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Oligoclonal myelin-reactive T-cell infiltrates derived from multiple sclerosis lesions are enriched in Th17 cells

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Abstract In this study, acute and chronic brain and spinal cord lesions, and normal appearing white matter (NAWM), were resected *post-mortem* from a patient with aggressive relapsing-remitting multiple sclerosis (MS). T-cell infiltrates from the central nervous system (CNS) lesions and NAWM were separated and characterized *in-vitro*. All infiltrates showed a proliferative response against multiple myelin peptides. Studies of the T-cell receptor (TCR)Vβ and Jβ usage revealed a very skewed repertoire with shared complementarity-determining region (CDR)3 lengths detected in all CNS lesions and NAWM. In the *acute lesion*, genomic profiling of the infiltrating T-cells revealed up-regulated expression of TCRα and β chain, retinoic acid-related orphan nuclear hormone receptor C (RORC) transcription factor, and multiple cytokine genes that mediate Th17 cell expansion. The differentially expressed genes involved in regulation of Th17 cells represent promising targets for new therapies of relapsing-remitting MS.

Abbreviations: NAWM, normal appearing white matter; MS, multiple sclerosis; CNS, central nervous system; TCR, T-cell receptor; CDR, complementarity-determining region; RORC, retinoic acid-related orphan nuclear hormone receptor C; PBMC, peripheral blood mononuclear cells; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; NMO, neuromyelitis optica; AP, acute pontine; LFB, luxol fast blue; CP, chronic periventricular; CTh, chronic thoracic; PHA, phytohemagglutinin; EAE, experimental autoimmune encephalomyelitis; PLP, proteolipid protein; SI, stimulation index; CNPase, cyclic nucleotide phosphodiesterase; MOG, myelin oligodendrocyte glycoprotein; MBP, myelin basic protein; CC, chronic cervical; GA, gene array; IR, immune response; DEGs, differentially expressed genes; FOXP3, forkhead box protein P3; BTLA, B and T lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PA, protein array; PCR, polymerase chain reaction.

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Introduction

Inflammatory cells play a central role in MS lesions development [1,2]. Therefore, their detailed characterization in the CNS lesions at various stages of progression provides a framework for better understanding the pathogenesis of the disease [3]. Studies of *human* CNS lesions, and particularly of the infiltrating inflammatory cells, have been hampered by the limited availability of tissue from *active* MS lesions, the small number of infiltrating inflammatory cells, and technical challenges involved in their separation and *in-vitro* expansion [4].

Longitudinal studies of auto-reactive cells in the peripheral circulation have determined that the chronic inflammatory response in MS is mediated by oligoclonal T-cell populations [5]. Studies of the TCRVβ repertoire of peripheral blood mononuclear cells (PBMC)s and cerebrospinal fluid (CSF)-derived T-cells from MS patients have identified a "skewed" repertoire, suggesting that a limited number of T-cell clones drive the chronic immune response in MS [6]. These auto-reactive cells persist in the circulation for years and expand during clinical relapses [7]. As the blood brain barrier becomes permeable, those selected T-cells migrate to the multiple CNS lesions during disease exacerbations.

The present study has identified, for the first time, the presence of myelin-reactive T-cells in CNS demyelinating lesions and NAWM, a skewed TCRVβ repertoire dominated by a limited number of T-cell clones in all CNS regions, and gene and protein expression profiles of T-cells infiltrating CNS MS lesions at various stages of progression.

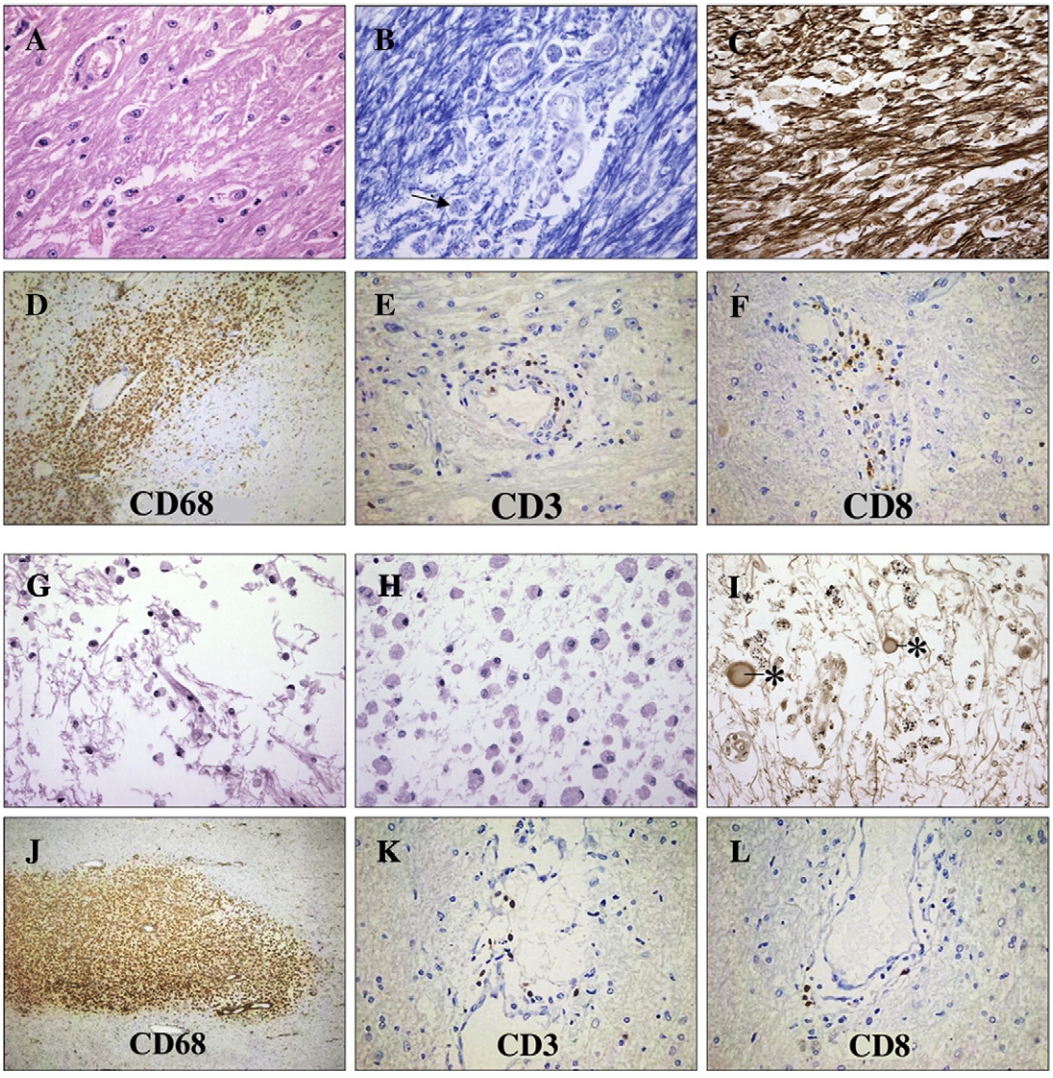

Figure 1 Histopathological characteristics of the AP and CP MS lesions. (A) Hematoxylin and Eosin staining of the AP lesion detected perivascular inflammatory infiltrates, activated microglia and a decreased number of oligodendrocytes. (B) LFB staining revealed active demyelination with phagocytosed LFB-positive myelin within macrophages (arrow). (C) Axonal staining showed a relatively preserved axonal network. (D–F) The perivascular inflammatory infiltrate was composed of CD68+ macrophages and predominantly CD8+ lymphocytes. (G) The CP lesion showed cystic changes with scattered mononuclear cells. (H) Myelin staining revealed complete demyelination. (I) Axonal staining showed profound axonal loss with axonal swelling and spheroids (asterisk). (J–L) The residual inflammatory infiltrate, which was less prominent when compared to the AP lesion, was composed of CD68+ macrophages and CD3+ lymphocytes.