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Distinct T cell recognition of naturally processed and cryptic epitopes within the immunodominant 35–55 region of myelin oligodendrocyte glycoprotein

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Abstract

We have assessed the complexity in T cell recognition of the immunodominant 35–55 region of myelin oligodendrocyte glycoprotein (MOG) in C57BL/6 mice. Immunization with the p35–55 peptide generated two types of T cell, recognizing either a cryptic, or a naturally-processed epitope. Clear differences in the recognition of residues within a core sequence of 40-48 were observed. The majority of the p35–55-reactive repertoire in vivo appeared responsive to the intact autoantigen, supporting the notion of a failure of central tolerance to this region of MOG. Our data also provide a basis for exploring the requirements for antigen processing of MOG.

Keywords: T cell; Autoimmunity; Multiple sclerosis; Tolerance; Antigen processing

1. Introduction

The use of self Ag to specifically modulate the activity of autoaggressive T cells holds promise as an effective therapeutic approach to human autoimmune disorders (Larche and Wraith, 2005). The use of animal models of disease has confirmed this using various Ag-specific approaches. We and others have administered soluble peptides containing the individual epitopes recognized by autoreactive T cells to abrogate disease (Anderton, 2001; Larche and Wraith, 2005). One potential problem with using peptides is ensuring the peptide binds to the appropriate MHC molecule in a manner that mimics the "natural" peptide-MHC (pMHC) complex that is generated by processing of the intact self Ag. Using murine experimental autoimmune encephalomyelitis (EAE) induced with myelin basic protein (MBP) we have previously reported that a peptide identified as containing the immunodominant encephalitogenic epitope of MBP can be bound to a single MHC class II molecule in multiple registers when given as a peptide. The optimal fit for the MBP peptide produced a pMHC complex that was not generated as a result of natural processing of intact MBP and consequently the peptide was unable to tolerize the most pathogenic T cells that, as would be predicted, were those able to recognize processed MBP (Anderton et al., 2002). In an attempt to increase specificity, many autoimmune models have now been adapted to use synthetic peptides rather than whole myelin Ag for immunization. It seems therefore to be important to determine how closely the peptide-induced T cell repertoire fits with the repertoire generated by intact autoantigen.

Here we have studied the T cell response to myelin oligodendrocyte glycoprotein (MOG) in C57BL/6 mice.

Abbreviations: APL, altered peptide ligand; HEL, hen egg lysozyme; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; PLNC, primed lymph node cell; p35–50, the 35–50 peptide of mouse MOG; p35–55, the 35–55 peptide of mouse MOG; rMOG, the recombinant extracellular domain of mouse MOG.

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This is a well characterised EAE model with the encephalitogenic epitope contained within residues 35–55 (Mendel et al., 1995). Previous studies have indicated that the crucial residues for T cell recognition of this region are contained within the residues 40-48 (Mendel et al., 1996). We generated panels of T cell lines and hybridomas to assess the fine specificity of the anti-MOG repertoire. As would be predicted, immunization with MOG generated only T cells capable of responding to MOG, whereas priming with the p35–55 peptide resulted in two populations that either did, or did not respond to processed MOG. We used an overlapping nested set of peptides to determine the core T cell epitopes. This revealed that both types of T cell contacted residues within the 40-48 core sequence. However, those recognizing the natural epitopes focused on C terminal residues, whereas those recognising only the cryptic epitope (i.e. not generated from processing of intact rMOG) focused on residues at the N terminus. This allowed us to use APL that preferentially stimulated T cells seeing either the natural or the cryptic epitope to induce tolerance in vivo. We conclude that the majority of the p35–55 T cell repertoire recognizes the natural epitope, further underlining the lack of central tolerance to this immunodominant region of MOG.

2. Materials and methods

2.1. Mice, Ags and immunizations

C57BL/6 mice were bred under specific pathogen free conditions at the University of Edinburgh. 6–8 weeks old, sex-matched mice were used for all experiments. The recombinant extracellular domain of murine MOG (rMOG) was expressed and purified as described previously (Fillatreau et al., 2002). Peptides p35–55 (MEVGWYRSPFSRVVHLYRNGK) and p35–50 were synthesized by the Advanced Biotechnology Centre, Imperial College London. Two panels of overlapping 15mer peptides were generated. The first shifted by 5 residues at a time and covered MOG 1-15 to 106-120. The second panel shifted by one residue and covered MOG 30-44 to 46-60. A third panel of peptides were APL based on p35–50, with Ala substitutions at individual residues (35Ala, 36Ala, through to 50Ala). The above three panels of peptides were synthesized by the laboratory of Prof. D. Wraith, University of Bristol, UK.

For experiments analyzing T cell activation, mice were immunized s.c. with 100 μg of either p35–55 or rMOG emulsified in CFA (Sigma, Poole, GB). After 10 days, draining inguinal and para-aortic LN were removed, disaggregated and used as a source of primed lymphoid populations.

2.2. Tolerance induction and assessment of PLNC recall responses

Mice received 200 μg of peptide in 0.5 ml PBS (or PBS alone) intraperitoneally on days 8, 6 and 4 prior to 100 μg of p35–55 in CFA subcutaneously on day 0. After 10 days, LN populations were isolated and tested for recall responses to p35–55.

Lymphoid cell suspensions were cultured in 96-well flat-bottom microtitre plates (Becton Dickinson, Oxford, UK) at 6×10^5 LN cells/well using X-vivo 15™ serum free medium (BioWhittaker, Maidenhead, UK) supplemented with 2 mM L-glutamine and 5×10^{-5} 2-ME (both from Gibco, Life Technologies, Paisley, UK). Cultures were stimulated with dose ranges of p35–55 or rMOG, or 10 μM of overlapping peptides for 48 h prior to addition of ^{3}H-thymidine (0.5 Ci/well) (Amersham, Amersham, UK). After a further 18 h, cultures were harvested and dThd incorporation measured using a liquid scintillation β counter (LKB Wallac, Turku, Finland). Results are expressed as mean cpm of triplicate cultures.

2.3. T cell lines

T cell lines (TCL) were generated using repeated restimulation and expansion cycles as described previously (Anderton et al., 1998). LN populations were initially stimulated with 10 μM p35–55 in X-vivo 15™ serum-free medium. After 3 days viable cells were purified by density gradient centrifugation using Nycoprep 1.077 animal™ (Nycomed, Oslo, Norway). Blasts were then expanded for 7 days in RPMI 1640 medium containing 2 mM L-Glutamine, 5×10^{-5} M 2-ME, 100 μg/ml penicillin, 100 U/ml streptomycin (Gibco) supplemented with 5% FCS (RPMI-5) and further supplemented with 5% supernatant from Con A-stimulated rat splenocytes (as a source of T cell growth factors). T cells were then restimulated with peptide and irradiated syngeneic spleen APC and the cycle repeated. Once established, the dose of peptide used for restimulation of T cell lines was lowered to 1 μM. All TCL were $CD4^+$.

Proliferation assays were performed using flat-bottom 200 μl microtiter wells (Becton Dickinson). TCL (2×10^4 per well) were cultured with irradiated (30 Gy) syngeneic splenocytes (3×10^5 per well) in the presence or absence of Ag (peptide or rMOG) for a total of 72 h. Cultures were pulsed for the final 18 h with ^{3}H-thymidine and incorporation measured as above. Results are expressed as mean cpm of triplicate cultures.

2.4. Generation of p35–55-reactive T cell hybridomas

Draining LN populations (from p35–55- or rMOG-primed mice) were stimulated in vitro with 10 μM p35–55 as for the generation of TCL above. After 3 days, blasts were fused with the TCR-deficient thymoma BW5147 as described previously (Anderton et al., 2001). All hybrids were cloned and re-cloned by limiting dilution. Hybridoma activation was tested as IL-2 secretion in response to Ag presented the A^b-expressing B cell line LB27.4. In some experiments anti-A^b (clone Y3P) or anti-D^b (clone 28-14-8) antibodies were added to the cultures at 10 μg/ml. Supernatants were harvested after 24 h of culture and their IL-2 content was measured either as proliferation of the IL-2 dependent cell