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Monoclonal MOG-reactive autoantibody from progressive EAE has the characteristics of a natural antibody

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Abstract

A.SW mice sensitized with myelin oligodendrocyte glycoprotein $(MOG)_{92-106}$ is an animal model for progressive multiple sclerosis (MS). We isolated MOG-reactive monoclonal antibodies that were immunoglobulin (Ig)M and polyreactive, similar to natural autoantibodies. Upon analysis of the variable (V) light chains and the diversity (D) and joining (J) regions of V heavy chains, we found they were identical to germ line $V_{\kappa}19/28$, $J_{\kappa}5$, DFL16.1^{e} and J_H4, respectively. The sequence of the V_H region had 99.7% and 100% identity at the nucleotide and amino acid levels, respectively, compared with the germ line encoded antibody, P3, of the Q52 family. Although A strain mice have been reported to have an insertion in BAFF-R, the receptor for BAFF (B cell activation factor from the tumor necrosis factor family), which could explain our results, A.SW mice have no mutations in BAFF-R.

Keywords: Autoimmunity; Autoimmune diseases; B lymphocyte gene rearrangement; Demyelinating diseases; Genetic polymorphism; Experimental autoimmune encephalomyelitis

1. Introduction

Experimental allergic encephalomyelitis (EAE) in the mouse is an experimental animal model of multiple sclerosis (MS) (Tsunoda and Fujinami, 1996). EAE can be induced by the administration of central nervous system (CNS) proteins or peptides in complete Freund's adjuvant (CFA) with or without the supplementation of *Bordetella pertussis* (*BP*). Myelin oligodendrocyte glycoprotein (MOG) is one of many CNS proteins for which whole proteins and specific encephalitogenic peptides are known to induce EAE. Previously, we established an animal model for primary or secondary progressive MS using A.SW mice ($H\text{-}2^s$) sensitized with MOG_{92-106} (Tsunoda et al., 2000). The mice developed progressive disease and showed immunoglobulin (Ig) deposition in the CNS and high titers of circulating MOG-reactive antibody. Histologically, large confluent plaque-like demyelinating lesions were frequently observed, suggesting MOG-reactive antibodies play an important role in their development. Interestingly, SJL/J mice, whose H2 haplotype are also $H\text{-}2^s$, developed relapsing-remitting (RR) EAE with low levels of MOG-reactive antibody, when sensitized with MOG_{92-106}.

To further examine the role played by MOG-reactive antibodies in MOG-induced EAE, we generated MOG-specific B cell hybridomas. Two hybridoma clones, A4ac and A4cd, were selected for further work. The identification of targets, both within and outside the CNS, and the characterization of the MOG-reactive antibodies are important in understanding how the antibodies may deposit in tissues and contribute to disease progression. The MOG-reactive monoclonal antibodies, A4ac and A4cd, were IgM with κ light chains. The antibodies reacted not only with MOG but also other antigens, such as gangliosides, histone, blood, liver and kidney proteins (Peterson et al., 2005). The polyreactivity and IgM isotype are characteristics of natural antibodies, which have been shown to be encoded by germ line DNA sequences (Asakura and Rodriguez, 1998). We

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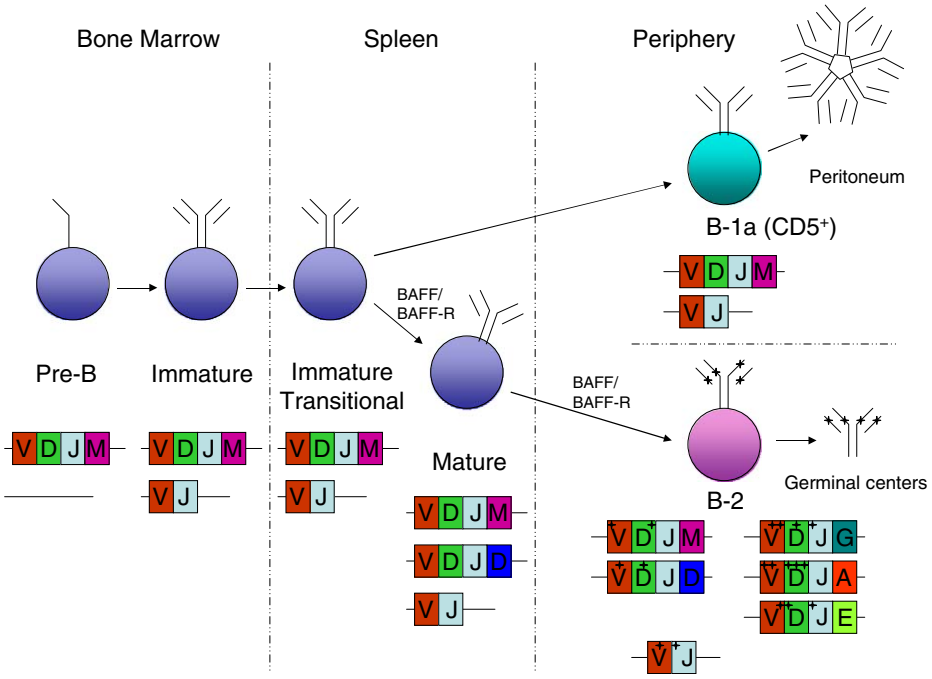

Fig. 1. The role of BAFF/BAFF-R in B cell maturation and survival. In the bone marrow, pre-B cells, which have only somatically recombined the immunoglobulin (Ig) heavy chain, progress into immature B cells where both the heavy and light chains have undergone somatic recombination resulting in the expression of IgM on the cell surface. In the spleen, BAFF/BAFF-R interactions influence immature transitional B cells to mature into IgM and IgD surface expressing B cells. In the germinal centers in the periphery, these mature IgM and IgD expressing B cells become B-2 cells which switch from surface bound to secretion of antibodies, undergo class switching resulting in expression of IgG, IgA and IgE and are subject to somatic hypermutation (represented by the stars) resulting in diverse, high-affinity, mono-specific, non-germ line-encoded antibodies. BAFF/BAFF-R may also play a role in the survival of B-2 cells. Conversely, B-1a cells develop in a BAFF/BAFF-R independent manner. These B cells, which are found mainly in the peritoneum and are $CD5^{+}$, do not undergo class switching and are not subject to somatic hypermutation, thus they mainly produce polyreactive, germ line-encoded natural autoantibodies of the IgM isotype. In the background strain of A.SW mice, A/WySnJ mice that have a mutation in the *BAFF-R* gene, the number of mature peripheral B-2 cells is significantly reduced but B-1a cells are not. B-1a cells have also been proposed to be derived from precursors of fetal origin which differs from conventional B-2 cells (not shown in this figure).

hypothesized that the MOG-reactive antibodies could be natural antibodies, and that the differences in non-major histocompatibility complex (MHC) genes between A.SW mice versus SJL/J mice favor production of natural autoantibody in A.SW mice.

BAFF–B cell activation factor from the tumor necrosis factor (TNF) family–is expressed on cells of the myeloid lineage (monocytes, activated T cells, and possibly dendritic cells), and along with its receptor, BAFF-R (expressed on B lymphocytes), is a principle regulator of peripheral B cell fate and survival (Defrance et al., 2002; Melchers, 2003; Waldschmidt and Noelle, 2001). BAFF/BAFF-R are essential for optimal humoral immunity; dysregulation may result in immune deficiency or autoimmunity (Waldschmidt and Noelle, 2001). Both BAFF-deficient and BAFF-R mutant mice show normal B cell development in the bone marrow and have early transitional B cells in the spleen; however, all of the peripheral B cell subsets, with the exception of B-1 cells, are markedly reduced (Fig. 1) (Lentz et al., 1996; Schiemann et al., 2001). B-1 cells are maintained in a BAFF independent manner (Gross et al., 2001; Waldschmidt and Noelle, 2001). B-1 cells are distinguished from other B cells by their surface phenotype, which is CD45R $(B220)^{lo}$, IgM^{hi}, $CD23^{-}$, $CD43^{+}$, IgD^{lo}, FSC^{hi}, and either $CD5^{+/-}$, determining whether they are B-1a ($CD5^{+}$) or B-1b ($CD5^{-}$) cells (Berland and Wortis, 2002; Trowbridge and Thomas, 1994). B-1a cells are the primary source of natural antibody, which is produced in the absence of exogenous antigenic stimulation, and is polyreactive and autoreactive.

A/WySnJ mice, the background strain of A.SW mice, have mutations in the *BAFF-R* gene (Amanna et al., 2003; Gross et al., 2001; Schiemann et al., 2001; Thompson et al., 2001; Yan et al., 2001). The genomic defect in the A/WySnJ mice was localized to chromosome 15 between 27 and 56 centimorgans (Hoag et al., 2000). Thompson et al. (2001) localized the mouse *BAFF-R* gene to this region on chromosome 15. In the mouse, the MHC occupies a central region of chromosome 17 (Margulies, 1999). The chromosomal location of the *BAFF-R* gene and the phenotype of A/WySnJ mice prompted us to look for a mutation in the *BAFF-R* gene of A.SW mice. We hypothesized that A.SW mice could have a similar mutation in the *BAFF-R* gene, since A.SW mice have the genetic background of A/WySnJ mice, except for the MHC locus.