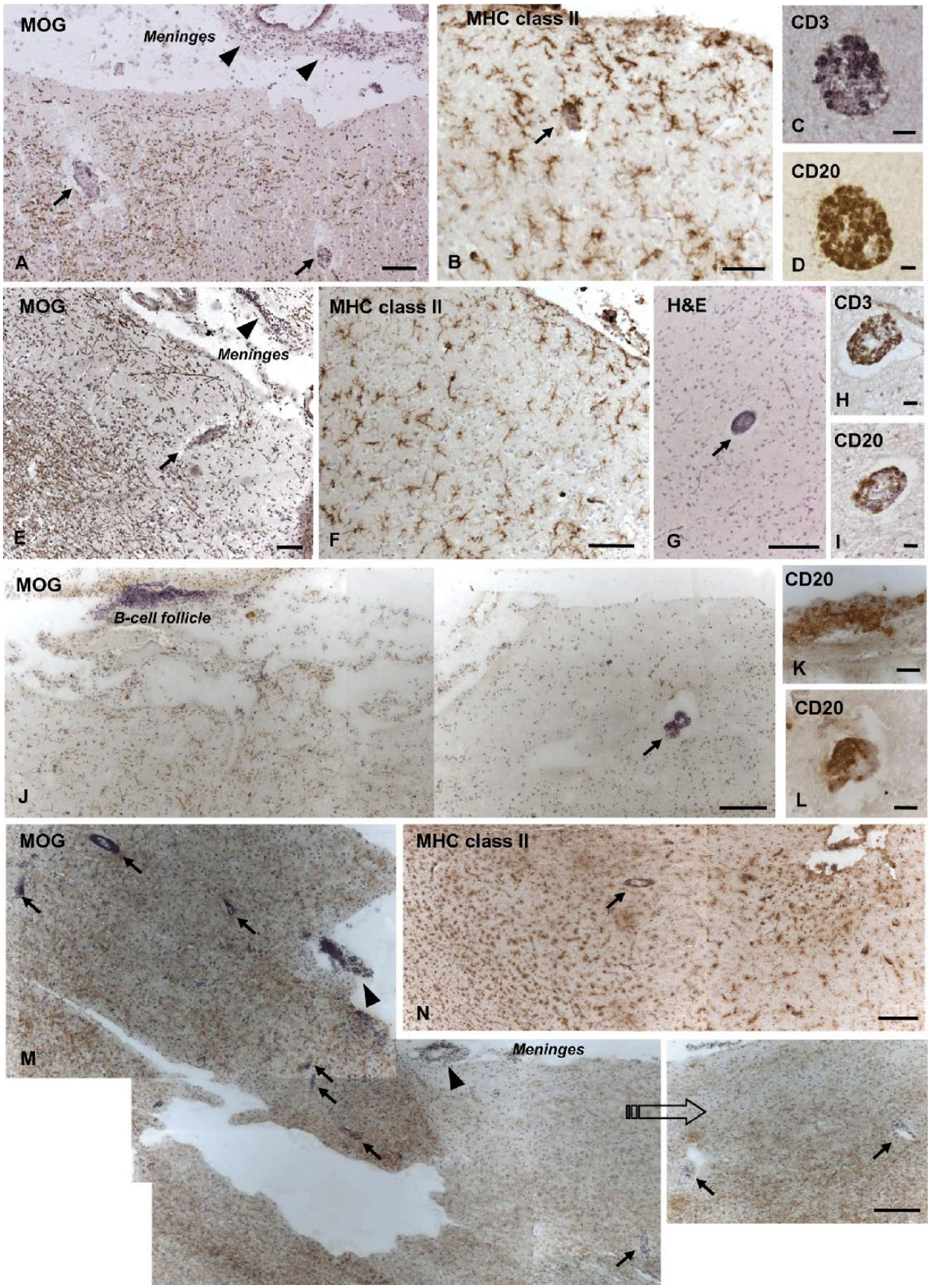
MS Case	Sex/Age at Death, years	Disease Duration, years	Age at Wheelchair (EDSS = 7)	Cause of Death/ Immunotherapy	Degree of Perivascular WM Inflammation/ Meningeal Inflammation*	Postmortem Delay, hours	No. Tissue Blocks With Infiltrated Cortical Lesions/Total No. Tissue Blocks Analyzed
MS79	F/49	24	36	Bronchopneumonia, MS†/none	+/++	7	1/8
MS92	F/37	7	28	MS /none	+/++	26	2/4
MS121	F/49	14	45	MS/none	++/++	24	1/5
MS153	F/50	32	47	MS/ACTH for 30 months (at age 42 years)	+/+	12	1/5
MS154	F/35	12	30	Pneumonia/none	++/++	12	2/4
MS176	M/37	27	21	Intestinal obstruction, MS/none	++/+	12	1/5
MS180	F/44	18	39	MS/none	++/++	9	4/7
MS342	F/35	5	34	MS/none	++/+	10	1/2
MS352	M/43	18	33	Bronchopneumonia, MS/ Campath-01H (at age 33 years)	+/+	26	1/5
MS356	F/45	16	45	MS/none	++/+	10	1/3
MS407	F/44	19	34	Septicemia, pneumonia/none	+/++	22	1/4

*Intraparenchymal and meningeal inflammation were scored as +, moderate; ++, abundant.
†MS is stated as a cause of death where death occurred as a direct result of MS or any related disability.
EDSS, Expanded Disability Status Scale; F, female; M, male.

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TABLE 3. Immunologic Activity and Expression of EBV Markers in Infiltrated MS Cortical Lesions

MS Case	Tissue Processing	Block Number	No. and Immunologic Activity of Infiltrated Cortical Lesions	No. Cortical Infiltrates	EBER	EBV Lytic Proteins
MS79	Fixed frozen	L10B3	1 Chronic active	1	Positive	Np
MS92	Fixed frozen	R4A2	1 Active	2	Np	Np
		R9D1	1 Active	1	Np	BZLF1-positive
MS121	Snap frozen	P2C6	1 Active	1	Np	BFRF1-positive
MS153	Snap frozen	P5B5	1 Chronic active	1	Np	BFRF1-negative
MS154	Fixed frozen	P2A4	1 Chronic active	1	Np	BFRF1-negative BZLF1-negative
		P2A3	1 Active	2	Np	BZLF1-positive
MS176	Fixed frozen	R1E2	1 Active	1	Np	Np
MS180	Fixed frozen	L4D5	1 Active	1	Np	BZLF1-positive
		R9A1	1 Active	3	Positive	BFRF1-positive BZLF1-positive
	Snap frozen	A1D7	1 Chronic active	1	Positive	Np
		AIC4	1 Active	1	Np	Np
MS342	Fixed frozen	P4C6	1 Active	2	Np	BFRF1-positive
			1 Chronic active	1	Np	Np
MS352	Snap frozen	P5C3	1 Active	1	Np	BFRF1-positive
	Fixed frozen	A1B7	1 Active	1	Np	Np
MS356	Snap frozen	A1B3	1 Active	7	Np	BFRF1-positive
MS407	Snap frozen	P2B6	1 Chronic active	1	Np	Np

Np, not performed.

cases. Among these, 26 (n = 113 brain tissue blocks analyzed) were classified as F+ SPMS cases based on prominent meningeal inflammation with B-cell follicle-like structures, and 18 were classified as F- SPMS cases because meningeal inflammation was absent or negligible, and no B-cell follicle-like structures were detected in the brain tissue blocks analyzed (n = 68). Compared with the F- SPMS cases, the F+ SPMS cases also had a higher frequency of immunologically active lesions in the WM, a higher frequency of immunologically active and chronic active cortical lesions, and younger ages of disease onset, wheelchair dependence, and death (10, 11, 14) (Table 1). Among the 26 F+ SPMS cases, 11 cases (42%) had subpial cortical lesions that contained dense perivascular immune infiltrates (Table 2), whereas none of the tissue blocks from the 18 F- SPMS cases contained infiltrated cortical lesions. The pathologic and clinical features of the 11 F+ SPMS cases with active cortical inflammation did not differ substantially from those of the whole F+ SPMS population, except that this F+ subgroup had a significantly higher frequency of chronic active WM lesions than the F- SPMS cases (Table 1).

In the subgroup of 11 F+ SPMS cases with active cortical inflammation, the frequency of infiltrated cortical lesions ranged from 1 in 8 tissue blocks in Case MS79 to 4 in 7 tissue blocks in Case MS180, a case characterized by massive inflammation in both the WM and meninges (Table 2).

FIGURE 1. Identification of perivascular immune infiltrates in MS cortical lesions. Examples of perivascular immune infiltrates in active and chronic active subpial cortical lesions from different SPMS cases are shown. (**A**–**D**) Case MS92. Two dense perivascular immune infiltrates (arrows) are present in Layer II of the cortex within a partially demyelinated subpial lesion (myelin-oligodendrocyte glycoprotein [MOG] immunostaining in **[A]**). This lesion is adjacent to an area of diffuse meningeal inflammation (arrowheads in **[A]**) and contains activated microglia with short thick processes (immunostaining for MHC class II molecules in **[B]**). The perivascular cuff on the right side in **(A)** is composed of CD3-positive T cells **(C)** and tightly packed CD20-positive B cells **(D)**. **(E, F)** Case MS342. There is a dense perivascular immune infiltrate (arrow) in Layer II of the cortex within a partially demyelinated subpial lesion (MOG immunostaining in **[E]**) containing activated microglia (immunostaining for MHC class II in **[F]**). The meningeal infiltrate adjacent to this lesion is indicated by an arrowhead in **(E)**. Note the absence or marked reduction of MOG immunoreactivity in the parenchyma immediately adjacent to the perivascular cuffs shown in **(A)** and **(E)**. **(G**–**I)** Case MS154. An infiltrated blood vessel (H&E in **[G]**) is present in Layer III of the cortex within a demyelinated chronic active subpial lesion (characterization of this lesion is not shown) and contains numerous CD3-positive T cells **(H)** and relatively fewer CD20-positive B cells **(I)**. **(J**–**L)** Case MS180. There is a perivascular cuff (arrow) within a large demyelinated subpial cortical lesion (MOG immunostaining in **[J]**) that is adjacent to a meningeal B-cell follicle. Both the B-cell follicle and the intracortical perivascular cuff are crowded with B cells (CD20 immunostaining in **[K]** and **[L]**, respectively). (**M, N**) Case MS356. Numerous perivascular immune infiltrates (n = 9, arrows in **[M]**) are present in Layers II and III of the cortex within a very large subpial cortical lesion characterized by partial demyelination (MOG immunostaining in **[M]**; adjacent areas of the same lesion are shown in the left and right parts of this panel) and by massive microglia activation (MHC class II immunostaining in **[N]**; the area shown in this panel corresponds to the area shown in the left side of **[M]**). Meningeal infiltrates are indicated by arrowheads in **(M)**. All sections shown were counterstained with hematoxylin. Scale bars = **(A, E, G, J, M, N)** 200 μm; **(F, K, L)** 100 μm; **(B, C, D)** 50 μm; **(H, I)** 20 μm.

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All infiltrated cortical lesions identified in this study were adjacent to areas of diffuse or lymphoid-like meningeal inflammation (Fig. 1). Of the 18 infiltrated cortical lesions, 12 were only partially demyelinated (Fig. 1A, E, M) and were classified as active lesions based on high density of intraparenchymal MHC class II–positive cells, mainly activated microglia with short and thick processes (Table 3; Fig. 1B, F, N). Only 1 active subpial cortical lesion in Case MS180 contained intraparenchymal MHC class II–positive cells with a macrophage-like morphology (Fig. 2F). The remaining infiltrated cortical lesions (n = 6) were almost completely demyelinated (Fig. 1J) and were classified as chronic active because of a higher density of MHC class II–positive microglia at the lesion edge (Table 3).

One to 3 dense intracortical perivascular cuffs were generally observed in a section (Table 3). Only Case MS356

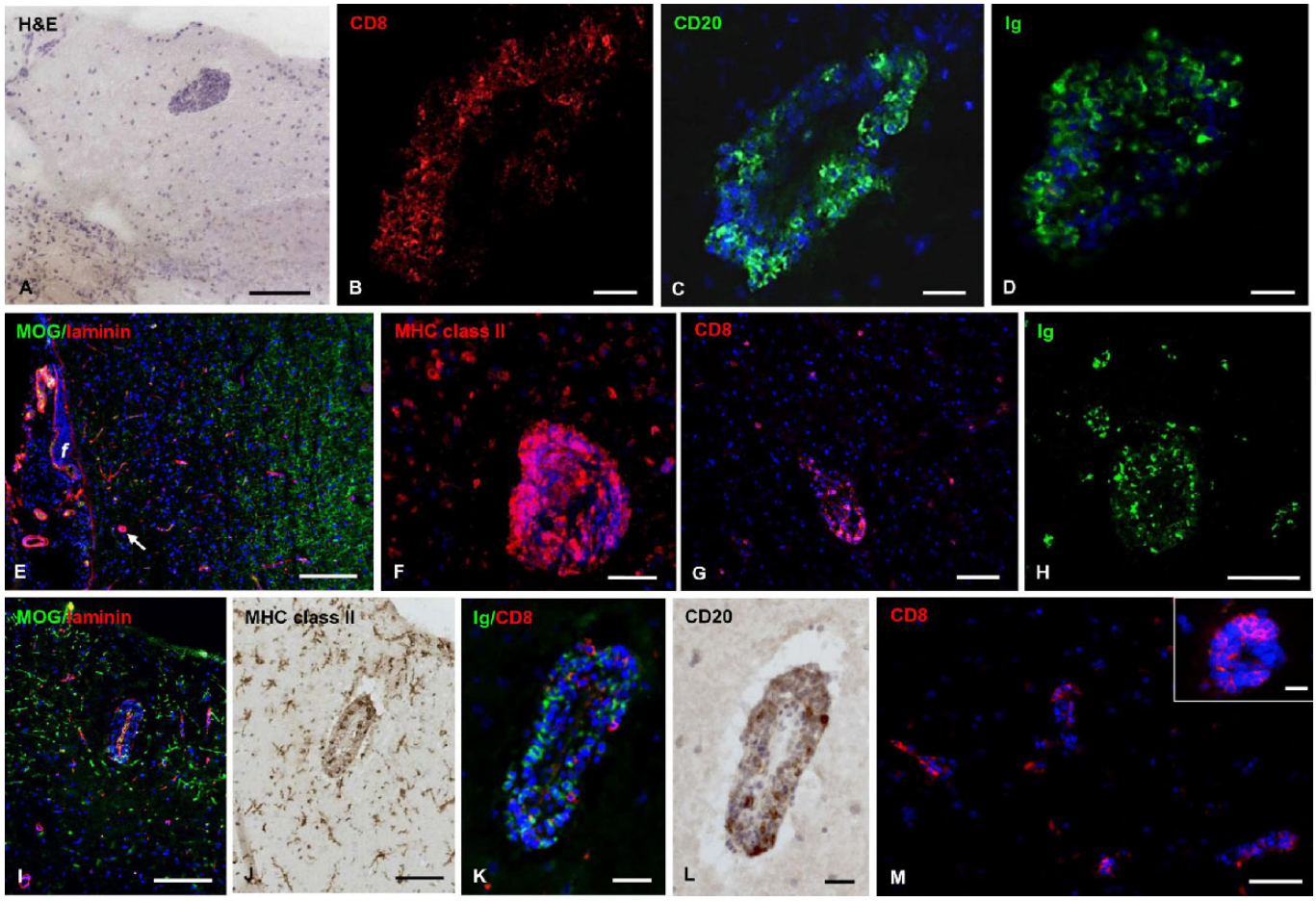


FIGURE 2. Components of perivascular immune infiltrates in MS subpial cortical lesions. **(A–D)** A perivascular immune infiltrate in an active subpial cortical lesion from Case MS121 (H&E stain in **[A]**) is crowded with CD8-positive T cells (CD8 immunostaining in **[B]**), B cells (CD20 immunostaining [green] and DAPI stain [blue] in **[C]**), and plasmablasts/plasma cells (Ig immunostaining [green] and DAPI stain [blue] in **[D]**). **(E–H)** Partially demyelinated subpial cortical lesion close to a meningeal lymphocytic aggregate, identified as an ectopic B-cell follicle (*f*) in an adjacent section, from Case MS180 (double immunostaining for myelin-oligodendrocyte glycoprotein [MOG] [green] and laminin [red] in **[E]**). Immunostaining performed in adjacent brain sections reveal that the blood vessel identified by an arrow in **[E]** shows heavy infiltration by MHC class II–positive cells (immunostaining for MHC class II [red] and DAPI stain [blue] in **[F]**), CD8-positive T cells (CD8 immunostaining and DAPI staining in **[G]**), and plasmablasts/plasma cells (Ig immunostaining in **[H]**). The area surrounding this infiltrated blood vessel contains numerous intraparenchymal MHC class II–positive cells with a macrophage-like morphology suggestive of active phagocytosis and smaller blood vessels infiltrated by CD8-positive T **(G)** and Ig-positive **(H)** cells. **(I–L)** A perivascular immune infiltrate in another partially demyelinated subpial cortical lesion of Case MS180 (double immunostaining for MOG [green] and laminin [red] and DAPI staining [blue] in **[I]**) is surrounded by many activated ramified microglia (MHC class II immunostaining in **[J]**) and contains numerous plasmablasts/plasma cells and CD8-positive T cells (double immunostaining for Ig [green] and CD8 [red] and DAPI staining [blue] in **[K]**) and fewer B cells (CD20 immunostaining in **[L]**). (**M**) Perivascular accumulation of CD8-positive T cells in the numerous infiltrated blood vessels present in the cortex of Case MS356 (Fig. 1M) (CD8 immunostaining [red] and DAPI staining [blue] in **[M]**). Staining in **(A–D)**, **(F–H)**, and **(I–L)** were performed in serial brain sections. Scale bars = **(E, I)** 200 μm; **(A, G, J, M)** 100 μm; **(H, F)** and inset in **(H)** 50 μm; **(B–D, K, L)** 20 μm.

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had numerous intracortical immune infiltrates in a large partially demyelinated subpial area with massive microglia activation (Fig. 1M, N). The perivascular immune infiltrates in the MS cerebral cortex typically formed around small venules in Layers II and III and contained a maximum of 150 to 200 cells per section. These observations indicate that intracortical inflammation mainly involves small veins draining the more superficial regions of the cortex or the superficial branches of large veins draining all cortical layers or the cortex and the subcortical WM (1, 27). In Case MS342, we also detected a highly infiltrated vein passing through the innermost and intermediate cortical layers (Figure, Supplemental Digital Content 1, http://links.lww.com/NEN/A418).

All intracortical inflammatory cell infiltrates contained CD3-positive T cells (Fig. 1C, H) and a high density of CD8-positive T cells (31.6% ± 11.3% of perivascular inflammatory cells), particularly in the most active lesions (Fig. 2B, G, K, M). Accumulation of CD20-positive B cells (Fig. 1D, I, L; Fig. 2C, L) and Ig-positive plasmablasts/plasma cells (Fig. 2D, H, K) was also prominent in infiltrated cortical lesions of all F+ SPMS cases analyzed (33.2% ± 17.6% and 24.6% ± 8.3% of perivascular inflammatory cells, respectively), except Case MS356. In that case, the numerous intracortical perivascular cuffs were crowded with CD8-positive T cells (Fig. 2M), and only a few contained B-lineage cells (<10% of total perivascular cells).

Detection of EBV-Infected Cells in Infiltrated MS Cortical Lesions

Because we have shown previously that B cells accumulating in the MS brain support an active EBV infection (19, 28), the presence of B cell/plasma cell–rich cuffs in cortical lesions of a subset of SPMS cases led us to verify whether these infiltrated brain areas also harbored EBV. To detect EBV-infected cells, we used in situ hybridization for EBERs, untranslated viral RNAs expressed during EBV latent infection (29). After checking for the RNA quality of the brain tissues using GAPDH in situ hybridization, 3 tissue blocks with well-preserved RNA from 2 MS cases (MS79, MS180) were selected for analysis of EBER transcripts. Using the PNA probe labeled with 5-carboxy-fluorescein and detection kit from Dako, as in previous studies (20, 28), we observed EBER-positive cells in 3 of 3 intracortical perivascular infiltrates analyzed, 2 of which localized in chronic active cortical lesions and one in an active cortical lesion (Table 3; Fig. 3A, B). In

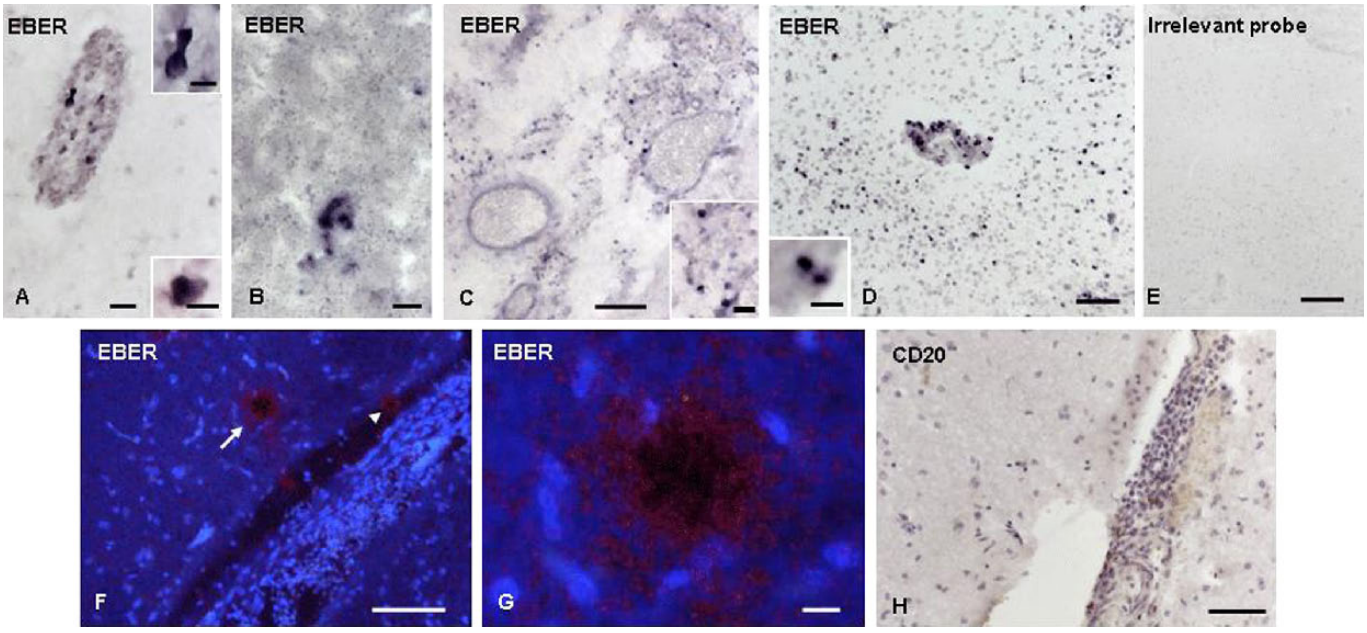


FIGURE 3. Detection of encoded small RNA (EBER)–positive cells in infiltrated MS cortical lesions and meninges. **(A, B)** Using in situ hybridization with a PNA/fluorescein EBER probe, several EBER-positive cells (blue-black nuclei) are detected in 2 intracortical perivascular immune infiltrates in Case MS180; the localization and cellular composition are of these infiltrates are shown in Figure 2I–L and Fig. 1J, L, respectively (**[A, B]** bright-field with hematoxylin stain). The insets in **(A)** show EBER-positive cells at high magnification; the dividing EBER-positive cell in the top inset suggests local proliferation of EBV-infected cells. (**C**–**E**) The EBER-positive cells in a diffuse inflammatory cell infiltrate in the meninges (**C**) and in an active WM lesion (**D**) from Case MS180. Insets show EBER-positive cells at high magnification. No EBER-positive cells are detected in the same case when using an irrelevant probe (**E**). **(F**–**H)** In situ hybridization with radioactive EBER probes reveals a cluster of EBER-positive cells (arrow) in the GM of Case MS79 and an isolated EBER-positive cell (arrowhead) in the adjacent inflamed meninges (**[F, G]** darkfield with DAPI stain; **[G]** shows the intracortical EBER-positive immune infiltrate at high magnification). Lack of CD20-positive cells in the meningeal infiltrate **(H)**, which is stained for EBER in **(F)** and is consistent with detection of only one meningeal EBER-positive cell. In darkfield, silver grains accumulating over EBER-positive cells appear as red dots or black spots in higher density areas; EBER-negative cells are only positive for DAPI nuclear staining. Scale bars = **(C**–**F)** 100 μm; **(H)** 50 μm; **(A, G)** and inset in **(C)** 20 μm; **(B)** and insets in **(A)** and **(D)** 10 μm.

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the same cases, EBER-positive cells were also detected in the adjacent inflamed meninges (Fig. 3C) and in WM lesions (Fig. 3D). The EBER signals were typically nuclear (insets in Fig. 3A, C, D), whereas sections incubated with an irrelevant probe were negative (Fig. 3E).

Because of questions regarding the specificity of the EBER signals obtained with the PNA/fluorescein EBER probe and chromogen-based detection system (22), we also hybridized brain sections from Case MS79 with αP^{33}-labeled EBER probes validated in previous studies (25). A small cluster of EBER-positive cells was observed in the only infiltrated cortical lesion identified in this case (Fig. 3F, G). A few scattered EBER-positive cells were also present in the adjacent inflamed meninges (Fig. 3F, H).

Because EBV reactivates in plasma cells (30), we used immunohistochemical techniques to investigate the presence of EBV proteins that are expressed during the immediate early (BZLF1) and early (BFRF1) phases of the viral lytic cycle (29, 31). The MS brain sections were incubated with anti-EBV antibodies alone or in combination with anti-Ig antibodies to stain plasmablasts/plasma cells. Perivascular cells showing nuclear immunoreactivity for BZLF1 were detected in all infiltrated active cortical lesions analyzed (n = 4) but not in a chronic active cortical lesion (Table 3; Fig. 4A–C).

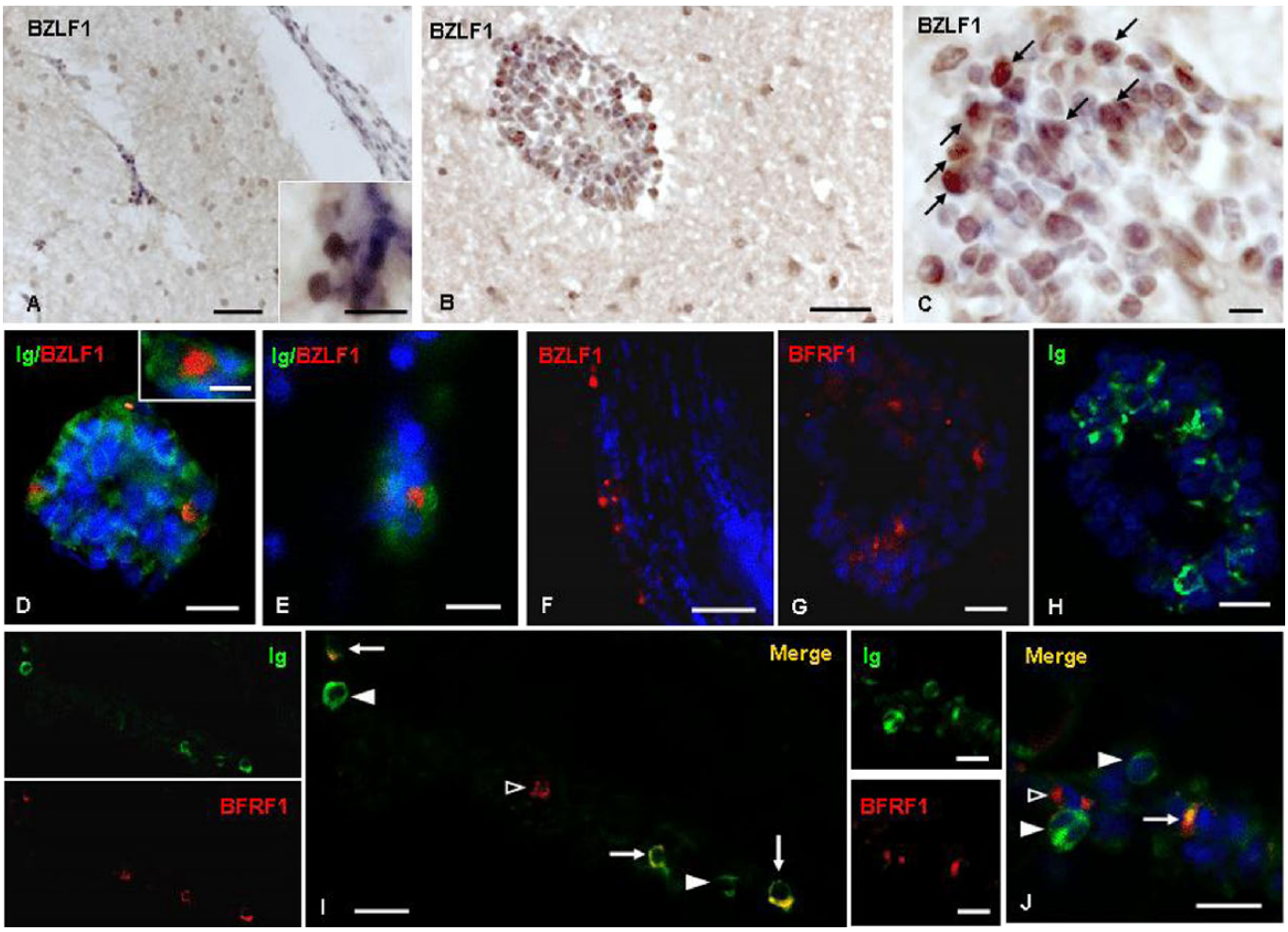


FIGURE 4. Detection of EBV lytically infected plasmablasts/plasma cells in infiltrated MS cortical lesions and meninges. **(A–C)** Cells expressing the EBV immediate early lytic protein BZLF1 are present in cortical immune infiltrates in Cases MS92 **(A)** and MS180 **(B, C)** (immunostaining for BZLF1 and hematoxylin stain in **[A–C]**). Inset in **(A)** and **(C)** highlight the nuclear localization of BZLF1 protein. The arrows in **(C)** point to the numerous BZLF1-positive cells in the large perivascular cuff shown in **(B)**. (**D, E**) Expression of BZLF1 protein in some plasmablasts/plasma cells present in intracortical immune infiltrates in Cases MS180 (**D**) and MS92 (**E**) (double immunofluorescence staining for Ig [green] and BZLF1 [red]). The inset in **(D)** shows the nuclear localization of BZLF1 in an Ig-positive cell at high magnification. **(F)** Several BZLF1-positive cells in the meninges of Case MS92. **(G–J)** Single immunostaining for the EBV early lytic protein BFRF1 **(G)** and Ig **(H)** performed in serial brain sections of Case MS352 reveals several BFRF1-positive cells in an intracortical perivascular cuff enriched in Ig-positive plasmablasts/plasma cells. Double immunofluorescence staining for Ig (green) and BFRF1 (red) reveals the presence of BFRF1-positive/Ig-positive cells (yellow, arrows), BFRF1-negative-/Ig-positive cells (white arrowheads), and BFRF1-positive/Ig-negative cells (empty arrowheads) in the meninges and in an active WM lesion of the same MS case (**[I]** and **[J]**, respectively). Nuclei are stained with DAPI in panels **(D–H)** and **(J)**. Scale bars = **(A, B, F)** 50 μm; **(D, E, G–J)** 20 μm; **(C)** and insets in **(A)** and **(D)** 10 μm.

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Double immunofluorescence staining confirmed that BZLF1 was expressed in a fraction (10%–20%) of intracortical Ig-producing cells (Fig. 4D, E). The BZLF1-positive cells were also detected in the meninges adjacent to the infiltrated cortical lesions (Fig. 4F) and in active WM lesions present in 2 of 3 MS cases analyzed (data not shown).

The EBV early antigen BFRF1 was detected in 5 of 5 active cortical lesions but not in 2 chronic active lesions (Table 3). The BFRF1 immunoreactivity had perinuclear localization and was expressed in a substantial fraction of Ig-positive plasmablasts/plasma cells accumulating in the cortical perivascular cuffs (Fig. 4G, H) in the adjacent meninges (Fig. 4I) and in WM lesions (Fig. 4J). To summarize, 8 of 8 active cortical lesions analyzed were positive for either or both EBV lytic antigens, whereas neither antigen was detected in 2 chronic active lesions.

Cytotoxicity Toward EBV Lytically Infected Cells in MS Cortical Lesions

Because CD8-positive T cells are essential to eliminate EBV infected cells and respond vigorously to EBV early lytic antigens (32), we searched for evidence of interactions between Ig-positive or BFRF1-positive cells and cytotoxic CD8-positive T cells within perivascular cuffs of cortical lesions. For this set of experiments, active cortical lesions positive for EBV lytic antigens from 5 MS cases (MS121, MS180, MS342, MS352, MS356) were analyzed. We consistently observed CD8-positive cells with an activated lymphoblast-like morphology that established tight contacts with or adhered to Ig-positive cells (Fig. 5A-D). Immunoreactivity for the lytic enzyme granzyme B was detected in a substantial fraction (20%–40%) of intracortical CD8-positive cells (Fig. 5E–G), whereas granzyme B–positive CD8-negative cells were rare (Fig. 5E). Most importantly, we observed close contacts between CD8-positive cells and BFRF1-positive cells (Fig. 5H–O), as well as granzyme B–positive CD8-positive cells adjacent to BFRF1-positive cells, displaying granzyme B immunoreactivity on their surface (Fig. 5K, O).

When performing double immunostaining for perforin and Ig, we observed clusters of perforin granules polarized toward Ig-positive cells (Fig. 6A–D). Furthermore, the degranulation marker CD107a (33) was detected on the surface of some CD8-positive cells in inflammatory cortical lesions and adjacent meninges (Fig. 6E–G). In line with our previous findings in WM lesions (19), these observations suggest that EBV lytically infected plasma cells accumulating in MS cortical lesions might be the target of a cytotoxic immune response.

DISCUSSION

This study demonstrates that dense perivascular lymphocytic infiltrates are detectable in the cerebral cortex of SPMS cases characterized by substantial CNS inflammation at the time of death, thus indicating that immune cell infiltration is not only seen in cortical lesions during the earliest stages of MS, as suggested recently (17). Our data also extend to the cerebral cortical GM the controversial hypothesis that B-lineage cells infected with EBV may sustain an immunopathologic response in the MS brain (20, 22, 34, 35).

The findings that both meningeal and intracortical immune infiltrates can be found in postmortem brain tissue from SPMS patients with more severe CNS inflammation, cortical damage and clinical course support the view that persistent inflammation is directly involved in the exacerbation of cortical pathology during MS progression. With only one exception (36), the available evidence indicates that meningeal inflammation is associated with extensive subpial demyelination (10, 11, 14–17). We have shown that in SPMS, prominent meningeal inflammation can be accompanied by formation of ectopic B-cell follicles (10, 19), a pathological feature shared with other chronic inflammatory diseases (37). Furthermore, we have shown that meningeal inflammation associates with a gradient of glial and neuronal cell loss from the external to the internal cortical layers (10). This finding strongly suggests that diffusible cytotoxic factors released from the inflamed meninges may contribute to the development of subpial cortical lesions. Because intracortical immune infiltration was detected in about 40% of the SPMS cases with meningeal B-cell follicles, but in none of the SPMS cases with negligible meningeal inflammation, and predominantly involved small veins draining the II and III cortical layer, a direct link between meningeal and intracortical inflammation is plausible. It is envisaged that leukocyte transmigration across superficial cortical veins is promoted by the active immunologic processes taking place in the nearby meninges and cerebrospinal fluid, possibly through upregulation of adhesion molecules and chemokines.

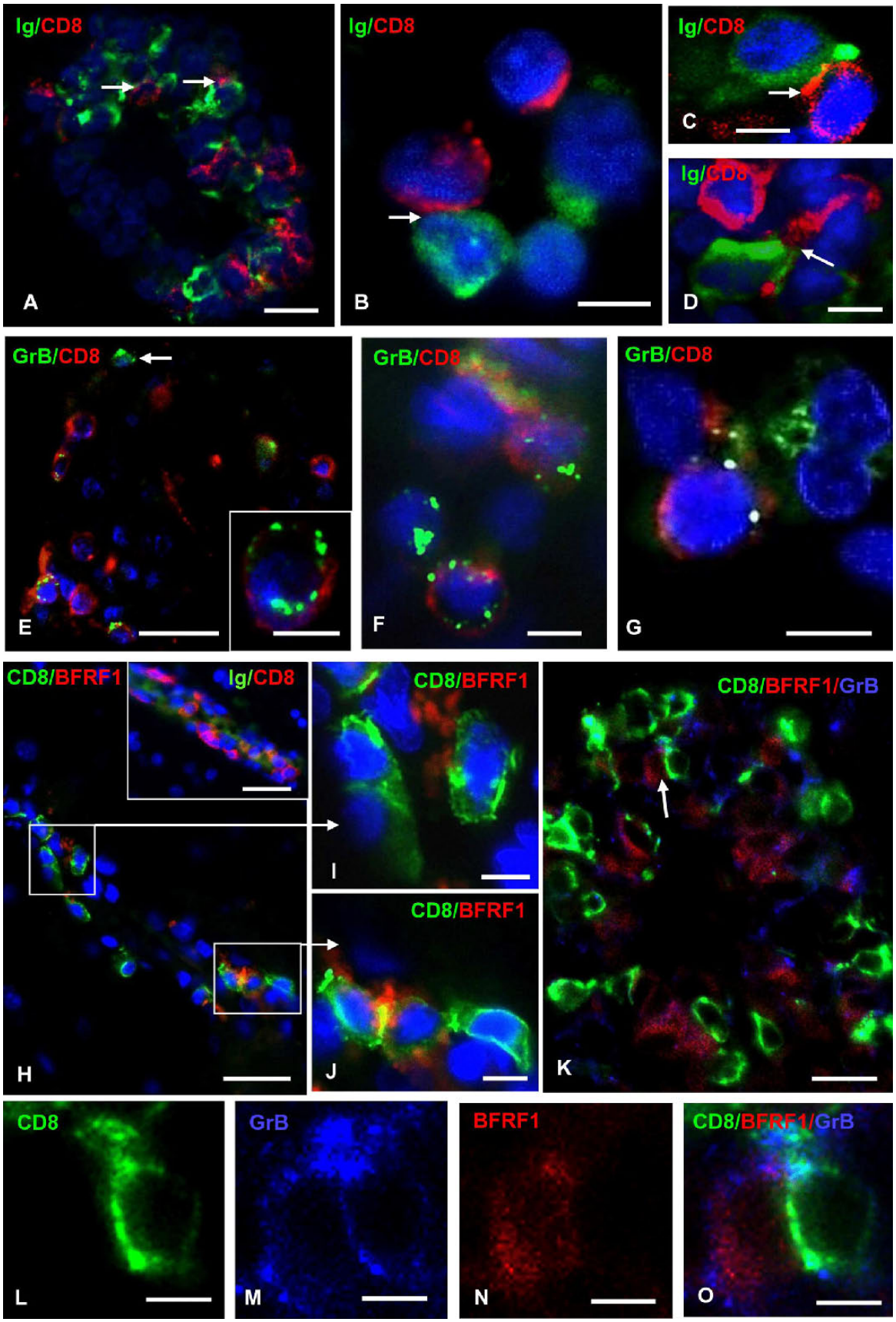
The fact that previous studies did not report substantial perivascular infiltration in purely intracortical lesions in postmortem brain from patients with long-standing progressive MS (6, 12, 13) could be a matter of sample size (i.e. small number of brain tissue blocks and sections stained for each MS case) and sparse inflammatory activity in the MS brains analyzed. Indeed, the findings described here required extensive cutting and analysis of several brain tissue blocks for each MS case and were restricted to a subset of MS cases with more prominent CNS inflammation. The discrepant findings on intracortical inflammation in progressive MS recall the controversy on the presence of meningeal B-cell follicles and association of meningeal inflammation with cortical demyelination, both of which have been challenged in previous studies (35, 36, 38). Even our extensive, but still very limited analysis (4–8 brain tissue blocks were usually examined for each MS case) could have underestimated the frequency of B-cell follicles and inflammatory cortical lesions in SPMS. The fact that meningeal and cortical inflammation is frequently observed in small brain biopsies from patients with early MS (17) but is more difficult to detect in postmortem brain samples from cases with long-standing progressive MS (10, 11, 14, 35, and this study) is consistent with a slowing down of the inflammatory process in the CNS with increasing disease duration (18).

Persistence of intracortical inflammation during the progressive phase of MS may aid in the interpretation of GM abnormalities that are detectable with the most advanced MRI techniques and show a strong correlation with progression of disability (2–4). Such GM abnormalities could be caused by meningeal and intracortical inflammation, which, however, is still undetectable on MRI. Inability to detect

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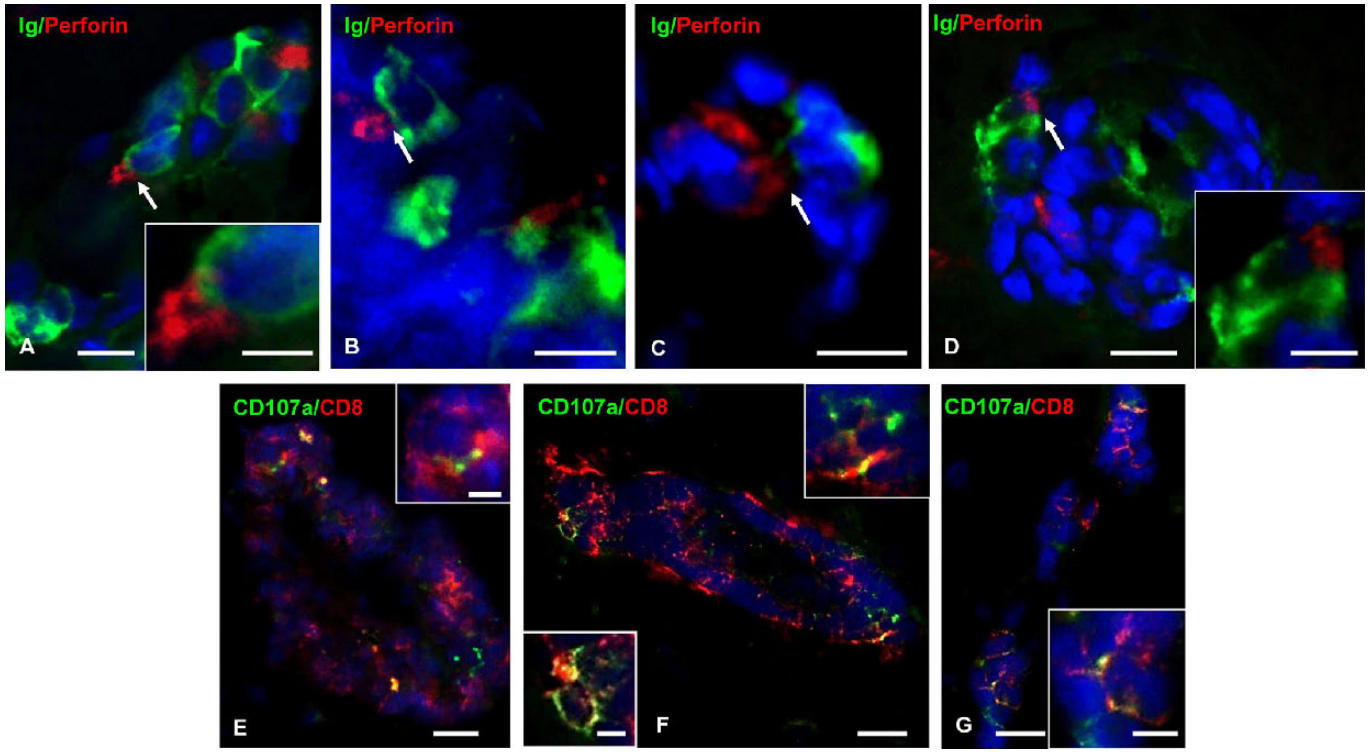


FIGURE 6. Active release of cytolytic granules in infiltrated MS cortical lesions. (**A**–**D**) Polarization of perforin granules toward Ig-positive plasmablasts/plasma cells (arrows) in intracortical perivascular cuffs from Cases MS180 **(A)**, MS121 **(B, C)**, and MS356 **(D)** (double immunofluorescence staining for Ig [green] and perforin [red]). **(E**–**G)** CD8-positive cells display surface immunoreactivity for the degranulation marker CD107a in intracortical perivascular cuffs from Cases MS352 **(E)** and MS356 **(F)** and in the meninges of Case MS356 **(G)** (double immunofluorescence staining for CD107a [green] and CD8 [red]). Nuclei are stained with DAPI. Scale bars = **(E**–**G)** 20 μm; **(A**–**D)** 10 mm; 5 μm in the insets.

gadolinium-enhancing lesions in the GM on MRI could be caused by the smaller caliber of inflamed venules and hence relatively less perivascular inflammation in the GM compared with that in the WM. In line with the inflammatory origin of MS cortical lesions, it has been shown that the monoclonal antibody natalizumab, which blocks leukocyte entry into the CNS, significantly reduces cortical lesion accumulation and progression of cortical atrophy in relapsing-remitting MS patients (39). By showing that intracortical inflammation is not restricted to early disease phases, this study suggests that immunosuppressive therapy could limit accumulation of cortical damage also during or after the shift to SPMS, at least in patients with more active disease.

The B-cell and plasma cell enrichment observed in infiltrated cortical lesions parallels that already reported in meningeal B-cell follicles and WM lesions of the same SPMS cases (10, 11, 14, 19). Taken together, these observations are consistent with a key role for B cells in CNS lesion development and with the well-known fact that intrathecal B-cell activation persists throughout MS (40, 41). Because epidemiologic and serologic studies have found a strong association between the B-lymphotropic herpesvirus EBV and MS

FIGURE 5. Interaction between cytotoxic CD8-positive T cells and EBV lytically infected, Ig-producing cells and granzyme B (GrB) expression in infiltrated MS cortical lesions. **(A**–**D)** The CD8-positive cells with lymphoblast-like morphology establish close contacts with or adhere to (arrows) Ig-positive plasmablasts/plasma cells in intracortical perivascular cuffs from Cases MS352 **(A)**, MS342 **(B)**, and MS180 **(C, D)** (double immunofluorescence staining for Ig [green] and CD8 [red]). **(E**–**G)** Expression of GrB in a substantial fraction of intracortical perivascular CD8-positive cells in Cases MS180 **(E)** and MS342 **(F, G)** (double immunofluorescence staining for GrB [green] and CD8 [red]). The arrow in **E** points to a rare GrB-positive CD8-negative cell. **(H**–**J)** Close contacts between lymphoblast-like CD8-positive T cells and BFRF1-positive EBV lytically infected cells in a cortical immune infiltrate of Case MS342 (double immunofluorescence staining for CD8 [green] and BFRF1 [red]). Inset in **(H)** shows colocalization of CD8-positive T cells and plasmablasts/plasma cells in the same infiltrate (double immunofluorescence staining for Ig [green] and CD8 [red]). Panels **(I)** and **(J)** show the areas marked with frames in **(H)** at higher magnification. **(K**–**O)** Granzyme B–positive/CD8-positive cells with a lymphoblast-like shape contacting BFRF1-positive cells that display GrB on their surface in a cortical immune infiltrate of Case MS180 (triple immunofluorescence staining for CD8 [green], GrB [blue], and BFRF1 [red]) **(K)**. The arrow in **(K)** points to stained cells shown at high magnification in **(L**–**O)**. Nuclei are stained with DAPI in **(A**–**J)**. Scale bars = **(A, E, H, K)** and inset in **(H)** 20 μm; **(B**–**D, F, G, I, J, L**–**O)** and inset in **(E)** 5 μm.

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(42, 43), it has long been suspected that EBV infection of CNS-infiltrating B cells may drive MS pathology (44, 45). However, this remains a controversial issue because we (20, 28) and others (46) have found high frequencies of EBV-infected B cells in inflammatory WM lesions and meninges, whereas several other groups reported absence of EBV infection in the MS brain (22, 38, 47–51). In this study, we show that B-cell colonization of cortical lesions is also accompanied by the presence of EBER-positive cells. Furthermore, we demonstrate that expression of the EBV lytic proteins BZLF1 and BFRF1 is restricted to plasma cells accumulating in active cortical lesions, thus confirming previous observations in active WM lesions and meningeal B-cell follicles (20). This finding is of particular interest because EBV early lytic antigens elicit very strong CD8-mediated immune responses and the latter have highly detrimental effects in several EBV-associated diseases (32). The CD8-positive T-cell response to EBV antigens has been studied in MS and found to be more robust than in control individuals (52, 53), particularly during the early phases of the disease (54). Furthermore, accumulation of EBV-specific CD8-positive T cells has been observed in the cerebrospinal fluid of MS patients (55). The demonstration of contacts between lymphoblastoid CD8-positive T cells engaged in cytotoxic activity and EBV lytically infected plasma cells in infiltrated MS cortical lesions is consistent with a scenario in which CNS B cells may act as a source of highly immunogenic viral antigens. Although it can be argued that cell proximity alone is not indicative of a functional cytotoxic attack, altogether, the presence of granzyme B within CD8-positive cells and on the surface of the adjacent EBV lytically infected (BFRF1-positive) cells, the polarization of perforin granules toward Ig-positive plasma cells, and the surface localization of CD107 on CD8-positive T cells indirectly suggest active immune surveillance to EBV in inflammatory cortical lesions. The above features were not a sporadic finding but were consistently observed in the perivascular immune infiltrates of immunologically active MS cortical lesions supporting the occurrence of a florid cytotoxic immune response at these sites. It is interesting to note that in the MS case showing the highest density of intracortical inflamed blood vessels and massive microglia activation (Case MS356), the perivascular space was crowded with CD8-positive T cells but contained only a few B cells/plasma cells, leading us to speculate that the extent of acute cortical inflammation might positively correlate with a substantial reduction in the frequency of EBV-infected B cells/plasma cells. Taken together with similar observations in active WM lesions and meninges (20), these findings suggest that EBV reactivation and the ensuing antiviral cytotoxic response may drive acute inflammation in WM, GM, and meningeal compartments.

In summary, the present results support the inflammatory nature of cortical lesions in MS and the key role played by CNS B cells and plasma cells in MS pathogenesis. Because rituximab, a monoclonal antibody selectively depleting CD20-positive B cells, has demonstrated efficacy in reducing disease activity in the WM in relapsing-remitting MS (56), it can be envisaged that it might also be effective in limiting accumulation of GM abnormalities. Of note, there is some evidence that rituximab may affect disease progression in patients with primary progressive MS and SPMS, particularly those with younger age and more active disease (57, 58). Finally, by confirming that an active EBV infection can spread across the CNS through infection of B cells and reactivation in plasma cells, this study supports further a causal link between the increased immune response to EBV found in MS patients and inflammatory CNS pathology (42, 54, 59–63).

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REFERENCES

1. Kidd D, Barkhof F, McConnell R, et al. Cortical lesions in multiple sclerosis. Brain 1999;122:17–26
2. De Stefano N, Matthews PM, Filippi M, et al. Evidence of early cortical atrophy in MS: Relevance to white matter changes and disability. Neurology 2003;60:1157–62
3. Geurts JJ, Barkhof F. Grey matter pathology in multiple sclerosis. Lancet Neurol 2008;7:841–51
4. Calabrese M, Filippi M, Gallo P. Cortical lesions in multiple sclerosis. Nat Rev Neurol 2010;6:438–44
5. Geurts JJ, Stys PK, Minagar A, et al. Gray matter pathology in (chronic) MS: Modern views on an early observation. J Neurol Sci 2009;282:12–20
6. Peterson JW, Bø L, Mørk S, et al. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001;50:389–400
7. Bø L, Vedeler CA, Nyland HI, et al. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol Exp Neurol 2003;62:723–32
8. Vercellino M, Plano F, Votta B, et al. Grey matter pathology in multiple sclerosis. J Neuropathol Exp Neurol 2005;64:1101–7
9. Wegner C, Esiri MM, Chance SA, et al. Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. Neurology 2006;67:960–67
10. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089–104
11. Magliozzi R, Howell OW, Reeves C, et al. A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol 2010;68:477–93
12. Bø L, Vedeler CA, Nyland H, et al. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. Multiple Sclerosis 2003;4:323–31
13. Brink BP, Veerhuis R, Breij EC, et al. The pathology of multiple sclerosis is location dependent: No significant complement activation is detected in purely cortical lesions. J Neuropath Exp Neurol 2005;64:147–55
14. Howell O, Reeves C, Nicholas R, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 2011;134:2755–71
15. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005;128:2705–12
16. Lassmann H, Brück W, Lucchinetti CF. The immunopathology of multiple sclerosis: An overview. Brain Pathol 2007;17:210–18
17. Lucchinetti CF, Popescu BF, Bunyan RF, et al. Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011;365:2188–97
18. Compston A, Coles A. Multiple Sclerosis. Lancet 2008;372:1502–17

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19. Serafini B, Rosicarelli B, Magliozzi R, et al. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 2004;14:164–74
20. Serafini B, Rosicarelli B, Franciotta D, et al. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med 2007;204: 2899–912
21. Pender MP. Does Epstein-Barr virus infection in the brain drive the development of multiple sclerosis? [Review] Brain 2009;132:3196–98.
22. Lassmann H, Niedobitek G, Aloisi F, et al. Epstein-Barr virus in the multiple sclerosis brain: A controversial issue-report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. Brain 2011;134:2772–86
23. Reynolds R, Roncaroli F, Nicholas R, et al. The neuropathological basis of clinical progression in multiple sclerosis. Acta Neuropathol 2011;122: 155–70
24. Serafini B, Magliozzi R, Rosicarelli B, et al. Expression of TWEAK and its receptor Fn14 in the multiple sclerosis brain: Implications for inflammatory tissue injury. J Neuropathol Exp Neurol 2008;67:1137–48
25. Niedobitek G, Young LS, Lau R, et al. Epstein-Barr virus infection in oral hairy leukoplakia: Virus replication in the absence of a detectable latent phase. J Gen Virol 1991;72:3035–46
26. Centonze D, Muzio L, Rossi S, et al. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. J Neurosci 2009;29:3442–52
27. Duvernoy HM, Delon S, Vannson JL. Cortical blood vessels of the human brain. Brain Res Bull 1981;7:519–79
28. Serafini B, Severa M, Columba-Cabezas S, et al. Epstein-Barr virus latent infection and BAFF expression in B cells in the multiple sclerosis brain: Implications for viral persistence and intrathecal B-cell activation. J Neuropathol Exp Neurol 2010;69:677–93
29. Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, Griffin DE, et al eds. *Fields Virology*. Philadelphia, PA: Lippincott-Williams & Wilkins Publishers, 2007:2655–700
30. Laichalk LL, Thorley-Lawson DA. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. J Virol 2005;79:1296–307
31. Farina A, Santarelli R, Gonnella R, et al. The BFRF1 gene of Epstein-Barr virus encodes a novel protein. J Virol 2000;74:3235–44
32. Hislop AD, Taylor GS, Sauce D, et al. Cellular responses to viral infection in humans: Lessons from Epstein-Barr virus. Annu Rev Immunol 2007;25:587–617
33. Betts MR, Brenchley JM, Price DA, et al. Sensitive and viable identification of antigen-specific CD8-positive T cells by a flow cytometric assay for degranulation. J Immunol Methods 2003;281:65–78
34. Maghzi AH, Marta M, Bosca I, et al. Viral pathophysiology of multiple sclerosis: A role for Epstein-Barr virus infection? Pathophysiology 2011; 18:13–20
35. Aloisi F, Serafini B, Magliozzi R, et al. Detection of Epstein-Barr virus and B-cell follicles in the multiple sclerosis brain: What you find depends on how and where you look. Brain 2010;133:e157
36. Kooi EJ, Geurts JJ, van Horssen J, et al. Meningeal inflammation is not associated with cortical demyelination in chronic multiple sclerosis. J Neuropathol Exp Neurol 2009;68:1021–28
37. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol 2006;6:205–17
38. Peferoen LA, Lamers F, Lodder LN, et al. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain 2010;133:1–4
39. Rinaldi F, Calabrese M, Seppi D, et al. Natalizumab strongly suppresses cortical pathology in relapsing-remitting multiple sclerosis. Mult Scler May 8, 2012 [epub ahead of print] doi:10.1177/1352458512447704
40. Franciotta D, Salvetti M, Lolli F, et al. B cells and multiple sclerosis. Lancet Neurol 2008;7:852–58
41. Disanto G, Morahan JM, Barnett MH, et al. The evidence for a role of B cells in multiple sclerosis. Neurology 2012;78:823–32
42. Ascherio A, Munger KL. Epstein-Barr virus infection and multiple sclerosis: A review. J Neuroimmune Pharmacol 2010;5:271–77
43. Pender MP. The essential role of Epstein-Barr virus in the pathogenesis of multiple sclerosis. Neuroscientist 2011;17:351–67
44. Warner HB, Carp RI. Multiple sclerosis and Epstein-Barr virus. Lancet 1981;2:1290
45. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol 2003;24:584–88
46. Tzartos JS, Khan G, Vossenkamper A, et al. Association of innate immune activation with latent Epstein-Barr virus in active MS lesions. Neurology 2012;78:15–23
47. Hilton DA, Love S, Fletcher A, et al. Absence of Epstein-Barr virus RNA in multiple sclerosis as assessed by in situ hybridization. J Neurol Neurosurg Psychiatry 1994;57:975–76
48. Opsahl ML, Kennedy PGE. An attempt to investigate the presence of Epstein Barr virus in multiple sclerosis and normal control brain tissue. J Neurol 2007;254:425–30
49. Willis SN, Stadelmann C, Rodig SJ, et al. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain 2009;132: 3318–28
50. Torkildsen O, Stansberg C, Angelskar SM, et al. Upregulation of immunoglobulin-related genes in cortical sections from multiple sclerosis patients. Brain Pathol 2010;20:720–29
51. Sargsyan SA, Shearer AJ, Ritchie AM, et al. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology 2010;74:1127–35
52. Höllsberg P, Hansen HJ, Haahr S. Altered CD8-positive T cell responses to selected Epstein-Barr virus immunodominant epitopes in patients with multiple sclerosis. Clin Exp Immunol 2003;132:137–43
53. Cepok S, Zhou D, Srivastava R, et al. Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis. J Clin Invest 2005;115:1352–60
54. Jilek S, Schluep M, Meylan P, et al. Strong EBV-specific CD8-positive T-cell response in patients with early multiple sclerosis. Brain 2008;131: 1712–21
55. Jaquiéry E, Jilek S, Schluep M, et al. Intrathecal immune responses to EBV in early MS. Eur J Immunol 2010;40:878–87
56. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 2008;358:676–88
57. Hawker K, O'Connor P, Freedman MS, et al. Rituximab in patients with primary progressive multiple sclerosis: Results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol 2009;66: 460–71
58. Rommer PS, Patejdl R, Winkelmann A, et al. Rituximab for secondary progressive multiple sclerosis: A case series. CNS Drugs 2011;25: 607–13
59. Sumaya CV, Myers LW, Ellison GW, et al. Increased prevalence and titer of Epstein-Barr virus antibodies in patients with multiple sclerosis. Ann Neurol 1985;17:371–77
60. Levin, LI, Munger KL, Rubertone MV, et al. Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 2005;293:2496–500
61. Farrell RA, Antony D, Wall GR, et al. Humoral immune response to EBV in multiple sclerosis is associated with disease activity on MRI. Neurology 2009;73:32–8
62. Lünemann JD, Edwards N, Muraro PA, et al. Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. Brain 2006;129:1493–506
63. Lünemann JD, Tintoré M, Messmer B, et al. Elevated Epstein-Barr virus-encoded nuclear antigen-1 immune responses predict conversion to multiple sclerosis. Ann Neurol 2010;67:159–69

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