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Clonal expansions of pathogenic CD8+ effector cells in the CNS of myelin mutant mice

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Tissue damage in the CNS is critically influenced by the adaptive immune system. Primary oligodendrocyte damage (by overexpression of PLP) leads to low-grade inflammation of high pathological impact, which is mediated by CD8+ T cells. To yield further insight into pathogenesis and nature of immune responses in myelin mutated mice, we here apply a detailed immunological characterization of CD8+ T cells in PLP-transgenic and aged wild type mice.

We provide evidence that T effector cells accumulate in the CNS of PLP-transgenic and wild-type mice and show a higher level of activation in mutant mice, indicated by surface markers and clonal expansions, as demonstrated by T cell receptor CDR3-spectratype analysis. Vβ–Jβ similarities suggest specificity against a common antigen, albeit we could not find specific responses against myelin-antigen-derived peptides. The association of primary oligodendrocyte damage with secondary expansions of pathogenic cells underlines the role of adaptive immune reactions in neurodegenerative and neuroinflammatory diseases.

Keywords: PLP; Oligodendrocyte damage; Inflammation; CD8+ T-cells; Clonal expansion; Multiple sclerosis

Introduction

The interplay between adaptive as well as innate immune reactions and the immune privileged central nervous system (CNS) has been known to play a critical role in the development and course of many neuroinflammatory and neurodegenerative diseases. Multiple sclerosis (MS) for example is an acquired CNS disorder of young adults and characterized by inflammatory demyelination as well as axonal degeneration. A variety of findings support the hypothesis that in some subtypes of MS the immune system may not be the initial trigger but suggest that inflammation is a secondary response to a primary degenerative stimulus in the CNS. Recent functional studies elucidating the interactions of the immune system with CNS structures have provided novel insights into the molecular mechanisms of inflammatory damage. The detailed histopathological classification of MS lesions demonstrated their remarkable heterogeneity. In general, MS lesions can be divided into four different patterns (patterns I–IV) (Lucchinetti et al., 2000). While cellular and humoral immune components are the prevailing elements found in pattern I and II, a "primary oligodendropathy" with less inflammation dominates in pattern III and IV lesions. On the basis of histopathological analysis of a small set of MS cases, a recently published study postulates a primarily oligodendroglial damage as initiator of MS lesions in general (Barnett and Prineas, 2004). Whereas the concept(s) of CNS lesion development are

Abbreviations: CD, cluster of differentiation; CDR3, complementarity determining region 3; CM, complete medium; CNS, central nervous system; CTL, cytotoxic T lymphocyte; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; FACS, fluorescence activated cell sorting; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; IFNγ, interferon-γ; IL-2, interleukin-2; MBP, myelin basic protein; MS, multiple sclerosis; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PE, phycoerythrin; PerCP, peridinium chlorophyllic protein; PLP, proteolipid protein; RAG, recombination activating gene; TCR, T cell receptor; Tg, transgenic; TNFα, tumor necrosis factor α; Wt, wild type.

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currently under controversial discussion (Lassmann, 2005), there is consent that molecular epitopes related to the myelin sheath play important roles as immunological targets. This is of particular pathological and clinical relevance since the myelin sheath is important not only for mediating the saltatory conduction of axonal action potentials but also for the maintenance of axonal integrity and survival (Bjartmar et al., 1999; Edgar et al., 2004; Frei et al., 1999; Griffiths et al., 1998; Lappe-Siefke et al., 2003; Martini, 2001; Samsam et al., 2003; Wrabetz et al., 2000; Yin et al., 1998; Yin et al., 2000).

We have recently investigated a mouse myelin mutant over-expressing proteolipid protein (PLP) in oligodendrocytes leading to myelin degeneration and late onset axonal degeneration that was accompanied by an elevation of CD11b+ macrophages and CD8+ T lymphocytes in the central nervous system (Ip et al., 2006). By crossbreeding these mutants with RAG-1 deficient

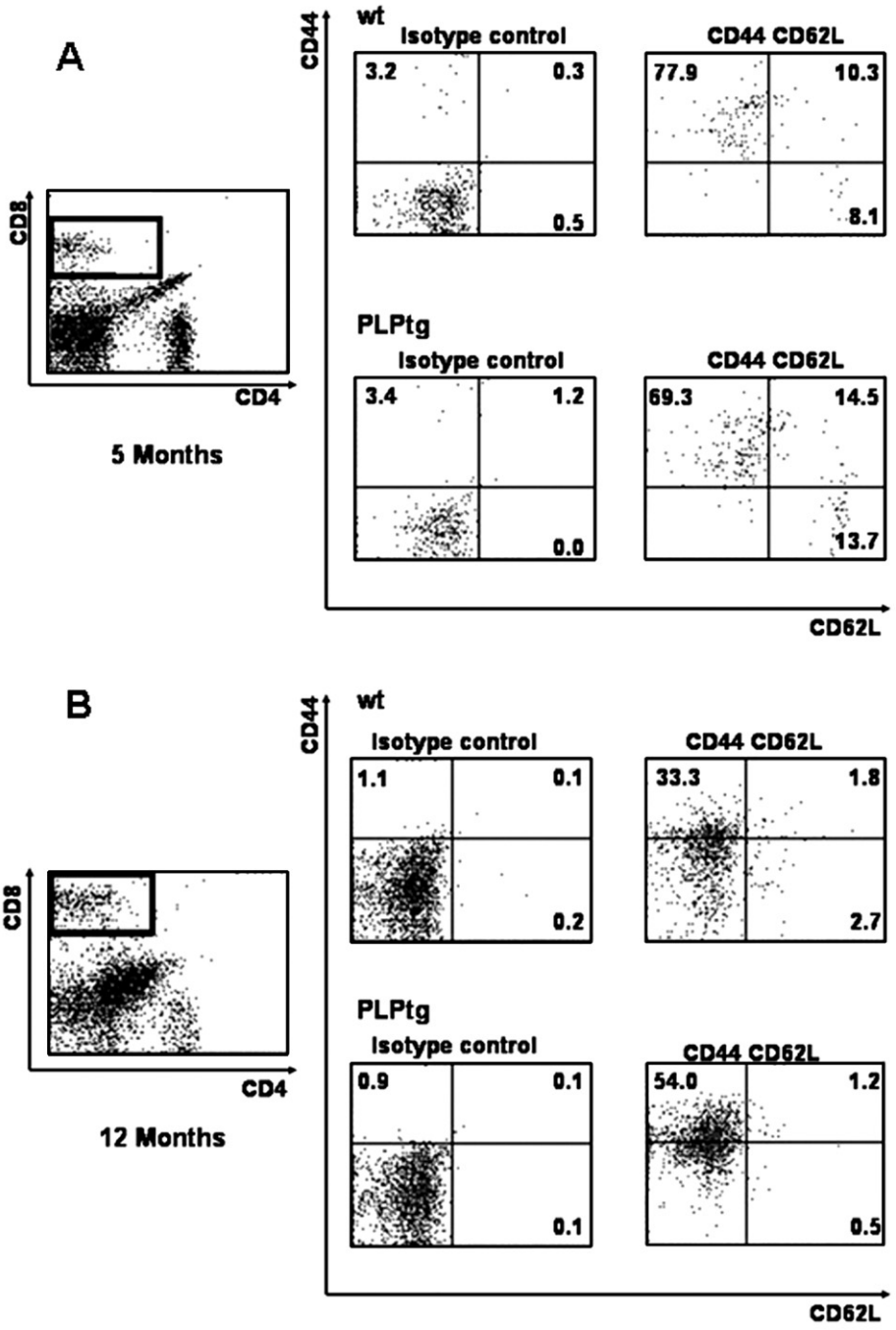

Fig. 1. Flow cytometry-based phenotyping of CD8+ T cells derived from CNS of wild-type and PLP-transgenic mice (5 months of age (A), 12 months of age (B)). Isolated lymphocytes were stained for CD8, CD44 and CD62L. After collecting all events of the stainings, the data were analyzed by gating on CD8+ cells and subsequently displaying their activation status by showing CD44 and CD62L expression.