Accumulation of meningeal lymphocytes correlates with white matter lesion activity in progressive MS
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Supplemental Figures:

Supplementary figure 1. The density of meningeal T cells positively correlates with the density of meningeal B cells in MS. Spearman correlation coefficient between meningeal CD3+ T cell count and meningeal CD20+ B cell count of MS donors. Each data point represents the mean meningeal cell count in all fields analyzed per case.
Supplementary figure 2. Cortical subpial demyelination in MS. a-c. Immunohistochemical staining with anti-myelin proteolipid protein (PLP) of MS cortex with subpial grey matter demyelination (arrows in a). Close up of MS cortex showing a subpial (type III) grey matter lesion (GML) (b) or normal appearing grey matter (NAGM) (c) adjacent to meninges (M). In a, scale bar represents 5mm; In b, c, scale bars represent 1mm.
Supplementary figure 3. Meningeal T cells and B cells in the ‘low’ donor group are not enriched in proximity of cortical subpial demyelinated lesions. Representative immunohistochemical staining for CD3 (a and b, arrows) and CD20 (c and d, arrows) in meninges adjacent to a subpial grey matter lesion (GML) or adjacent to normal appearing gray matter (NAGM) in MS donors with low CD3+ or CD20+ meningeal cell count. e, f. Representative immunostaining for CD3 and CD20 in Lymph Nodes used as technical positive controls. In a-d, scare bars represent 100μm. In e, f, scare bars represent 200μm.
Supplementary figure 4. Enrichment of meningeal T cells and B cells is not linked to disease duration. Disease duration in MS donors with high vs low (a) CD3$^+$ or (b) CD20$^+$ meningeal cell count. Data shown as mean±SD. Statistically significant differences were tested by the non-parametric Mann Whitney test (p<0.05).
Supplementary figure 5. Enrichment of meningeal T cells and B cells is not linked to the proportion of cortical subpial demyelinated grey matter lesions. Quantification of (a, b) number of cortical grey matter lesions (GMLs), (c, d) proportion of leukocortical (type I) GMLs, (e, f) proportion of intracortical (type II) GMLs and (g, h) proportion of subpial (type III) GMLs in MS donors with high vs low meningeal CD3⁺ T cells (a, c, e, g) or CD20⁺ B cell (b, d, f, h) count. Each data point represents the proportion of GMLs in all tissue blocks analyzed per case. Data shown as mean±SD. Statistically significant differences were determined by the non-parametric Mann Whitney test.
Supplementary figure 6. Enrichment of meningeal T cells and B cells is not linked to the total number of subcortical white matter lesions. Total number of subcortical white matter lesions in MS donors with high vs low (a) CD3+ or (b) CD20+ meningeal cell count. Data shown as mean±SD. Statistically significant differences were tested by the non-parametric Mann Whitney test (p<0.05).
Supplementary figure 7. Characterization of mixed active-inactive white matter lesions (WLM) in progressive MS cases. A mixed active-inactive WML in progressive MS case, showing loss of luxol fast blue (LFB) staining (a), loss of immunoreactivity for the proteolipid protein (PLP, in b) and loss of immunoreactivity for the myelin oligodendrocyte glycoprotein (MOG, in c) within the lesions, with intracellular inclusions of MOG+ myelin products inside ionized calcium-binding adapter molecule-1 (IBA1)+ macrophages at the lesion edge (d), indicative of demyelinating activity. Immunoreactivity for the CD68 lysosomal marker is abundant throughout the lesion and accumulates at the lesion edge (e) in a staining pattern consistent with enlarged lysosomes within phagocytic cells (f). Human leukocyte antigen (HLA-DR)+ myeloid cells accumulate at the lesion edge (g) and show enlarged morphology (h), consistent with the active phenotype. CD3+ T cells are found in the parenchyma (i) and as perivascular “cuffs”, expanding within the Virchow-Robbin space of capillaries (j), located within the lesion or at the peri-lesional areas. CD20+ B cells are occasionally found in the parenchyma (k) and more often observed as perivascular “cuffs” (l), located within the lesion or at the peri-lesional areas.
Supplementary figure 8. Characterization of inactive WML in progressive MS cases. Inactive WML in a progressive MS case, showing loss of luxol fast blue (LFB) staining (a), loss of immunoreactivity for the proteolipid protein (PLP, in b) and loss of immunoreactivity for the myelin oligodendrocyte glycoprotein (MOG, in c) with no evidence of intracellular inclusions of MOG+ myelin products inside ionized calcium-binding adapter molecule-1 (IBA1)+ macrophages (d), indicative of no demyelinating activity. Immunoreactivity for the CD68 lysosomal marker is scarce within the lesion and more obvious at the lesion edge (e) in a staining pattern consistent with inactive, gliotic cells (f). A paucity of human leukocyte antigen (HLA-DR)+ microglia can be found at the lesion edge (g) and show small ramified morphology (h), consistent with the inactive, resting phenotype. (i-l) CD3+ T cells and CD20+ B cells are occasionally found in the parenchyma (i, k) and perivascular space (j, l), located within the lesion or at the perilesional area.
Supplementary figure 9. The density of meningeal IBA1⁺ myeloid cells positively correlates with the density of meningeal CD68⁺ cells in MS. Spearman correlation coefficient between meningeal IBA1⁺ myeloid cell count and meningeal CD68⁺ cell count of MS donors. Each data point represents the mean meningeal cell count in all fields analyzed per case.
Supplementary figure 10. Enrichment of meningeal myeloid cells is not linked to disease duration. Disease duration in MS donors with high vs low IBA1⁺ meningeal cell count. Data shown as mean±SD. Statistically significant differences were determined by the non-parametric Mann Whitney test (p<0.05).
Supplementary figure 11. Meningeal myeloid cells are not topographically linked to subpial demyelination in MS. Representative immunohistochemical staining for IBA1 (arrows) in meninges adjacent to a subpial grey matter lesion (GML) or adjacent to normal appearing gray matter (NAGM) in MS donors with high (a, b) or low (c, d) meningeal IBA1⁺ myeloid cell count. In a-d, sace bars represent 100μm.
Supplementary figure 12. Enrichment of meningeal myeloid cells is not linked to the total number of subcortical white matter lesions. Total number of subcortical white matter lesions in MS donors with high vs low IBA1⁺ meningeal cell count. Data shown as mean±SD. Statistically significant differences were determined by the non-parametric Mann Whitney test (p<0.05).
## Supplemental Tables:

### Supplementary table 1. Donor demographics

| Case     | Sex | Age (years) | PMD (h:min) | Type of MS | DD (years) | No. tissue blocks analysed for lesion characterization | COD                                                                 |
|----------|-----|-------------|-------------|------------|------------|--------------------------------------------------------|----------------------------------------------------------------------|
| MS       |     |             |             |            |            |                                                        |                                                                      |
| 2010-034 | F   | 57          | 08:40       | SPMS       | 26         | 41                                                    | Respiratory insufficiency to (uro)sepsis                               |
| 2015-006 | F   | 57          | 10:40       | SPMS       | 29         | 30                                                    | Euthanasia                                                            |
| 2015-011 | F   | 57          | 07:30       | SPMS       | 34         | 27                                                    | Euthanasia                                                            |
| 2013-047 | F   | 48          | 11:50       | PPMS       | 22         | 32                                                    | Respiratory failure with end stage MS                                 |
| 2012-084 | M   | 63          | 08:15       | SPMS       | 30         | 23                                                    | Pneumonia, cachexia and dehydration                                   |
| 2015-047 | F   | 50          | 09:05       | SPMS       | 18         | 23                                                    | Euthanasia                                                            |
| 2015-082 | F   | 47          | 08:35       | PPMS       | 29         | 34                                                    | Aspiration pneumonia                                                  |
| 1996-039 | F   | 57          | 05:45       | PPMS       | 22         | 15                                                    | Sepsis                                                               |
| 2004-017 | F   | 41          | 08:25       | SPMS       | 11         | 8                                                     | Natural death                                                        |
| 2010-025 | M   | 51          | 11:00       | SPMS       | >12        | 38                                                    | Exact cause unknown, infection 2 days prior to death                   |
| 2010-040 | F   | 57          | 08:40       | PPMS       | 29         | 31                                                    | Euthanasia                                                            |
| 2011-080 | F   | 56          | 08:25       | SPMS       | 34         | 27                                                    | Respiratory insufficiency secondary to pneumonia                       |
| 2011-089 | M   | 64          | 07:30       | -          | -          | 28                                                    | -                                                                   |
| 1997-006 | F   | 62          | 06:45       | PPMS       | 29         | 5                                                     | Cardiac asthma                                                       |
| 2003-020 | F   | 68          | 07:30       | SPMS       | 39         | 23                                                    | Bronchitis/ aspiration pneumonia                                      |
| 2012-113 | M   | 59          | 10:45       | RRMS       | 22         | 34                                                    | Euthanasia                                                           |
| 2002-023 | F   | 75          | 08:00       | SPMS       | 42         | 32                                                    | Pneumonia                                                            |
| 2008-021 | M   | 55          | 06:20       | PPMS       | 32         | 33                                                    | Respiratory insufficiency                                             |
| 2015-070 | F   | 77          | 10:05       | SPMS       | 26         | 18                                                    | Cardiovascular event and dehydration                                  |
| 2014-058 | F   | 74          | 07:50       | SPMS       | 50         | 72                                                    | Euthanasia                                                           |
| 2012-113 | M   | 59          | 10:45       | RRMS       | 22         | 34                                                    | Euthanasia                                                           |
| 2011-100 | F   | 71          | 07:05       | SPMS       | 34         | 26                                                    | Cachexia with slowly progressive MS and metastatic breast cancer       |
| 2012-045 | F   | 81          | 09:35       | PPMS       | 22         | 25                                                    | Cardiac asthma                                                       |
| 2012-057 | F   | 81          | 04:35       | SPMS       | 34         | 30                                                    | Aspiration pneumonia                                                  |
| 2012-078 | M   | 58          | 09:15       | -          | -          | 30                                                    | -                                                                   |
| 2012-122 | M   | 60          | 07:30       | PPMS       | 32         | 30                                                    | Respiratory failure due to pneumonia                                  |
| 2014-007 | F   | 51          | 09:45       | SPMS       | 35         | 34                                                    | Euthanasia                                                           |
| Non-neurological Controls |     |             |             |            |            |                                                        |                                                                      |
| 2011-069 | M   | 49          | 06:15       | -          | -          | -                                                     | Euthanasia with Hopkin’s lymphoma                                     |
| 2009-021 | F   | 99          | 04:15       | -          | -          | -                                                     | -                                                                   |
| 1994-114 | M   | 63          | 26:45       | -          | -          | -                                                     | Pulmonary infection                                                  |
| 2010-070 | F   | 60          | 07:30       | -          | -          | -                                                     | Infection e.c.i                                                      |
| 2005-019 | M   | 74          | 05:00       | -          | -          | -                                                     | -                                                                   |
| 1998-061 | F   | 64          | 06:00       | -          | -          | -                                                     | Cachexia                                                            |
| 2005-034 | M   | 56          | 14:00       | -          | -          | -                                                     | Heart failure                                                       |
| 1994-119 | F   | 51          | 7:40        | -          | -          | -                                                     | -                                                                   |
| 2003-002 | M   | 51          | 7:44        | -          | -          | -                                                     | -                                                                   |

MS, multiple sclerosis; F, Female; M, Male; PMD, post-mortem delay; DD, disease duration; h:min, hours:minutes; COD, cause of death.
### Supplementary table 2. Primary antibodies, dilution, source

| Antigen                                      | Clone                  | Dilution/Concentration | Source                                      |
|----------------------------------------------|------------------------|------------------------|---------------------------------------------|
| Proteolipid protein (PLP)                    | Monoclonal (plpc1)     | 1:3000<sup>a</sup>     | Serotec (Puchheim, Germany)                 |
| CD3                                          | Monoclonal (SP7)       | 1:500<sup>b</sup>      | Dako (Glostrup, Denmark)                    |
| CD20                                         | Monoclonal (L26)       | 1:1000<sup>b</sup>     | Dako                                        |
| IBA1                                         | Monoclonal (EPR16589)  | 1:8000<sup>c</sup>     | Abcam                                       |
| CD68                                         | Monoclonal (PGM1)      | 1:200<sup>b</sup>      | Dako                                        |
| Human leukocyte antigen (HLA)-DR             | Monoclonal (CR3/43)    | 8.6μg/ml<sup>a</sup>   | Dako                                        |

Antigen retrieval of paraffin sections was performed by heat in <sup>a</sup> 0.05 M Tris buffered saline pH 7.6; <sup>b</sup> 10 mM Tris/1 mM EDTA buffer pH 9 or 10mM Sodium Citrate pH 6.0<sup>c</sup>