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Review

Epitope Spreading: a Mechanism for Progression of Autoimmune Disease

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Abstract. Autoimmune diseases are typically characterized by a persistent inflammatory self-recognition process that ultimately leads to chronic progressive disability. Over the past several years we have addressed the fundamental question of why autoimmune diseases are chronic. Our working hypothesis in these studies has been that autoimmunity involves a continuous acquisition of new self-recognition events, thereby providing an inflammatory steady-state that leads to chronicity. This acquired T cell neoautoreactivity is commonly referred to as epitope spreading. By studying multiple sclerosis (MS) and its related animal model, experimental autoimmune encephalomyelitis (EAE), we have found that chronic progression of autoimmune disease is invariably linked to the development of an epitope-spreading process that manifests as a cascade of inflammatory T cell neoautoreactivities to a sequential series of predictable new target self-antigens. However, our most recent observations indicate that the emergence of epitope spreading is accompanied by a concurrent regression of the established primary autoreactivity associated with disease onset. Thus, our studies indicate that progression of autoimmune disease involves a shifting of T cell autoreactivity from primary initiating self-determinants to defined cascades of secondary determinants that sustain the inflammatory self-recognition process during progression to chronicity. Our data support the view that the natural development of self-recognition during autoimmune disease may best be understood when considered in the temporal context of an "epitope *du jour*" and "moving target" perspective.

Key words: autoimmunity; EAE/MS; T cell; myelin; demyelination.

Introduction

Much of our current understanding of self-recognition in multiple sclerosis (MS) stems from studies on experimental autoimmune encephalomyelitis (EAE), an animal model with many clinical and histopathologic similarities to MS[11, 22]. EAE is induced by immunization of animals with either whole central nervous system (CNS) tissue, myelin or myelin, proteins such as myelin basic protein (MBP), myelin proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG). EAE is mediated by $CD4^+$ T cells of the Th1 phenotype (IL-2, IFN-γ, TNF-β) that recognize peptide determinants of CNS myelin proteins in the context of major

Abbreviations used: CDMS – clinically definite multiple sclerosis, CNS – central nervous system, EAE – experimental autoimmune encephalomyelitis, IMDS – isolated mono-symptomatic demyelinating syndrome, MBP – myelin basic protein, MOG – myelin oligodendrocyte glycoprotein, MRI – magnetic resonance imaging, MS – multiple sclerosis, PBMC – peripheral blood mononuclear cells, PLP – myelin proteolipid protein, MHC – major histocompatibility complex.

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histocompatibility complex (MHC) class II molecules and initiate a cascade of events leading ultimately to paralysis accompanied by perivascular mononuclear infiltrates and demyelination in CNS white matter[11, 19].

SWXJ mice develop a relapsing form of EAE that progresses to chronicity. Following immunization with the immunodominant encephalitogenic determinant PLP 139-151, SWXJ mice develop acute EAE followed by a relapsing-remitting clinical course, with each relapse being progressively more severe and each remission leaving the mice progressively more impaired. Within 4 months after immunization, the majority of SWXJ mice reach a chronic-progressive stage of EAE characterized by a gradual inability to initiate any self-directed movement and by severe CNS demyelination, particularly pronounced in the spinal cord[21, 25].

A growing number of studies indicate that relapse and progression of EAE may be due to the acquisition of T cell responses to new myelin protein determinants during the course of disease. This acquired T cell neoautoreactivity is commonly referred to as epitope or determinant spreading and is presumably due to endogenous priming with new self-antigens presented during the inflammatory destruction of tissue to an infiltrating immune system[24, 30]. The first definitive report of intramolecular epitope spreading may be attributed to McCarron et al.[12], who showed that (SJLxPL)F_1 mice acquired a new class II MHC-restricted response to MBP during relapse that was not present during the primary inflammatory attack. Lehmann et al.[10] extended this finding and found that the specificity of the T cell repertoire in (SJLxB10.PL)F_1 mice was restricted to the priming immunodominant MBP 1-11 determinant during the inductive phase of MBP-induced EAE, but broadened with increasing time to include reactivity to the MBP cryptic determinants p35-47, p81-100 and p121-140. Most importantly, the observed epitope spreading did not require immunization with whole MBP but occurred even when the priming immunogen was the MBP 1-11 peptide determinant. Shifts in MBP, epitope recognition during the course of EAE have also been observed in SJL/J and B10.PL parental mouse strains[3] and in Lewis rats[14].

Intermolecular spreading was described by Perry et al.[18] who showed proliferative responses and delayed-type hypersensitivity to PLP during the course of EAE in (SJLxPL)F_1 mice immunized with MBP. The pathogenicity of intermolecular spreading was established by Cross et al.[5], who were able to transfer EAE with PLP 139-151 stimulated spleen cells from SJL/J mice that had previously developed passive disease mediated by the immunodominant MBP 87-99 determinant. McRae et al.[13] showed that progression of PLP 139-151-induced EAE could be inhibited by inducing tolerance to whole PLP after recovery from the primary attack. The induction of tolerance inhibited the development of neoautoreactivity directed toward the encephalitogenic PLP 178-191, thereby providing functional evidence, that epitope spreading is a pathogenic process that may be targeted therapeutically with peptide-specific treatment after disease onset. The following account details our experimental evidence, which provides support for the view that epitope spreading is a pathogenic mechanism for progression to chronicity in autoimmune disease.

Epitope Spreading in EAE

We have systematically assessed the pattern of intra- and intermolecular determinant spreading as a function of time in SWXJ mice immunized with the immunodominant PLP 139-151[32]. We found that the order in which new determinants are recognized during the course of disease follows an ordered, predictable, sequential pattern. After immunization of SWXJ mice with PLP 139-151, a sequential cascade of self-recognition occurred. At 4, 8 and 12 weeks after immunization, responses to PLP 249-273, MBP 87-99 and PLP 178-191, respectively, emerged. Thus, at 12 weeks after priming with PLP 139-151, SWXJ mice consistently showed a chronic accumulation of neurologic deficit accompanied by severe CNS demyelination and a cascading accumulation of neoautoreactivity involving PLP 139-151 $\rightarrow$ PLP 249-273 $\rightarrow$ MBP 87-99 $\rightarrow$ PLP 178-191. Similar patterns of temporally spaced neoautoreactivity were observed following priming of SWXJ mice with other encephalitogenic determinants, including PLP 104-117 and PLP 178-191 (Fig. 1a, b).

In addition, we observed an invariant relationship between the development of relapse/progression and the spreading of self-recognition to new immunodominant encephalitogenic determinants. Moreover, we found that peptide-specific tolerance to spreading, but not to "nonspreading", determinants after disease onset prevents subsequent chronic progression of EAE. Finally, we showed that $CD4^+$ Th1 T cells generated *in vivo* to spreading determinants are autonomously pathogenic, since they adoptively transfer EAE into naive recipients even after photolytic elimination of T cells responding to the priming immunogen[33]. Thus, our data indicate that determinant spreading is a pathogenic process that leads to relapse and chronic-progression of autoimmune disease, and that the predictable sequential

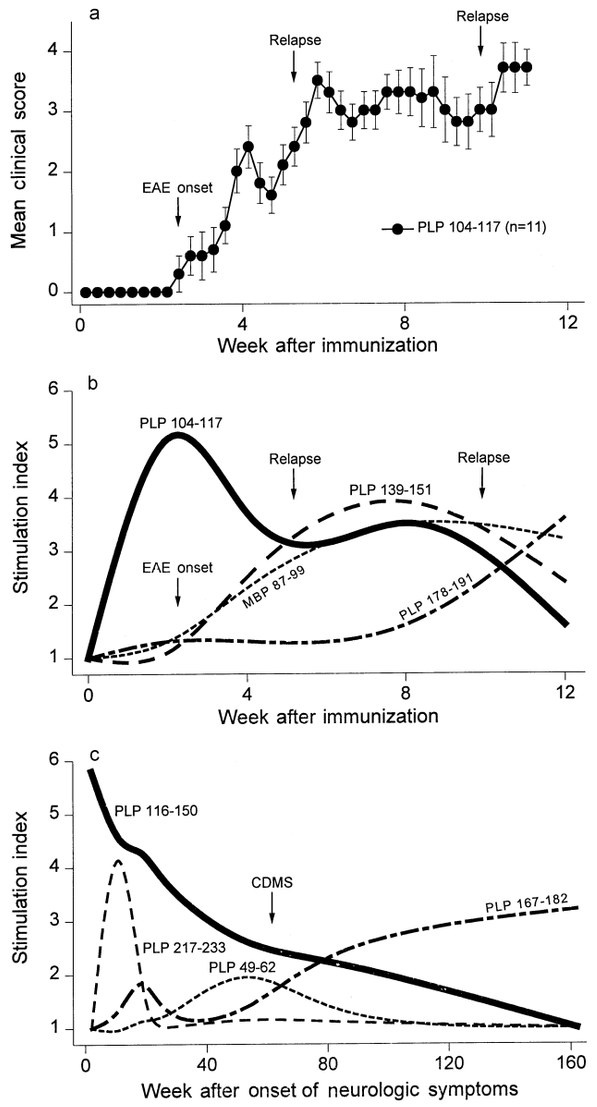

Fig. 1. The epitope-spreading cascade accompanies progression of EAE and MS: a – immunization of SWXJ mice with PLP 104-117 results in a relapsing-remitting course of EAE that progresses to chronicity. Each relapse is followed by an incomplete recovery that results in a progressive accumulation of neurologic deficit. Similar clinical profiles occur following immunization with PLP 139-151 and PLP 178-191. Error bars show ±SEM; b – a predictable sequential epitope-spreading cascade accompanies progression to chronicity in EAE. Induction of EAE by immunization of SWXJ mice with PLP 104-117 results in a temporally spaced cascade of neoautoreactivity involving PLP 104-117 → PLP 139-151→ MBP 87-99 → PLP 178-191. Similar, and perhaps more pronounced, sequential cascades occur during progression of EAE induced with other encephalitogenic PLP determinants, including PLP 139-151 and PLP 178-191. It should be noted that EAE progression continues long after responses to the priming PLP 104-117 determinant have peaked, regressed, and virtually disappeared. The data are represented as spline curve plots of mean splenocyte proliferative responses of at least three mice tested at each 0, 2, 4, 8 and 12 week time points; c – progression of IMDS → CDMS is accompanied by the spontaneous regression of the proliferative autoreactivity associated with initial IMDS symptoms and by the concurrent emergence of epitope spreading. IMDS patient DL showed IMDS → CDMS progression 60 weeks after the initial onset of IMDS symptoms at week 0. Disease progression involved a sustained neoautoreactivity to PLP 167-182 accompanied by a regression and eventual disappearance of proliferative responses to the PLP 116--150 determinant associated with disease onset. The data are represented as spline curve plots of the mean proliferative responses of PBMC on each day tested

pattern of the determinant-spreading cascade provides a basis for peptide-specific therapy after the onset of clinical disease.

Epitope Spreading in MS

We next asked the question whether epitope spreading occurs during the development and progression of MS[26]. Since MS typically persists for many years before a definitive diagnosis can be made, we focused our studies on patients at the earliest stage of the disease process that ultimately leads to clinically definite MS (CDMS). Patients with isolated monosymptomatic demyelinating syndromes (IMDS) have distinct clinical disorders often associated with the eventual progression to CDMS[2, 7, 15]. By magnetic resonance imaging (MRI) analysis, IMDS patients showing only the CNS lesion responsible for acute symptoms (monocentric monophasic IMDS) are widely viewed to represent the earliest stage at which inflammatory demyelination can be followed from the genuine onset of disease.

Our approach was to determine serial changes over a 12-18 month period in response to an epitope-mapping series of 265 12-mer PLP peptides by PBMC from IMDS patients. We found that an extensive array of PLP peptides could elicit autoreactivity. Moreover, differential autoreactive patterns were evident within IMDS patient subpopulations distinguished by the absence or presence of old inactive lesions. Monocentric monophasic IMDS patients with no evidence of prior subclinical disease typically showed fully sustained autoreactivity, characterized by extensive plasticity, epitope focusing, shifting and spreading of responses to new self-determinants (Fig. 1c). In contrast, multicentric monophasic IMDS patients with putative evidence of prior asymptomatic lesion formation typically showed partially sustained autoreactivity, characterized by abrupt abrogation of responses to an extensive array of self-determinants. No sustained autoreactivity was observed in normal control subjects or in patients with other neurologic diseases. Our results indicate that self-

-recognition associated with the development of MS is characterized by autoreactive diversity, plasticity and instability, with epitope spreading being a core manifestation of the observed plasticity.

We have also found that the neoautoreactivity occurring during the progression of MS involves HLA--DP-restricted responses of autoreactive $CD4^+$ T cells[34]. In our human studies, we have never observed a consensus determinant recognized by patients sharing common HLA-DR and -DQ class II genes, including several study subjects expressing the DRB1*1501/ /DRB5*0101/DQB1*0602 haplotype associated with increased risk for developing MS[1, 8]. However, we did observe epitope spreading to PLP 50-69 in each of two study subjects showing complete disparity at HLA-DR and HLA-DQ but identity at the HLA-DP class II allele DPB1*0301. Upon further analysis we found that peptide-specific proliferation of T cell lines generated from both patients was inhibited by treatment at the initiation of culture with monoclonal antibody specific for HLA--DP, but not with antibody specific for either HLA-DR or -DQ. Moreover, the derived T cell lines showed peptide-specific lytic activity against homozygous-typing B cells expressing the DPB1*0301 class II allele, but not against target cells compatible at HLA-DR and -DQ alleles. Our data indicate that self-presentation by HLA-DP may play an important role in the spreading and propagation of autoreactivity during the clinical progression of MS.

Regression, Spread and Progression of Autoimmune Disease

Recently, we have directly addressed the relative roles of primary autoreactivity and secondary epitope spreading in the progression of both EAE and MS. We serially evaluated the development of several epitope--spreading cascades in SWXJ mice primed with distinctly different encephalitogenic PLP determinants and in monophasic monocentric IMDS patients as their disease progressed to CDMS. Our results showed that in both EAE (Fig. 1a, b) and MS (Fig. 1c), primary proliferative autoreactivity associated with onset of clinical disease invariably regressed with time and was often undetectable during periods of disease progression. In contrast, the emergence of sustained secondary autoreactivity to spreading determinants was consistently associated with disease progression in both EAE and MS. Thus, chronic progression of EAE and MS actually involves a definitive shift of autoreactivity from primary initiating self-determinants to defined cascades of secondary determinants that sustain the self-recognition process during disease progression[27, 28]. A shifting, rather than an accumulation, of autoreactivites implies that the T cell repertoire associated with the progression of autoimmune disease is dramatically different from that associated with disease onset. Moreover, it becomes increasingly more difficult to explain how disease progression can result from an undetectable initiating primary autoreactivity.

The repeated failure to demonstrate memory responses to self-determinants indicates that autoimmune memory has features not observed in traditional memory responses to environmental antigens. This apparent anomaly may find some explanation from recent studies that showed complete deletion of the memory repertoire to viral antigens following high-load inoculation with virus[9]. A similar outcome seems feasible in autoimmunity when one considers the virtually infinite antigen load of constantly synthesized self-proteins. Thus, chronic self-stimulation may result in T cell exhaustion and the peripheral apoptotic deletion of the autoreactive repertoire associated with disease onset, leaving only the developing, endogenously primed neoautoreactive repertoire to sustain the inflammatory self-recognition process[17, 23, 35]. Alternatively, regression of autoreactivity may be due to anergy[6, 4, 20, 21] or suppression[16, 29] of the established mature autoreactive repertoire. Experiments designed to determine the relative contributions of deletion, anergy and suppression toward the observed spontaneous regression of self-recognition are currently in progress. The outcome may, one hopes, explain the fundamental mechanism(s) responsible for the inherent instability of the self-recognition process responsible for progression of autoimmune disease.

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References

1. ALLEN M., SANDBERG-WOLLHEIM M., SJOGREN K., ERLICH H. A., PETTERSON U. and GYLLENSTEN U. (1994): Association of susceptibility to multiple sclerosis in Sweden with HLA class II DRB1 and DQB1 alleles. Hum. Immunol., **39**, 41–48.
2. BECK R. W., CLEARY P. A., TROBE J. D., KAUFMAN D. I., KUPERSMITH M. J., PATY D. W. and BROWN C. H. (1993): The effect of corticosteroid for acute optic neuritis on the subsequent development of multiple sclerosis. N. Engl. J. Med., **329**, 1764–1769.
3. BHARDWAJ V., KUMAR V., GREWAL I. S., DAO T., LEHMANN P. V., GEYSEN H. M. and SERCARZ E. E. (1994): T cell determinant structure of myelin basic protein in B10. PL, SJL/J, and their F_1s. J. Immunol., **152**, 3711–3719.
4. CORREALE J., GILMORE W., LOPEZ J., LI S. Q., MCMILLAN M. and WEINER L. P. (1996): Defective post-thymic tolerance

mechanisms during the chronic progressive stage of multiple sclerosis. Nat. Med., **2**, 1354–1360.
5. CROSS A. H., TUOHY V. K. and RAINE C. S. (1993): Development of reactivity to new myelin antigens during chronic relapsing autoimmune demyelination. Cell. Immunol., **146**, 261–269.
6. DE SILVA D. R., URDAHL K. B. and JENKINS M. K. (1991): Clonal anergy is induced *in vitro* by T cell receptor occupancy in the absence of proliferation. J. Immunol., **147**, 3261–3267.
7. FILIPPI M., HORSFIELD M. A., MORRISSEY S. P., MACMANUS D. G., RUDGE P., MCDONALD W. I. and MILLER D. H. (1994): Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. Neurology, **44**, 635–641.
8. FRANCIS D. A., THOMPSON A. J., BROOKES P., DAVEY N., LECHLER R. I., MCDONALD W. I. and BATCHELOR J. R. (1991): Multiple sclerosis and HLA: Is the susceptibility gene really HLA-DR or -DQ? Hum. Immunol., **32**, 119–124.
9. GALLIMORE A., GLITHERO A., GODKIN A., TISSOT A. C., PLUCKTHUN A., ELLIOTT T., HENGARTNER H. and ZINKERNAGEL R. (1998): Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. J. Exp. Med., **187**, 1383–1393.
10. LEHMANN P. V., FORSTHUBER T., MILLER A. and SERCARZ E. E. (1992): Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. Nature, **358**, 155–157.
11. MARTIN R., MCFARLAND H. F. and MCFARLIN D. E. (1992): Immunological aspects of demyelinating diseases. Annu. Rev. Immunol., **10**, 153–187.
12. MCCARRON R. M., FALLIS R. J. and MCFARLIN D. E. (1990): Alterations in T cell antigen specificity and class II restriction during the course of chronic relapsing experimental allergic encephalomyelitis. J. Neuroimmunol., **29**, 73–79.
13. MCRAE B. L., VANDERLUGT C. L., DAL CANTO M. C. and MILLER S. D. (1995): Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. J. Exp. Med., **182**, 75–86.
14. MOR F. and COHER I. R. (1993): Shifts in the epitopes of myelin basic protein recognized by Lewis rat T cells before, during, and after the induction of experimental autoimmune encephalomyelitis. J. Clin. Invest., **92**, 2199–2206.
15. MORRISSEY S. P., MILLER D. H., KENDALL B. E., KINGSLEY D. P., KELLY M. A., FRANCIS D. A., MACMANUS D. G. and MC DONALD W. I. (1993): The significance of brain magnetic resonance imaging abnormalities at presentation with clinically isolated syndromes suggestive of multiple sclerosis. Brain, **116**, 135–146.
16. OFFNER H., HASHIM G. A. and VANDENBARK A. A. (1991): T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. Science, **251**, 430–432.
17. PELFREY C. M., TRANQUILL L. R., BOEHME S. A., MCFARLAND H. F. and LENARDO M. J. (1995): Two mechanisms of antigen-specific apoptosis of myelin basic protein (MBP)-specific T lymphocytes derived from multiple sclerosis patients and normal individuals. J. Immunol., **154**, 6191–6202.
18. PERRY L. L., BARZAGA-GILBERT E. and TROTTER J. L. (1991): T cell sensitization to proteolipid protein in myelin basic protein-induced relapsing experimental allergic encephalomyelitis. J. Neuroimmunol., **33**, 7–15.
19. RAINE C. S. (1984): Biology of disease. Analysis of autoimmune demyelination: its impact upon multiple sclerosis. Lab. Invest., **50**, 608–635.
20. SCHWARTZ R. H. (1990): A cell culture model for T lymphocyte clonal anergy. Science, **248**, 1349–1356.
21. SOBEL R. A., TUOHY V. K. and LEES M. B. (1991): Parental MHC molecule haplotype expression in (SJL/JxSWR) F_1 mice with acute experimental allergic encephalomyelitis induced with two different synthetic peptides of myelin proteolipid protein. J. Immunol., **146**, 543–549.
22. STEINMAN L. (1996): Multiple sclerosis: A coordinated immunological attack against myelin in the central nervous system. Cell, **85**, 299–302.
23. TANAKA H., OTA K., IKUSAKA M., EJIMA M. and MARUYAMA S. (1995): Expression of Fas-antigen on T cells in multiple sclerosis (Japanese). Rinsho Shinkeigaku, **35**, 299–301.
24. TUOHY V. K., FRITZ R. B. and BEN-NUN A. (1994): Self-determinants in autoimmune demyelinating disease: Changes in T-cell response specificity. Curr. Opin. Immunol., **6**, 887–891.
25. TUOHY V. K. and THOMAS D. M. (1995): Sequence 104-117 of myelin proteolipid protein is a cryptic encephalitogenic T cell determinant for SJL/J mice. J. Neuroimmunol., **56**, 161–170.
26. TUOHY V. K., YU M., WEINSTOCK-GUTTMAN B. and KINKEL R. P. (1997): Diversity and plasticity of self-recognition during the development of multiple sclerosis. J. Clin. Invest., **99**, 1682–1690.
27. TUOHY V. K., YU M., YIN L., KAWCZAK J. A. and KINKEL R. P. (1999): Regression and spreading of self-recognition during the development of autoimmune demyelinating disease. J. Autoimmun., **13**, 11–20.
28. TUOHY V. K., YU M., YIN L., KAWCZAK J. A. and KINKEL R. P. (1999): Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. J. Exp. Med., **189**, 1033–1042.
29. VANDENBARK A. A., HASHIM G. and OFFNER H. (1989): Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature, **341**, 541–544.
30. VANDERLUGT C. J. and MILLER S. D. (1996): Epitope spreading. Curr. Opin. Immunol., **8**, 831–836.
31. VAN PARIJS L. V., PEREZ V. L., BIUCKIANS A., MAKI R. G., LONDON C. A. and ABBAS A. K. (1997): Role of interleukin 12 and costimulators in T cell anergy *in vivo*. J. Exp. Med., **186**, 1119–1128.
32. YU M., JOHNSON J. M. and TUOHY V. K. (1996): A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: A basis for peptide-specific therapy after onset of clinical disease. J. Exp. Med., **183**, 1777–1788.
33. YU M., JOHNSON J. M. and TUOHY V. K. (1996): Generation of autonomously pathogenic neoautoreactive Th1 cells during the development of the determinant spreading cascade in murine autoimmune encephalomyelitis. J. Neurosci. Res., **45**, 463–470.
34. YU M., KINKEL R. P., WEINSTOCK-GUTTMAN B., COOK D. J. and TUOHY V. K. (1998): HLA-DP: A class II restriction molecule involved in epitope spreading during the development of multiple sclerosis. Hum. Immunol., **59**, 15–24.
35. ZHANG J., VANDEVYVER C., STINISSEN P. and RAUS J. (1995): *In vivo* clonotypic regulation of human myelin basic protein-reactive T cells by T cell vaccination. J. Immunol., **155**, 5868–5877.

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