

Suppression of Autoimmunity via Microbial Mimics of Altered Peptide Ligands

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Abstract Molecular mimics of self-antigens can behave as altered peptide ligands and serve to ameliorate autoimmune disease. Analysis of experimental autoimmune encephalomyelitis with proteomic autoantibody microarrays reveals that there might exist a wide variety of microbes with features that mimic self-epitopes. Autoimmunity could therefore be modulated via microbial immunity, which may account for relapse and remission of ongoing disease.

Fujinami and Oldstone demonstrated molecular mimicry between myelin basic protein (MBP) and hepatitis B (Hep B) viral polymerase. When a common stretch of six amino acids shared between MBP and HepB polymerase was injected into rabbits, the animals developed inflammatory brain lesions characteristic of experimental autoimmune encephalomyelitis (EAE) (Fujinami and Oldstone 1985). This seminal paper provided the foundation for the idea that structural mimicry between microbes and self could lead to autoimmunity, when an immune response launched during an infection with a microbe cross-reacts with self.

Molecular mimicry refers to structural homologies between a self-protein and a microbial protein. The concept of molecular mimicry might have pathological consequences and provide a basis for the relapses and remissions so often characteristic of autoimmune disease. For example, molecular mimics

may actually modulate the course of multiple sclerosis (MS) and other autoimmune diseases. Consider these examples, based on the immune response in humans with MS to an epitope of MBP: A major epitope of MBP, p87–99 (VHFFKNIVTPRTP), induces EAE in rats and in mice and is a major target of the immune response in MS (Sakai et al. 1988; Bielekova et al. 2000; Kappos et al. 2000; Steinman 2001, 2004). Intravenous tolerization to the MBPp87–99 epitope in patients with MS leads to the abrogation of anti-MBP antibodies, an effect lasting for months after a single intravenous injection (Warren et al. 1997). In a placebo-controlled double-blinded study administration of an altered peptide ligand (APL) of MBPp87–99 reduced the number of active lesions enhancing with gadolinium on magnetic resonance scans (Kappos et al. 2000). These two examples from actual clinical trials with MS patients emphasize how an immune response to MBPp87–99 or to an APL based on MBPp87–99 can either provoke disease or tolerize during the course of ongoing disease. These clinical examples point to the potential relevance of the “molecular mimicry” hypothesis when applied to human disease.

1 Microbial Mimics Resembling Altered Peptide Ligands Modulate Animal Models of MS

The pentapeptide VHFFK contains the major residues for binding of this self-molecule to T cell receptor (TCR), to the HLA DR2 molecule of the major histocompatibility complex, and to anti-MBP antibodies from MS patients (Warren et al. 1995; Wucherpfennig et al. 1997; Smith et al. 1998). Peptides from papillomavirus strains containing the motif VHFFK induce T cells that are capable of transferring EAE (Ufret-Vincenty et al. 1998). In contrast, Ruiz et al. showed that APL peptides resembling MBPp87–99 peptide, but differing in certain key residues from the native VHFFK, suppressed EAE. Thus a peptide from human papillomavirus type 40 (HPV 40) containing VHFFR, and one from HPV 32 containing VHFFH, prevented EAE (Ruiz et al. 1999). The K residue was shown to be the major TCR contact site in SJL mice for MBPp87–99 (Brocke et al. 1996). Likewise, K at position 91 is the major TCR contact site in humans for T cell clones recognizing the native VHFFK bound to HLA DR2 (Vergelli et al. 1998; Smith et al. 1998).

In our EAE studies, in addition to the sequences from HPV 32 and HPV 40, a sequence from *Bacillus subtilis* (RKVVTDFFKNIPQRI) also prevented EAE. We also showed that T cell lines, producing IL-4 and specific for these microbial peptides including HPV40, HPV32, and *Bacillus subtilis*, suppressed EAE, probably by inducing a Th1 → Th2 shift (Ruiz et al. 1999). These findings

demonstrated that microbial peptides, differing from the core motif of the self-antigen, MBPp87-99, functioned as altered peptide ligands, and behaved as TCR antagonists, in the modulation of autoimmune disease.

2 Viral Damage, Subsequent Breakdown in Self-Tolerance, and Epitope Spreading In Animal Models of MS

When certain neurotropic viruses trigger inflammation in the central nervous system (CNS), immune cells in the inflammatory infiltrate attack neighboring myelin antigens in the CNS (Miller et al. 1997; Steinman and Conlon 1997). This immune response then spreads to various epitopes on various myelin antigens, a process known as epitope spreading (Lehmann et al. 1992; Miller et al. 1997). In the context of epitope spreading, molecular mimicry can either exacerbate or ameliorate disease. A virus that mimics one or more of these epitopes that are targeted by the immune system after epitope spreading may trigger a flare-up of demyelinating disease (Ufret-Vincenty et al. 1998), whereas APLs resembling the immunogenic portion of certain neurotropic viruses can suppress EAE (Ruiz et al. 1999, Fig. 1). Earlier work from my laboratory showed that administration of such APLs could lead to the widespread clearance of inflammatory infiltrates in the brain. Such infiltrates are comprised of a diverse collection of T cells and B cells in the brain (Brocke et al.

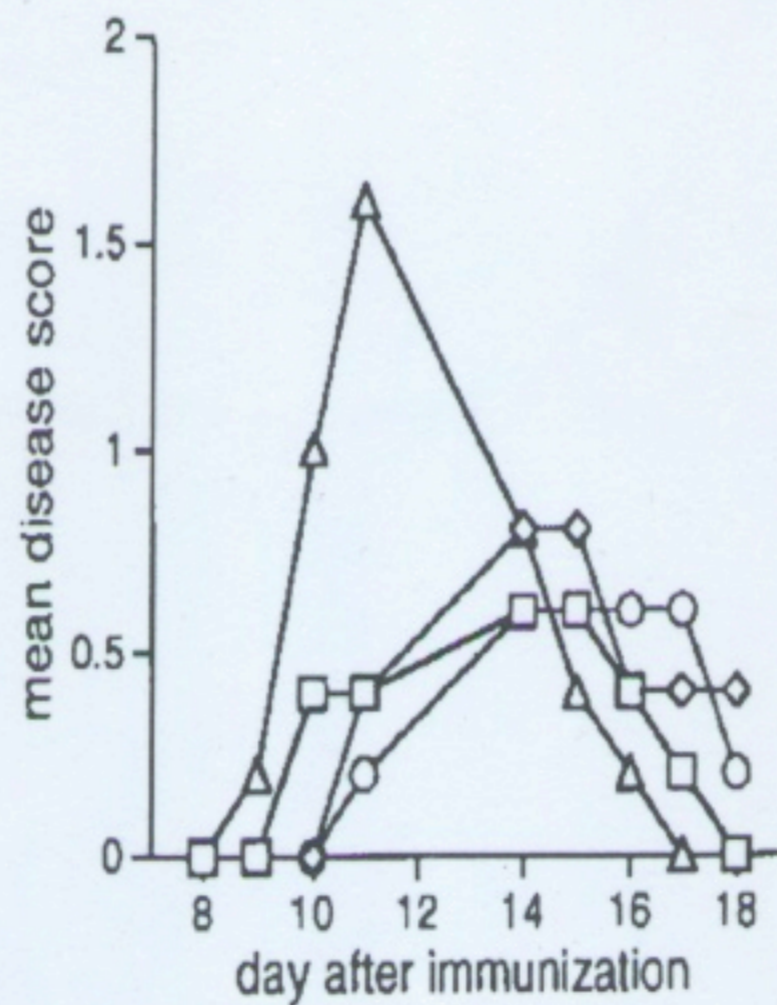


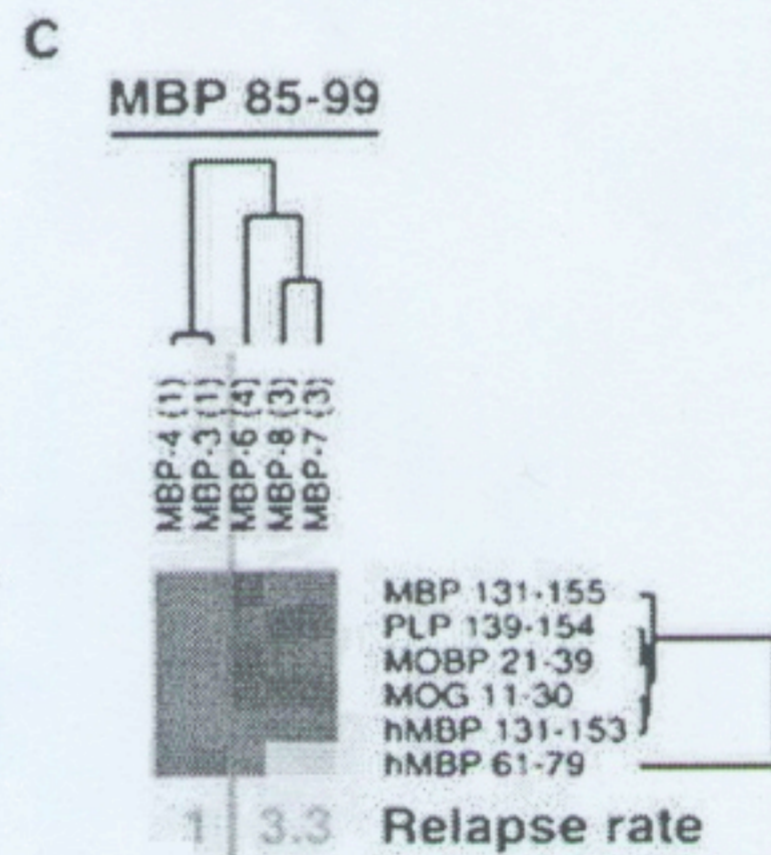
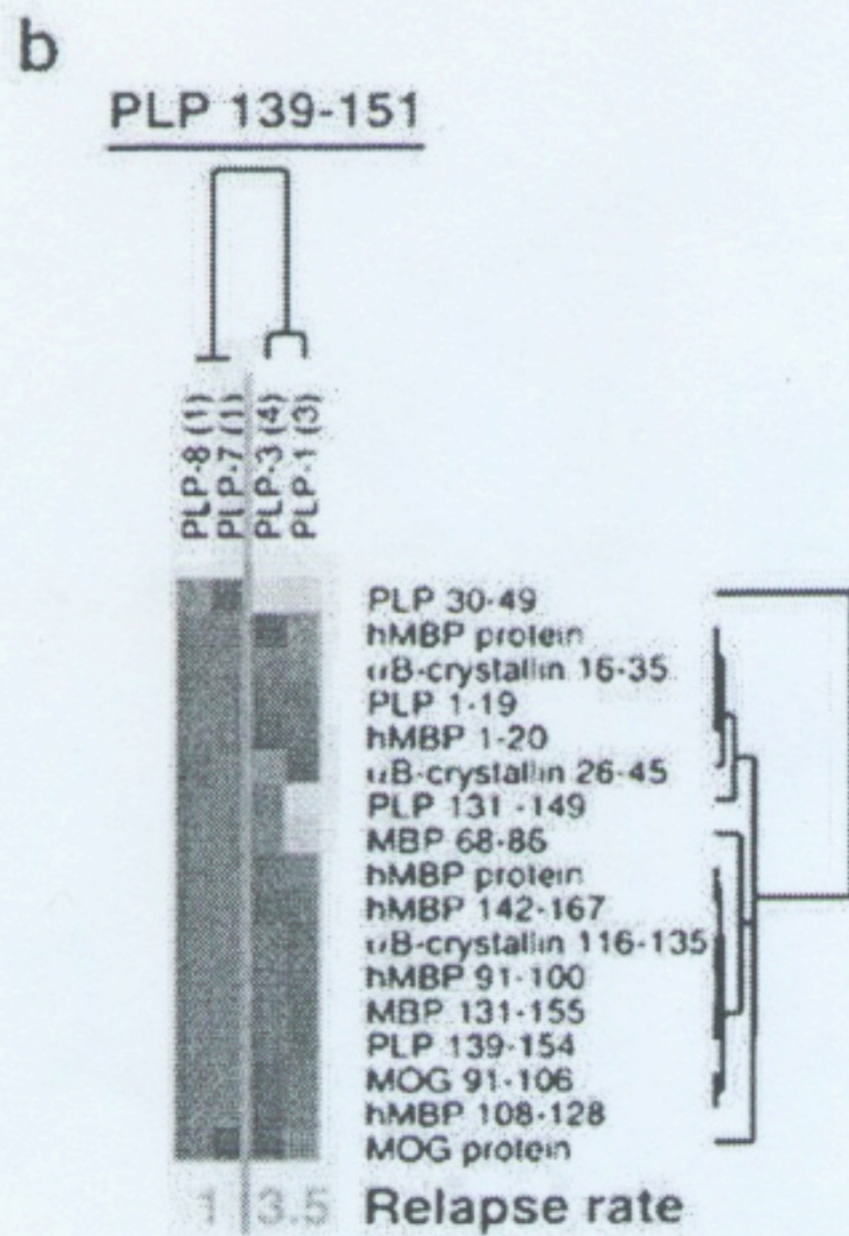
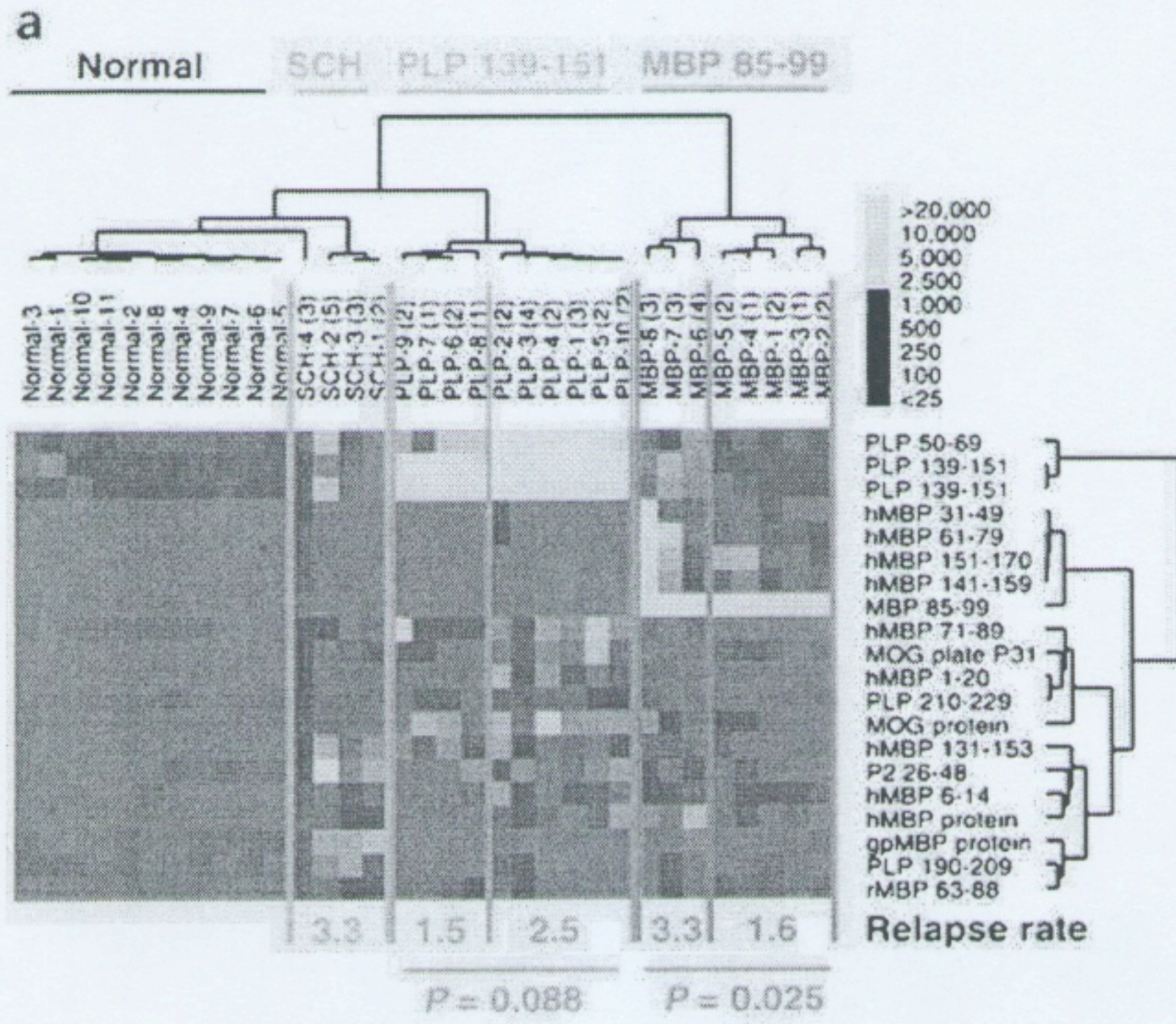
Fig. 1 (■ no legend given ■)

1996). An APL of MBP p87–99 was able to actually cause such a collection to disperse from areas of inflammation.

In EAE and MS we have constructed large-scale proteomic microarrays to assess the diversity of epitope spreading in the autoantibody response (Robinson et al, 2003). The 2,304-feature myelin proteome arrays contain 232 distinct antigens, including proteins and sets of overlapping peptides representing MBP, PLP, MOG, myelin-associated oligodendrocytic basic protein (MBOP), oligodendrocyte-specific protein (OSP), B-crystallin, cyclic nucleotide phosphodiesterase (CNPase) and Golli-MBP. These arrays contained 13 proteins and 219 synthetic peptides, including 4 proteins and 85 peptides from MBP, 3 proteins and 30 peptides from PLP, 3 proteins and 50 peptides from MOG, 2 peptides from MBOP, 1 protein and 16 peptides from B-crystallin, 20 peptides from CNPase, 1 protein and 11 peptides from peripheral myelin protein 2 (P2), 2 peptides from the acetylcholine receptor, and 4 nonmyelin peptides or proteins.

We used myelin proteome arrays to profile autoantibody responses in serum derived from mice with EAE, and images of representative arrays are presented in Fig. 2. A similar broad diversity of autoantibody responses is being detected in cerebrospinal fluid of MS patients and in the serum of patients

Fig. 2a–c a Hierarchical clustering of antigen features with statistically significant differences in myelin proteome array reactivity between sera derived from groups of normal control mice and from groups of mice on recovery from acute EAE induced with PLP(139–151) (day 17), MBP(85–99) (day 22), or SCH (day 25). Mice were later scored daily for 10 weeks to determine the number of relapses for each mouse (indicated in *parentheses*). The average relapse rates for mice included in the primary subnodes of the dendrogram, and *P* values by Mann-Whitney test for the differences in relapse rate between these nodes, are indicated. **b, c** Antigen features with statistically significant differences in array reactivity between subsets of mice with the greatest (three and four) and least (one) number of relapses within groups induced for EAE with PLP(139–151) or MBP(85–99). SAM (Robinson et al. 2003) was used to identify antigen features with statistically significant differences in array reactivity between groups of mice. A hierarchical cluster algorithm based on a pairwise similarity function was applied to order mice based on similarities in their array reactivities for the SAM-identified features (dendrograms depicting cluster relationships are displayed *above* the individual mice), and to order antigen features based on similarities in reactivities in the mice studied (dendrograms displayed to the *right*). Relationships between mice or antigen features are represented by tree dendrograms whose branch lengths reflect the degree of similarity in array reactivity determined by the hierarchical cluster algorithm. After clustering, labels were added above the dendrograms to indicate the location of clusters of mice induced for EAE with different encephalitogens. (Robinson et al. 2003)



with acute disseminated encephalomyelitis (Robinson et al., in preparation). These studies imply that there might exist a wide variety of microbes with features similar enough to some or even many of these myelin epitopes. If so, then modulation of autoimmunity might be triggered or modulated, similar to what was seen in animal studies of EAE (Wucherpfennig and Strominger 1995; Ufret-Vincenty et al. 1998). A search of the literature reveals that there are a reasonable number of microbes whose structures resemble many of the myelin epitopes that are targeted in EAE (Robinson et al. 2003), and in MS and ADEM (Robinson et al., in preparation).

First of all it is worth remembering that the homologies between a microbe and its mimic do not have to be extensive. We demonstrated that a polyalanine peptide with only five native MBP residues is able to induce EAE in (PLS_{JL/J})F1 mice (Gautam et al. 1995). Further analysis also showed that an 11-amino acid peptide, consisting mostly of alanines with only four native Ac1-11 residues, was able to induce T cell hybridoma proliferation. Taking the approach of introducing either D-amino acids or unnatural amino acids in place of L-amino acids into MBPpAc1-11 analogs, we showed that T cells recognize only a short stretch of six or seven amino acids. More importantly, this stretch contains only four native MBPpAc1-11 residues. We also tested T cell recognition in vivo, using EAE as a measure of activation. We showed that a short peptide of six amino acids with a core of only five native Ac1-11 amino acids induces EAE (Karin et al. 1998).

Molecular mimicry between Semliki Forest virus and MOG was shown to induce a chronic onset late EAE, with unusual characteristics including CNS vacuolation. (Mokhtarian et al. 1999). Hughes and coworkers showed that "antisera against MBP (residues 110-124) reacted with both *Acinetobacter* and *Pseudomonas* peptides from 4- and gamma-carboxymuconolactone decarboxylase, respectively. MOG (residues 43-57) antisera reacted with *Acinetobacter* peptide from 3-oxo-adipate-CoA-transferase subunit A" (Hughes et al. 2003). Lington and coworkers have shown interesting homologies between two immunoglobulin supergene family members, MOG and the milk protein butyrophilin (Guggenmos et al. 2004). Zhang and coworkers showed that "greater than 50% of T cells recognizing MBP(93-105) cross-reacted with and could be activated by a synthetic peptide corresponding to residues 1 to 13 of HHV-6 U24 in MS patients" [Tejada-Simon et al, 2003].

As we wrote in 1999, "the interaction of the immune system with microbes may allow the selection of viral and bacterial subtypes" (Ruiz et al. 1999). It is interesting to speculate, from our studies with HPV subtypes, "that attenuation of the immune response by a peptide derived from a papilloma viral subtype, containing an APL-like motif, may be desirable for viral survival. A virus capable of subverting the immune response against itself might be

selected because it could survive and persist, instead of being eradicated in the wake of an autoimmune response.” Arguing from the precedent of T cells specific for MBPp87–99, “there may be a delicate physiological interplay between self- and microbial antigens, allowing the modulation of autoimmune disease and the persistence and survival of mutant microbes. Attenuating inflammation in the brain may allow microbes to survive, sequestered within the central nervous system. It is remarkable that certain viral subtypes are mutated exactly at a main TCR contact site, and such mutations may represent an adaptive response of a virus, which then acts as an APL” (Ruiz et al. 1999). There are now numerous other examples of potential APL-like sequences in other microbial mimics for other epitopes that are targeted by immunity as epitope spreading involves in the course of demyelinating disease (Robinson et al. 2003).

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References

- Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, Gran B, Eaton J, Antel J, Frank JA, McFarland HF, Martin R (2000) Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med* 6(10):1167–1175
- Brocke S, Gijbels K, Allegretta M, Ferber I, Piercy C, Blankenstein T, Martin R, Utz U, Karin N, Mitchell D, Veromaa T, Waisman A, Gaur A, Conlon P, Ling N, Fairchild PJ, Wraith DC, O’Garra A, Fathman CG, Steinman L (1996) Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. *Nature* 379:343–345
- Fujinami RS, Olstone MBA (1985) Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 230(4729):1043–1045
- Gautam AM, Liblau R, Chelvanayagam G, Steinman L, Boston T (1998) A viral peptide with limited homology to a self-peptide can induce clinical signs of experimental autoimmune encephalomyelitis. *J Immunol* 161:60–64
- Guggenmos J, Schubart AS, Ogg S, Andersson M, Olsson T, Mather IH, Lington C (2004) Antibody cross-reactivity between myelin oligodendrocyte glycoprotein and the milk protein butyrophilin in multiple sclerosis. *J Immunol* 172(1):661–668
- Hughes LE, Smith PA, Bonell S, Natt RS, Wilson C, Rashid T, Amor S, Thompson EJ, Croker J, Ebringer A (2003) Cross-reactivity between related sequences found in *Acinetobacter sp.*, *Pseudomonas aeruginosa*, myelin basic protein and myelin oligodendrocyte glycoprotein in multiple sclerosis. *J Neuroimmunol* 144(1–2):105–115

- Kappos L, Comi G, Panitch H, Oger J, Antel J, Conlon P, Steinman L and the APL in Relapsing MS Study Group (2000) Induction of a non-encephalitogenic Th2 autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo controlled, randomized phase II trial. *Nat Med* 6(10):1176-1182
- Karin N, Binah O, Grabie N, Mitchell D, Felzen B, Solomon M, Conlon P, Gaur A, Ling N, Steinman L (1998) Short peptide-based tolerogens without self-antigenic or pathogenic activity reverse autoimmune disease. *J Immunol* 160:5188-5194
- Lehmann P, Forsthuber T, Miller A, Sercarz EE (1992) Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. *Nature* 358:155-157
- Miller SD, Vanderblugt CL, Begolks WS, Pao W, Yauch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat Med* 3:1133-1136
- Mokhtarian F, Zhang Z, Shi Y, Gonzales E, Sobel RA (1999) Molecular mimicry between a viral peptide and a myelin oligodendrocyte glycoprotein peptide induces autoimmune demyelinating disease in mice. *J Neuroimmunol* 95(1-2):43-54
- Robinson, WH, Fontoura P, Lee BJ, Neuman de Vegvar HE, Tom J, Pedotti R, DiGennaro C, Mitchell DJ, Fong D, Ho PK, Ruiz P, Maverakis E, Stevens D, Bernard CCA, Olsson T, Martin R, Kuchroo VK, van Noort JM, Genain CP, Utz PJ, Garren H, Steinman L (2003) Reverse genomics: Protein microarrays guide tolerizing DNA vaccine treatment of autoimmune encephalomyelitis. *Nat Biotechnol* 21:1033-1039
- Ruiz PJ, Garren H, Hirschberg DL, Langer-Gould AM, Levite M, Karpuj MV, Southwood S, Sette A, Conlon P, Steinman L (1999) Microbial epitopes act as altered peptide ligands to prevent EAE. *J Exp Med* 189:1275-1284
- Sakai K, Zamvil SS, Mitchell DJ, Lim M, Rothbard JB, Steinman L (1988) Characterization of a major encephalitogenic T cell epitope in SJL/J mice with synthetic oligopeptides of myelin basic protein. *J Neuroimmunol* 19:21-32
- Smith KJ, Pyrdol J, Gauthier L, Wiley DC, Wucherpfennig KW (1998) Crystal structure of HLA-DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human myelin basic protein. *J Exp Med* 188(8):1511-1520
- Steinman L (2001) Multiple sclerosis: a two stage disease. *Nat Immunol* 2:762-765
- Steinman L (2004) Immune therapy for autoimmune diseases. *Science* 305:212-216
- Steinman L, Conlon P (1997) Viral damage and the breakdown of self-tolerance. *Nat Med* 3:1085-1087
- Tejada-Simon MV, Zang YC, Hong J, Rivera VM, Zhang JZ (2003) Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis. *Ann Neurol* 53(2):189-197
- Ufret-Vincenty R, Quigley L, Tresser N, Pak SH, Gado A, Hausmann S, Wucherpfennig KW, Brocke S (1998) In vivo survival of viral antigen-specific T cells that induce experimental autoimmune encephalomyelitis. *J Exp Med* 188:1725-1738
- Vergelli M, Hemmer B, Utz U, Vogt A, Kalbus M, Tranquill L, Conlon P, Ling N, Steinman L, McFarland H, Martin R (1996) Differential activation of human autoreactive T cell clones by altered peptide ligands derived from myelin basic protein peptide (87-99). *Eur J Immunol* 26:2624-2634

- Warren KG, Catz I, Steinman L (1995) Fine specificity of the antibody response to myelin basic protein in the central nervous system in multiple sclerosis: The minimal B cell epitope and a model of its unique features. *Proc Natl Acad Sci USA* 92:11061-11065
- Warren KG, Catz I, Wucherpfennig KW (1997) Tolerance induction to myelin basic protein by intravenous synthetic peptides containing epitope P85 VVHFFKNIVTP96 in chronic progressive multiple sclerosis. *J Neurol Sci* 152(1):31-38
- Wucherpfennig KW, Catz I, Hausmann S, Strominger JL, Steinman L, Warren KG (1997) Recognition of the immunodominant myelin basic protein peptide by autoantibodies and HLA-DR2 restricted T cell clones from multiple sclerosis patients: Identity of key contact residues in the B-cell and T-cell epitopes. *J Clin Invest* 100:1114-1122
- Wucherpfennig KW, Strominger JL (1995) Molecular mimicry in T cell mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 80:695-705