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Amelioration of EAE by a cryptic epitope of myelin oligodendrocyte glycoprotein

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ABSTRACT

Previous work demonstrated that EAE induced by recombinant human MOG was B cell-dependent. Data presented here reveal a T cell response to MOG61–85 in human rMOG-immunized B cell$^{-/-}$ mice not observed in WT mice. Further study revealed this peptide to be a cryptic epitope in WT mice. Co-immunization of B cell$^{-/-}$ mice with MOG35–55 and MOG61–85 peptides led to less severe disease compared to mice immunized with MOG35–55 alone. Disease amelioration was associated with decreased production of Interferon-γ by lymph node cells. Thus, MOG61–85 represents a protective epitope to human rMOG induced EAE in B cell$^{-/-}$ mice.

1. Introduction

Multiple sclerosis (MS) is considered an autoimmune inflammatory demyelinating disease of the central nervous system (Furlan et al., 2009). The instrumental role of CD4^{+} Th1 and Th17 myelin-reactive T cells in mediating disease pathology is well accepted. A role of B cells and antibody in the disease process is also acknowledged. A pathologic role for antibody is suggested by the correlation of oligoclonal bands in the CSF of MS patients with disease severity (Avasarala et al., 2001). Furthermore, the importance of antibodies specific for conformational epitopes of myelin in the destruction of the myelin sheath is recognized (Brehm et al., 1999; Linington et al., 1988). Clinical benefits upon B cell-depletion support a role for B cells in the disease process (Hauser et al., 2008; Kappos et al., 2011; Naismith et al., 2010; Sorensen et al., 2014), although the exact role of B cells and their products in MS pathogenesis is still unclear.

Previous work from this group in the Experimental Autoimmune Encephalomyelitis (EAE) model of MS demonstrated that B cells and/or antibody were critical for the development of EAE when C57BL/6 (B6) mice were immunized with a recombinant form of human myelin oligodendrocyte glycoprotein (hrMOG) but not when immunized with the encephalitogenic peptide MOG35–55 (pMOG35–55) epitope (Lyons et al., 1999). Our subsequent studies revealed that adding back either B cells or serum isolated from hrMOG-primed wild-type (WT) B6 mice was able to reconstitute disease in B cell-deficient (B cell$^{-/-}$) mice. MOG-specific serum was as effective, if not more effective, in reconstituting disease in B cell deficient mice than was adding back B cells. This suggested a role for an antigen-specific soluble factor in promoting hrMOG-induced disease in WT mice (Lyons et al., 2002). However, the mechanism(s) by which B cells/antibody contribute to hrMOG-induced EAE remain under investigation.

Although our previous data demonstrated proliferative and cytokine responses to the encephalitogenic MOG35–55 epitope in hrMOG-immunized WT and B cell$^{-/-}$ mice, it is possible that additional epitopes are recognized in either the presence or absence of B cells or antibody that contribute to disease. We addressed this hypothesis using a panel of overlapping peptides spanning the extracellular portion of MOG, and discovered that T cells from hrMOG-immunized B cell$^{-/-}$ mice, but not WT mice, proliferated in response to MOG61–85. Subsequent experiments demonstrated that the MOG61–85 epitope inhibited disease initiation by the pMOG35–55 epitope.

2. Materials and methods

2.1. Mice

Female WT and B cell$^{-/-}$ B6 mice were purchased from Jackson Laboratory (Bar Harbor, ME) and immunized at 6–8 weeks of age. Mice were housed in specific pathogen free conditions according to NIH and University guidelines.

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2.2. Antigens

MOG aa35–55 (pMOG35–55; M-E-V-G-W-Y-R-S-S-F-S-R-V-V-H-L-Y-R-N-G-K); MOG aa 61–85 (pMOG61–85; Q-A-P-E-Y-R-G-R-T-E-L-L-K-D-A-I-G-E-G-K-V-T-L-R-I); and Hen's Egg Lysozyme (HEL) aa 46–61 (pHEL46–61; N-T-D-G-S-T-D-Y-G-I-L-Q-I-N-S-R). Initially, peptides were synthesized by Sigma-Genosys (The Woodlands, TX). More recently, MOG35–55 and MOG61–85 were synthesized by Genscript (Piscataway, NJ). Proteolipid Protein (PLP) aa 180–199 (pPLP280–199; W-T-T-C-Q-S-I-A-F-P-S-K-T-S-A-S-I-G-S-L) was synthesized by Genscript (Piscataway, NJ). The purity of all peptides was confirmed by HPLC. Human rMOG (hrMOG) consisting of the 120-amino acid extracellular domain of MOG, was isolated from the culture supernatant of High-5 insect cells infected with a recombinant baculovirus expressing the hrMOG protein, as previously described (Devaux et al., 1997).

2.3. Immunizations

Mice were immunized (s.c.) with pMOG35–55 (50 μg/mouse when peptides were synthesized by Sigma-Genosys; 100 μg/mouse when peptides synthesized by Genscript) and/or pMOG61–85 (50 μg/mouse [Sigma-Genosys]; 100 μg/mouse [Genscript]), or pHEL46–61 (50 μg/mouse) emulsified (1:1) in IFA containing 0.3 mg/mouse Mycobacterium tuberculosis, strain H37RA, 0.1 ml emulsion/mouse. Co-immunization experiments with pMOG35–55 and pPLP180–199 were performed with 100 μg of each peptide emulsified together in a single emulsion with CFA. Emulsions were prepared using an Omni-Mixer (Omni International, Warrenton, VA). Mice also received 300 ng pertussis toxin (List Biological Laboratories, Campbell, CA) intravenously at the time of immunization and 72 h later. As indicated, co-immunization was performed with both peptides in the same emulsion or in separate emulsions. When prepared in separated emulsions, peptides were administered at separate sites. The development of EAE was followed and graded on a scale of 0–5 by a blinded observer as previously described (Cross et al., 1993).

2.4. Serum injection

Polyclonal serum specific for MOG35–55 (mouse sequence) and MOG61–85 was raised in rabbits (Sigma Genosys, The Woodlands Texas), resulting in high titer serum (1:500,000) specific for each peptide. Serum was heated overnight at 56 °C to inactivate complement. Mice received 100 μl (i.p.) of a mixture of pMOG35–55 and pMOG61–85 serum or preimmune rabbit serum diluted 1:100 in normal mouse serum at the time of immunization and once daily for the following three days.

2.5. Proliferation assay

Lymph node and spleen cells were isolated from mice and cultured in quadruplicate at 2.5×10^6 cells/ml with antigen (pMOG35–55 or pMOG61–85) or mitogen (ConA, 1 μg/ml) and 5% FBS in complete RPMI-1640. Culture with irrelevant antigen (pHEL46–61) was included as a negative control. ^{3}H-thymidine (0.5 μCi/well) was added during the final 18 h, and its incorporation counted (Betaplate 1205; Wallac, Gaithersburg, MD). Results are reported as the Stimulation Index (SI; cpm with antigen/cpm with medium alone). SI > 2.0 was considered significant.

2.6. Epitope mapping experiments using hrMOG-primed LNC from WT vs. B cell$^{-/-}$ mice

T cells were isolated by negative selection from draining lymph nodes of hrMOG-primed mice immunized 10 days prior with hrMOG. T cells were cultured with the indicated peptides at 5–30 μg/ml, as indicated, and naïve spleen cells used as antigen presenting cells. Proliferation in response to a panel of overlapping 20–25 mer peptides spanning the length of the hrMOG protein was assessed by tritiated thymidine incorporation. A stimulation index (cpm with antigen/cpm with medium alone) >2.0 was considered significant.

2.7. Cytokine ELISAs

Lymph node and spleen cells were isolated from immunized mice and cultured at 2.5×10^6 cells/ml with the indicated peptide antigen at 10 μg/ml and 5% FBS in complete RPMI-1640. Cell culture supernatants were collected at the indicated times and frozen at −80 °C until assayed. Supernatant fluids were analyzed in duplicate for IFNγ (detection limit: 2 pg/ml), IL-10 (detection limit: 4 pg/ml), and IL-13 (detection limit: 1.5 pg/ml) by QuantikineM ELISA (R&D Systems, Minneapolis, MN) per manufacturer's instructions.

3. Results

3.1. Lymph node cells from B cell$^{-/-}$ mice, but not WT mice, reveal a second epitope within human MOG

To determine if epitopes other than the immunodominant 35–55 epitope within hrMOG elicited a proliferation response, mapping experiments were performed on lymph node cells isolated from hrMOG-primed B cell$^{-/-}$ and WT mice at 10 dpi. Lymph node cells were assayed for antigen-specific proliferation to 12 overlapping 20–25-mers of hrMOG (Fig. 1A). As expected, LNC from both strains responded to the immunogen hrMOG. Similarly, as we had shown previously, both strains responded to the known encephalitogenic epitope within

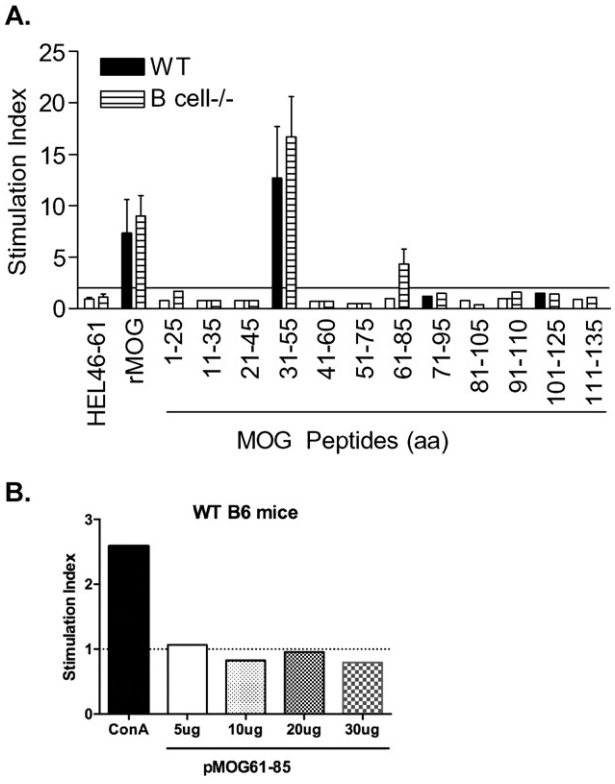

Fig. 1. B cell$^{-/-}$ mice, but not WT B6 mice, respond to MOG61–85, when immunized with hrMOG. WT and B cell$^{-/-}$ were immunized with hrMOG in CFA. (A). T cells were isolated by negative selection from draining lymph nodes 10 days post immunization, and proliferation to a panel of overlapping 20–25 mer peptides (10 μg/ml) spanning the hrMOG protein was assessed. Analysis revealed an additional response to MOG61–85 by cells isolated from B cell$^{-/-}$ mice that was not noted in WT mice. Stimulation index: (cpm with antigen) / (cpm with medium alone); dotted line: SI = 2; Error bars: S.D. of data from 3 separate experiments. (B). The failure of hrMOG-immunized WT B6 mice to generate a response to pMOG61–85 was confirmed using peptide concentrations ranging from 5 to 30 μg/ml. Data representative of 4 separate experiments.