Molecular Mimicry and Multiple Sclerosis: Degenerate T-Cell Recognition and the Induction of Autoimmunity

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Various mechanisms have been proposed for the initiation of autoimmune responses by autoreactive T-cell clones. One of these, the molecular mimicry hypothesis, postulates that myelin-reactive T-cell clones are activated by foreign antigens. Until recently, sequence homology between self- and foreign antigens was considered necessary for cross-recognition to occur in multiple sclerosis. This article reviews current progress in T-cell receptor immunology that led to modify this view and proposes a role for degenerate T-cell antigen recognition in the induction of autoimmunity.

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Both clinical and experimental evidence supports the hypothesis that immune mechanisms are involved in the pathogenesis of inflammatory demyelination in multiple sclerosis (MS) and that autoreactive T lymphocytes initiate the process of central nervous system (CNS) myelin damage.[1] Molecular mimicry has been proposed as a way by which an autoimmune response to myelin antigens may be initiated.[2,3] According to this model, self-reactive T cells may be activated by cross-reactivity with infectious agents that "mimic" or share immunological epitopes with the autoantigen. Sequence homology between a self-antigen such as myelin basic protein (MBP) and a foreign antigen such as a viral protein was initially considered a requirement for such cross-recognition. Recent studies on the mechanisms of T-cell activation have shown that, at least for some T-cell clones, antigen recognition is much more "degenerate" than previously appreciated, and that sequence homology is not necessary for cross-reactivity. This article will focus on current advances in basic T-cell receptor immunology that bear on the occurrence of autoimmunity in the CNS.

Evidence for Immunopathogenesis Comes from Different Lines of Research

Various fields of research, such as pathology, epidemiology, immunogenetics, pharmacology, brain imaging, and studies of experimental autoimmune encephalomyelitis (EAE), an animal model resembling MS, have contributed complementary evidence that MS is an immune-mediated disease.[1] The presence of perivenous inflammatory infiltrates of $CD4^+$, $CD8^+$, and γ/δ T-cell receptor$^+$ (TCR^+) T lymphocytes,[4,5] plasma cells,[6] and macrophages[4] suggests that these cell types contribute to myelin damage in MS lesions. The association with certain alleles of MHC class II genes (ie, DR15 Dw2 and DQw6 in whites,[7] DR2 and DR6 in Japanese,[8] and DR4 in Sardinians[9]) suggests a role of immunogenetic background in MS susceptibility, and was recently supported by two of three large-scale genome screenings.[10–12] This suggests a possible role of major histocompatibility locus (MHC) class II–dependent T-cell responses in the pathogenesis of MS. Immune system involvement is also suggested by the clinical and biological response to immunomodulatory and immunosuppressive treatments[13–15] as well as worsening of the disease by interferon-γ.[16] Although the combined evidence points to an immunological basis for MS, the origin of autoreactivity has remained uncertain. The molecular mimicry hypothesis, an important conceptual framework for how autoreactivity could be initiated, was first tested in experimental allergic encephalomyelitis (EAE), an animal model of

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MS that can resemble MS both clinically and pathologically.[17–19]

EAE, MS, and Molecular Mimicry

Discussing the immunology of MS is difficult without briefly recapitulating the relevant findings that have emerged from EAE research. EAE can be induced in susceptible animals by active immunization with brain homogenate, myelin antigens such as MBP or proteolipid protein (PLP) or peptides derived from these antigens. Because EAE resembles MS and is induced by $CD4^+$ T cells, it has stimulated research efforts to clarify the role of $CD4^+$ T cells in MS. This disease model has provided insight into the pathogenic "steps" that may be relevant to MS, including (1) genetic susceptibility, (2) priming and activation of myelin-specific T cells, (3) interaction of autoreactive T cells with endothelium and migration into the CNS, and (4) recognition of myelin antigens and initiation of inflammatory or demyelinating damage (Fig 1). Autoreactive T cells have been shown to be part of the mature immune repertoire of healthy, nonimmunized animals.[20] Genetic control of the frequency and function of such T cells in different animal strains may play an important role in disease susceptibility.[21] For potential autoreactivity to become overt autoimmunity, however, myelin-reactive T cells must be activated by immunization with myelin antigens or strong unspecific stimuli such as bacterial superantigens. The molecular mimicry hypothesis of autoimmunity proposes that cross-reactive foreign antigens can activate autoimmune T cells, and subsequently mediate pathological and clinical damage (Fig 2). Once activated in the periphery, autoreactive T cells can cross the blood–brain barrier (BBB),[22] infiltrate the CNS, recognize myelin antigens, and damage oligodendrocytes and the myelin sheath by various effector mechanisms.

With the exception of active immunization, similar pathogenic steps have been proposed for MS. Indeed, the presence of MBP- and PLP-reactive T cells has been extensively documented in the mature repertoire of both MS patients and healthy controls.[23–27] Based on epidemiological data linking viral infections to MS exacerbations[28,29] and possibly also to the cause of the disease,[30] viral antigens are attractive candidates for initiating autoimmune mechanisms through molecular mimicry.[31] Although it is as yet unknown how exactly a virus initiates autoimmunity, the molecular mimicry hypothesis provides an elegant conceptual framework for how autoreactivity may be triggered and will therefore be discussed herein.

Antigen Recognition by T Cells

One cannot understand molecular mimicry without describing the mechanisms underlying antigen recognition by T lymphocytes. Unlike antibodies, which react with complex protein or polysaccharide structures in particulate form or in solution, the TCR recognizes short peptide fragments derived from larger proteins in the context of self-MHC.[32] T lymphocytes respond to short peptides generated by intracellular proteolytic degradation of antigenic proteins by antigen-presenting cells (APCs). Ten- to 16-amino acid–long peptides are loaded onto MHC molecules and transported to the surface of APCs where they can be recognized as a complex by specific $CD4^+$ T cells (class II MHC-

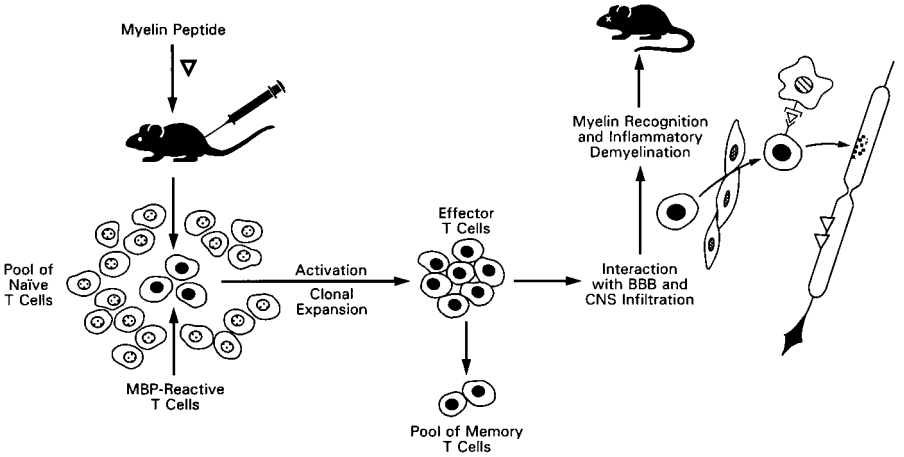


Fig 1. The experimental autoimmune encephalomyelitis animal model of multiple sclerosis. In susceptible animal strains, the injection of myelin antigens in the presence of adjuvant substances leads to priming of naive myelin-reactive T cells, which undergo clonal expansion and acquire effector functions. A portion of this population becomes part of the memory cell pool, which has lower activation requirements for subsequent antigenic challenge. Activated T cells express adhesion molecules that mediate interaction with cerebral endothelium and entry into the central nervous system (CNS) across the blood–brain barrier (BBB). Recognition of myelin antigens in the CNS initiates inflammatory damage in the target organ. MBP = myelin basic protein.

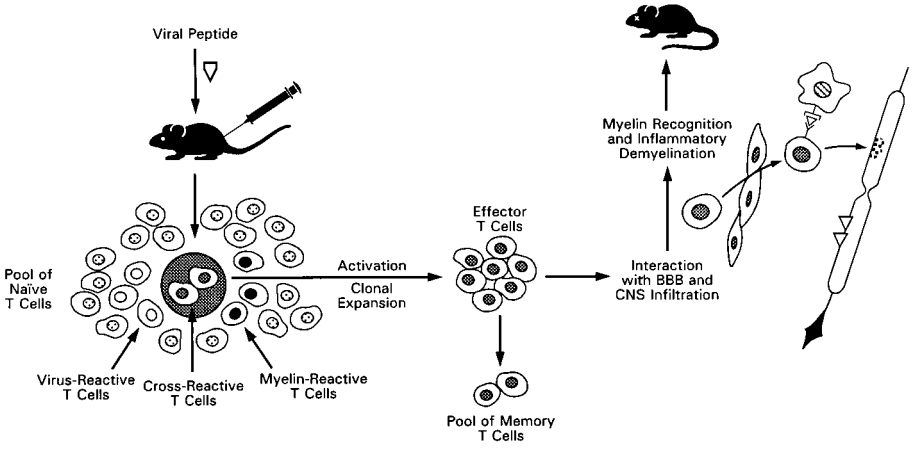


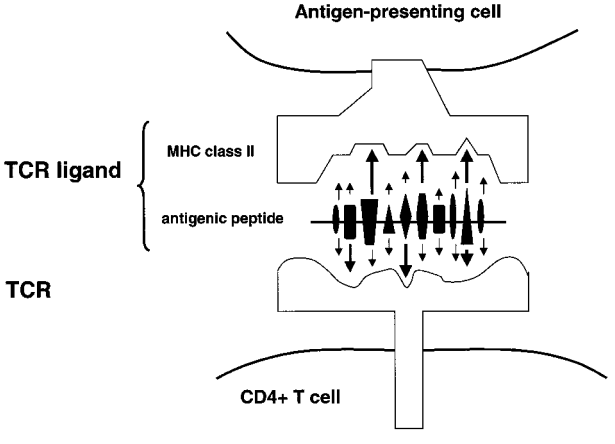
Fig 2. The molecular mimicry hypothesis of autoimmunity. Immunization with a viral ("mimic") peptide activates cross-reactive T cells that will also recognize a myelin antigen. After activation, clonal expansion, and passage of the blood-brain barrier (BBB), the T cells recognize the myelin antigen and initiate autoimmune inflammation. Part of the expanded, cross-reactive T cells will become part of the memory cell pool and will be more readily activated by new antigenic challenge. CNS = central nervous system.

restricted antigen recognition)[33] (Fig 3). AFter recognition of antigen/MHC, T cells become activated, undergo clonal expansion, and acquire effector functions such as cytokine production and cytotoxicity.[34] The production of cytokines such as interferon-γ and tumor necrosis factor-α and β by a subset of $CD4^+$ T cells (T-helper type 1 cells) may be important in mediating part of the immunological damage in MS.[35] After clonal expansion, subpopulations of the expanded cell clones enter the pool of circulating memory T cells, whose requirements for subsequent activation by antigenic challenge appear to be lower than those of naive T cells.[36]

Evolution of the Concept of Molecular Mimicry

Does molecular mimicry play a role in activating autoreactive T cells? Fujinami and Oldstone[2] observed that rabbits immunized with a hepatitis B polymerase peptide (ICG**YGSLPQ**E; one-letter code) that shared six amino acids with the sequence of MBP (TTH**YGSLPQ**K) developed an antibody response to MBP and, in some cases, CNS lesions reminiscent of EAE. Quite recently, Gautam and colleagues[37] induced clinical EAE by immunizing susceptible mice with a herpesvirus saimiri peptide (**AA**Q**RRPS**RPFA) that has five amino acids of discontinuous sequence homology to MBP (1–11 peptide, **AS**Q**K**R**PS**QRHG). Studies conducted in other animal models of autoimmune diseases such as adjuvant arthritis confirmed that mimicry of host antigens by infectious agents could lead to the development of disease by inducing cellular as well as humoral autoimmune responses.[38,39]

Fig 3. The trimolecular complex of T-cell antigen recognition. The antigenic peptide and the major histocompatibility locus (MHC) class II molecule on the surface of the antigen-presenting cells form the T-cell receptor (TCR) ligand. For a given trimolecular complex, certain amino acid side chains in the peptide sequence preferentially contribute to MHC binding (upward arrows = *MHC contacts*), *to TCR recognition* (downward arrows = *TCR contacts*), *or to both.*



What is the molecular basis of mimicry and what extent of sequence homology is required? The TCR, the antigenic peptide, and the MHC molecule form the trimolecular complex of T-cell antigen recognition (see Fig 3). Antigenic peptide and MHC molecule form the ligand that is recognized by antigen-specific T cells via their TCR. "Pockets" in the MHC peptide–binding groove preferentially "anchor" amino acids with certain chemical properties in specific positions of the antigenic peptides. Outside of the pockets, amino acid side chains that do not fit the MHC groove may have a strong negative influence and thus hinder binding.[33,40] When peptide–MHC complexes are formed based on such "MHC-binding motifs" and exposed on the surface of APCs, both components will be recognized by a specific TCR (see Fig 3). Certain amino acid positions in the peptide sequence are more critical than others for the interaction with the TCR. Allen and co-workers proposed that one amino acid residue in the antigenic peptide sequence is strictly required (primary TCR contact), so that even a conservative amino acid substitution at this position will abolish recognition. Amino acids in other TCR contact positions (secondary TCR contacts) modulate the interaction and can be substituted with amino acids that are

similar in charge, polarity, or size.[41,42] Thus, the antigenic peptide interacts with both MHC and TCR, and certain amino acids may be more important for contact in either direction. The MHC–TCR interface (ie, contacts formed between TCR and MHC directly rather than between TCR and peptide) is also crucial. In fact, the outcome of thymic selection of the mature T-cell repertoire is strongly influenced by the affinity of TCRs for self-MHC displayed on thymic epithelium.[43] This interaction is also relevant to the occurrence of autoimmunity, as will be discussed later.

Based on this theoretical background, amino acid residues critical for binding to the MS-associated class II molecules DR2 and DQ1 (MHC contacts), as well as for recognition by specific TCR (TCR contacts), have been defined for the MBP peptide preferentially recognized in the context of DR2.[44,45] Molecular mimicry motifs that would satisfy both MHC binding and recognition by specific TCR were used by Wucherpfennig and Strominger[46] to identify microbial peptides that were effective in activating three MBP-specific T-cell clones (TCCs) derived from MS patients (Table 1). Thus, experimental data confirmed the theoretical prediction that sequence homology was not required for cross-recognition of self- and foreign antigens. Indeed, only one of the stimulatory peptides could have been identified as a molecular mimic by sequence alignment, as opposed to structural criteria.

A further development of the molecular mimicry model originated from studies conducted in our laboratory to systematically dissect T-cell recognition of the above-mentioned immunodominant peptide MBP (83–99). Single amino acid substitutions in each position of the sequence were used to analyze the response to altered peptide ligands derived from MBP (83–99) that bind MHC with approximately the same strength. As expected, some substitutions were tolerated (they did not change the functional response of the clone) whereas others caused a reduction or abolition of the response.[47] A new and intriguing finding was that certain amino acid substitutions were not only tolerated, but actually generated superagonist peptides that were even more potent stimulators of TCC functions (proliferation, cytokine production, cytotoxicity, and early TCR-signaling events) than the native MBP peptide.[48] Thus, at least for some autoreactive TCCs, the immunodominant MBP peptide used to grow and expand the TCCs was not the optimal ligand. When multiple amino acid substitutions were introduced in the antigenic peptide, their combined effect on T-cell antigen recognition was largely predictable by the additive effect (ie, positive or negative) of single amino acid modifications. The effect of "negative" substitutions in virtually any position of the peptide sequence (those leading to a reduced functional response) could be compensated by "positive" substitutions in other positions (leading to more potent responses).[49,50] No amino acid seemed to be strictly necessary for antigen recognition, but rather to independently contribute to its recognition by the TCR. Based on these findings, it was even possible to design peptides that differed in all positions from the native antigenic sequence, and were still able to stimulate the TCCs.[49] These observations offer a new perspective on the concept of molecular mimicry; ie, sequence homology may not be required at all for cross-recognition. Indeed, all amino acid in corresponding positions of the peptides can differ (see Table 1).

Table 1. Evolution of the Concept of Molecular Mimicry: Extent of Sequence Homology Between Cross-Reactive Self-Peptides and Foreign or Synthetic Peptides

Antigens	Cross-Reacting Peptides	References
MBP (66–75)	TTH**YGSLPQ**K	2
HBV-P (589–598)	ICG**YGSLPQ**E	
MBP (83–97)	ENP**V**V**HF**F**K**NI**V**TPR	46
EBV-P (627–641)	TGG**V**Y**HF**V**K**KH**V**HES	
MBP (83–97)	ENPV**VHF**FKNIVTPR	46
HSV-T (153–167)	FRQL**VHF**VRDFAQLL	
MBP (87–99)	VHFFKNIVTPRTP	49
Predicted peptide	GGLLAHVISAKKA	
MBP (89–98)	FFKNIVTPRT	49
Predicted peptide	WYALLPSCKG	

Amino acid sequences of cross-reacting peptides are reported by using the one-letter code. Sequence homology, ie, identical amino acids in corresponding positions of cross-reacting peptides, are shown in boldfaced underlined characters.

MBP = myelin basic protein; HBV-P = hepatitis B virus polymerase; EBV-P = Epstein-Barr virus DNA polymerase; HSV-T = herpes simplex virus terminase.

Extensive Dissection of TCR Antigen Recognition by Synthetic Peptide Combinatorial Libraries

The use of synthetic peptide combinatorial libraries[51,52] has provided a novel, unbiased tool to further investigate the degeneracy of TCR antigen interactions. Synthetic peptide combinatorial libraries are highly complex mixtures of peptides in which the 20 natural amino acids occur in completely randomized order at each position in the sequence. For a given peptide length, they represent the complete set of peptides that can theoretically be built with the 20 naturally occurring amino acids (except cysteine to avoid secondary structures). For example, a completely randomized decapeptide library is made of 19^{10} different individual peptides. For the purpose of analyzing T-cell antigen recognition, the positional scanning approach was chosen.[51,53] Sets of "sublibraries" were used that are completely randomized, except for one defined position in the sequence. In the example of a decamer library, 200 sublibraries (20 amino acids $\times$ 10 positions in the se-

quence) are synthesized, each with only one fixed amino acid, whereas all other positions are completely randomized. If a clone preferentially responds to a complex mixture of peptides with one amino acid fixed in a defined position, that amino acid is considered optimal in that position of a hypothetical agonist peptide. By using the positional scanning approach, we defined the spectrum of stimulatory ligands for several autoreactive, MBP-specific TCCs and searched for cross-reactive, high-potency ligands.[54] For each position of the antigenic peptides, one or more optimal amino acids were defined and used to synthesize a series of high-potency ligands. These were found to be effective agonists at concentrations up to 5 orders of magnitude lower that the immunodominant MBP peptide. In addition, real protein sequences derived from both self- and foreign antigens were identified by database searches based on synthetic peptide combinatorial library predictions. Again, such peptides were synthesized and proved to be effective agonist ligands for the TCCs, in some cases even at lower concentrations than the MBP peptide.[53,54]

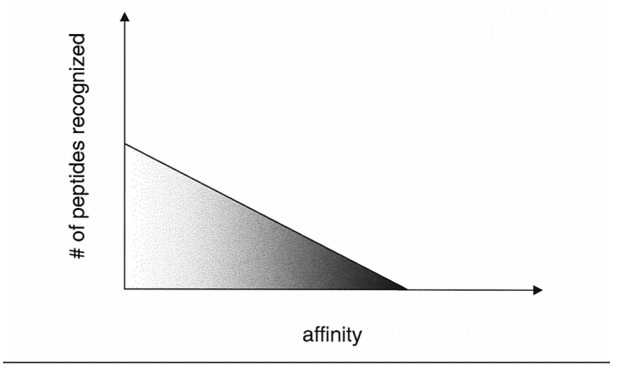
By extending the observations made with amino acid substitutions to their theoretical limits, the combinatorial chemistry approach supports a previously unrecognized level of TCR cross-reactivity.[53,54] It is noteworthy that this new concept is supported by other lines of experimental evidence (for review, see Mason[55]).

Degenerate TCR Antigen Recognition and T-Cell Development

How does this dissection of T-cell specificities help us to understand the induction of autoimmune diseases? Recognition of antigen by $CD4^+$ T cells is considered a crucial check point for the development of any kind of immune response. This is because of not only the specificity of interaction (a feature that also characterizes B-cell antigen recognition) but also the helper function that $CD4^+$ T cells exert on other cells of the immune system. The concept of T-cell antigen recognition has evolved from high specificity into high flexibility and, at least in some cases, extreme degeneracy.[55] The affinity between TCR and MHC–peptide ligands is crucial to understanding the implications of their interactions. As demonstrated for some autoreactive TCCs, ligands can be found that are several orders of magnitude more potent than those used to expand them in vitro. Experimental data clearly show that for the same TCCs, ligands with intermediate and low TCR affinity also exist.[54] The full spectrum of recognized ligands seems to be characterized by a continuum of affinity, from the highly specific ligands (high TCR affinity, full agonist activity) to the suboptimal ones (low TCR affinity, weak/partial agonists).[54,56–58] A model can be proposed in which for each clone an "affinity hierarchy" of recognition exists whereby a set of ligands defines the recognition potential of the clone and eventually the extent of its functional activation.[57] By assigning to each clone a limited set of optimal ligands (ie, those that will activate it at the lowest concentration) and a more extensive set of suboptimal ligands, this model reconciles the new findings on degenerate antigen recognition with the essential specificity that is necessary for immune function (Fig 4).

Degeneracy of TCR recognition may have relevance for the process of thymic selection, which may set the stage for the occurrence of autoimmune responses by shaping the immune repertoire.[59] The process involves the positive selection of T cells that are able to recognize a pool of self-peptides presented by self-MHC molecules. What appears to be crucial at this stage is the capacity to recognize such MHC–peptide ligands with a degree of affinity that is not extreme. T cells with very high affinity for self-MHC–peptide complexes are deleted by programmed cell death (ie, negative selection), whereas T cells with very low affinity do not expand (death by neglect; Fig 5).[60–63] Positive selection seems to occur at intermediate levels of affinity. The nature of the peptides displayed on thymic epithelial cells and their precise role in mediating the positive selection of the mature T-cell repertoire is currently under intense investigation.[64] Peptides derived from MHC class II molecules and other proteins abundantly available in the endocytic compartment have been eluted from thymic epithelial cells as well as other APCs in different organs.[44,65] It is noteworthy that a variety of self-antigens involved in the pathogenesis of autoimmune disease that were thought to be only expressed extrathymically, including MBP,[66–68] PLP,[67] uveitogenic retinal proteins,[69] and other proteins that are not "sequestered" by blood–organ barriers (thyroglobulin,[68,70] insulin, and glutamic acid decarboxylase[68]), have been shown to be expressed at the mRNA or protein level by thymic epithelial cells. These find-

Fig 4. Affinity hierarchy for recognition of antigens by a T-cell clone. For a given T-cell clone, a limited number of peptides can function as optimal, full agonists (high-affinity interaction). A higher number of weaker agonist peptides is recognized with lower affinity.



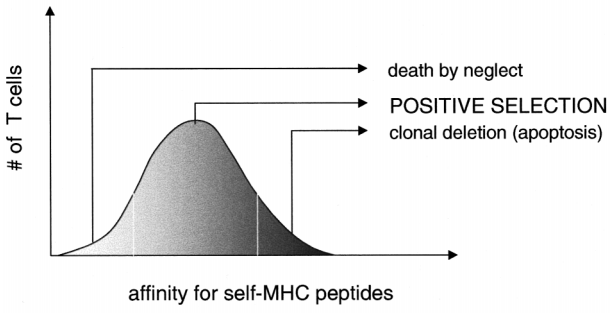


Fig 5. T-cell receptor affinity and thymic selection. Recognition of a pool of self-peptides in the context of self-MHC on the surface of specialized thymic antigen-presenting cells is crucial for selection of mature T lymphocytes. T cells with intermediate levels of affinity for self-MHC–peptides are positively selected and form the mature T-cell repertoire. MHC = major histocompatibility locus.

ings suggest that thymic presentation of peptides derived from these antigens may participate in shaping the mature immune repertoire by both negative and positive selection.[71] In some instances, thymic presentation of autoantigens correlates with resistance to autoimmune disease,[69] suggesting that resistant animal strains effectively establish central tolerance to such antigens. On the other hand, presentation of self-peptides in the thymus may also explain why autoreactive T cells are part of the normal mature immune system. In fact, incomplete clonal deletion may cause the "escape" of autoreactive clones from the thymus.[72,73] Although the importance of individual peptides in positive selection of T cells in the thymus is not yet completely understood,[64] it is clear that degenerate interactions between TCR and MHC–peptide ligands play an important role.[74] Indeed, animals that express a single MHC–peptide ligand select a remarkably diverse T-cell repertoire.[75] Conversely, different self-peptides have the capacity to promote the selection of a single TCR.[64,76] These mechanisms may ensure that the selected repertoire is diverse enough to respond to virtually any foreign antigen encountered in the periphery, but may also impose a risk for reactivity against self.

Another potential role for T-cell cross-reactivity is in the maintenance of the mature T-cell repertoire. If a high number of self-antigenic ligands are recognized with low affinity by mature T-cell clones, these ligands may provide the low degree of stimulation that is required for T-cell survival in the periphery.[77]

T-Cell Cross-Reactivity and Autoimmunity: A New Perspective

Results obtained with the use of peptide combinatorial libraries and other experimental approaches[55] suggest that a certain degree of degeneracy in recognition of antigens by the TCR is a normal feature of the immune system. A very important implication is that the potential for autoreactivity is very high, and any concept about autoimmunity must explain not only how autoreactivity is initiated, but also why it is rare. A model for how cross-recognition of foreign and self-peptides could initiate autoimmune responses is proposed, based on two fundamental requirements, the activation of cross-reactive T cells in the periphery, and the recognition of myelin antigens in the CNS. It is clear from the above considerations as well as from data obtained in other autoimmune diseases[78] that autoreactive T cells will always be part of the normal mature repertoire. Based on our current understanding of TCR antigen recognition, a subset of such cells is expected, for statistical reasons, to be cross-reactive to foreign antigens. In the course of infections, viral antigens may often activate cross-reactive T cells in the periphery. A fraction of such T cells may then cross the BBB and enter the CNS parenchyma.[22] If the viral peptide is a more potent agonist than the myelin antigen peptide (Fig 6), several other factors may be required to facilitate recognition of a myelin antigen that would normally be "seen" with lower affinity (Table 2). T-cell factors may include the increased expression of adhesion molecules and coreceptors that characterize

Fig 6. Activation of a cross-reactive T cell by a foreign antigen. A viral peptide is recognized in the periphery by a T cell that becomes activated. After migration into the central nervous system (CNS), the T cell encounters a peptide derived from myelin turnover and initiates inflammatory autoimmune damage. The affinity hierarchy for recognition of peptides by the cross-reactive T cell is represented in the lower panel. If the myelin peptide has lower stimulatory potency (lower affinity for the T-cell receptor) than the viral peptide, facilitating factors, such as local inflammation, are required to activate the cross-reactive T cell. APC = antigen-presenting cells.

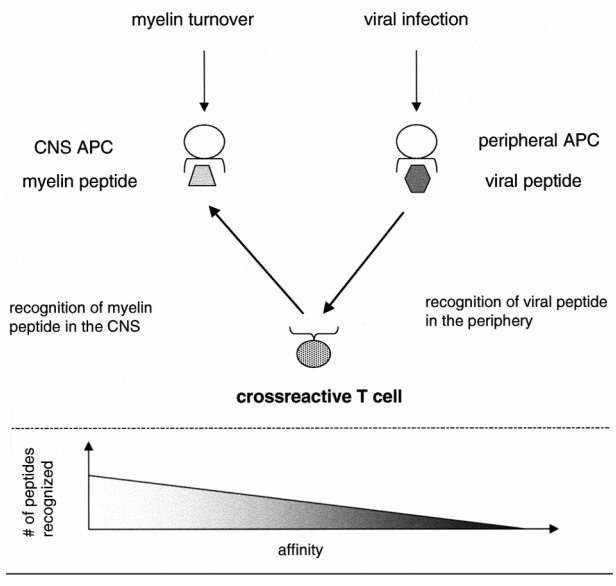


Table 2. Critical Factors in the Activation of Autoreactive T Cells in the CNS

	References
Effector/memory phenotype of infiltrating T cells	36, 79, 89
Up-regulation of adhesion molecules and coreceptors on T cells	90
Up-regulation of MHC and costimulatory molecules on CNS APCs and endothelium	91
Expression of viral antigens in the CNS	83
Release of self-antigens after initiation of tissue damage	92, 93

CNS = central nervous system; MHC = major histocompatibility locus; APCs = antigen-presenting cells.

the memory/effector phenotype.[79] In addition, up-regulation of MHC and costimulatory molecules on APCs in the CNS will increase the avidity of interaction with T cells.[80,81] It is interesting that the viral infection itself may cause local inflammation that will facilitate autoreactivity in the target organ. This has clearly been shown in a murine model of autoimmune diabetes, in which "bystander activation" of transgenic, β-islet antigen-specific T cells by local infection with coxsackievirus was sufficient to initiate pancreatic damage.[82] Although the viral infection is eventually cleared, the local release of self-antigen may lead to a self-perpetuating chronic inflammation.[83]

Another possibility is that peripheral activation of T cells by viral peptides may lead to cross-recognition of myelin antigens with the same or even higher affinity. In the scenario of highly degenerate TCR cross-recognition, this is indeed a possible event.[54,55] However, we favor the view that it may occur less frequently in vivo. In fact, consistent with the negative selection of the high-affinity autoreactive T-cell repertoire,[84] myelin-specific TCCs generated from both MS patients and healthy subjects are usually characterized by a relatively low affinity for their myelin peptide ligands (Martin and colleagues, unpublished data). Taken together, these considerations suggest that the concurrent effect of several factors may be required for "physiological cross-recognition" to become "dangerous mimicry" and frank autoimmunity. It is the need for several events to occur simultaneously that may help explain why autoimmune diseases are relatively rare. Compared with other target organs, the CNS may be protected from immune-mediated damage by the high selectivity of the BBB and the very low expression of MHC molecules.[85] Elegant studies of transgenic expression of viral peptides in the β-islet cell of the pancreas (viral peptides expressed as self) have shown that infection with the same virus is required to initiate autoimmunity even if most peripheral T cells are specific for "viral self" peptides.[86,87] When the same viral antigens were expressed as transgenes on oligodendrocytes, tissue damage induced by viral infection was less severe than in the pancreas, and only a second infection caused demyelination and obvious motor deficits. This elegant work also showed that the exacerbating effect of the second infection could be caused by an unrelated virus, a situation reminiscent of MS, where different viruses may play a role in disease exacerbations, but no single agent has been consistently associated with the disease.[88]

In summary, the data discussed in this article suggest that although the conceptual framework of molecular mimicry remains a valid hypothesis for the occurrence of autoimmunity, the requirements for cross-reactivity are more flexible than previously appreciated. New powerful tools are available for the study of these interactions. The application of these methods to T cells isolated from the CNS compartment will be important to obtain more direct evidence for a role of molecular mimicry in the induction of CNS autoimmunity in MS.

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References

1. Martin R, McFarland HF, McFarlin DE. Immunological aspects of demyelinating diseases. Annu Rev Immunol 1992;10:153–187
2. Fujinami RS, Oldstone MBA. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science 1985;230:1043–1045
3. Oldstone MBA. Molecular mimicry and autoimmune disease. Cell 1987;50:819–820
4. Traugott U, Reinherz EL, Raine CS. Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. J Neuroimmunol 1983;4:201–221
5. Hvas J, Oksenberg JR, Fernando R, et al. Gamma delta T cell receptor repertoire in brain lesions of patients with multiple sclerosis. J Neuroimmunol 1993;46:225–234
6. Esiri MM. Multiple sclerosis: a quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropathol Appl Neurobiol 1980;6:9–21
7. Vartdal F, Sollid LM, Vandvik B, et al. Patients with multiple sclerosis carry DQB1 genes which encode shared polymorphic aminoacid sequences. Hum Immunol 1989;25:103–110
8. Naito S, Kuroiwa Y, Itoyama T, et al. HLA and Japanese MS. Tissue Antigens 1978;12:19–24
9. Marrosu HG, Muntoni F, Murru MR, et al. Sardinian multiple sclerosis is associated with HLA-DR4: a serological and molecular analysis. Neurology 1988;38:1749–1753
10. Sawcer S, Jones HB, Feakes R, et al. A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. Nat Genet 1996;13:464–468
11. Haines JL, Ter-Minassian M, Bazyk A, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The Multiple Sclerosis Genetics Group. Nat Genet 1996;13:469–471

12. Ebers GC, Kukay K, Bulman DE, et al. A full genome search in multiple sclerosis. Nat Genet 1996;13:472–476
13. Paty DW, Li DKB, UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:662–667
14. The Interferon-Beta Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology 1993;43:655–661
15. Weiner HL, Mackin GA, Orav EJ, the NCMST. Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: final report of the Northeast Cooperative Multiple Sclerosis Treatment Group. Neurology 1993;43:910–918
16. Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology 1987; 37:1097–1102
17. Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathol 1996;6:259–274
18. Raine CS. The neuropathology of multiple sclerosis. In: Raine CS, McFarland HF, Tourtellotte WW, eds. Multiple sclerosis: clinical and pathogenetic basis. London, Chapman and Hall, 1997:151–171
19. Raine CS. The lesion in multiple sclerosis and chronic relapsing experimental allergic encephalomyelitis: a structural comparison. In: Raine CS, McFarland HF, Tourtellotte WW, eds. Multiple sclerosis: clinical and pathogenetic basis. London, Chapman and Hall, 1997:243–286
20. Schlüsener H, Wekerle H. Autoaggressive T lymphocyte lines recognize the encephalitogenic region of myelin basic protein; in vitro selection from unprimed rat T lymphocyte populations. J Immunol 1985;135:3128–3133
21. Fritz RB, Skeen MJ, Jen-Chou CH, et al. Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. J Immunol 1985;134:2328–2332
22. Wekerle H, Linington C, Lassmann H, Meyermann R. Cellular immune reactivity within the CNS. Trends Neurosci 1986;9: 271–277
23. Burns J, Rosenzweig A, Zweiman B, Lisak RP. Isolation of myelin basic protein-reactive T-cell lines from normal human blood. Cell Immunol 1983;81:435–440
24. Martin R, Jaraquemada D, Flerlage M, et al. Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. J Immunol 1990;145:540–548
25. Pette M, Fujita K, Kitze B, et al. Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. Neurology 1990;40:1770–1776
26. Zhang J, Markovic-Plese S, Lacet B, et al. Increased frequency of interleukin-2-responsive T cells specific for myelin basic protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994;179:973–984
27. Ota K, Matsui M, Milford EL, et al. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 1990;346:183–187
28. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet 1985;1:1313–1315
29. Panitch HS. Influence of infection on exacerbations of multiple sclerosis. Ann Neurol 1994;36(Suppl):S25–28
30. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev 1993;6:382–427
31. Talbot PJ, Paquette J-S, Ciurli C, et al. Myelin basic protein and human coronavirus 229E cross-reactive T cells in multiple sclerosis. Ann Neurol 1996;39:233–240
32. Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature 1974;248:701–702
33. Germain RN. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell 1994;76:287–299
34. Croft M. Activation of naive, memory and effector T cells. Curr Opin Immunol 1994;6:431–437
35. Olsson T. Cytokine-producing cells in experimental autoimmune encephalomyelitis and multiple sclerosis. Neurology 1995; 45(Suppl 6):S11–S15
36. Dubey C, Croft M, Swain SL. Naive and effector CD4 T cells differ in their requirements for T cell receptor versus costimulatory signals. J Immunol 1996;157:3280–3289
37. Gautam AM, Liblau R, Chelvanayagam G, et al. A viral peptide with limited homology to a self peptide can induce clinical signs of experimental autoimmune encephalomyelitis. J Immunol 1998;161:60–64
38. van Eden W, Hogervorst EJ, Hensen EJ, et al. A cartilage-mimicking T-cell epitope on a 65K mycobacterial heat-shock protein: adjuvant arthritis as a model for human rheumatoid arthritis. Curr Top Microbiol Immunol 1989;145:27–43
39. Davies JM. Molecular mimicry: can epitope mimicry induce autoimmune disease? Immunol Cell Biol 1997;75:113–126
40. Hammer J, Valsasnini P, Tolba K, et al. Promiscuous and allele-specific anchors in HLA-DR binding peptides. Cell 1993; 74:197–203
41. Evavold BD, Sloan-Lancaster J, Hsu BL, Allen PM. Separation of T helper 1 clone cytolysis from proliferation and lymphokine production using analog peptides. J Immunol 1993;150:3131–3140
42. Kersh GJ, Allen PM. Structural basis for T cell recognition of altered peptide ligands: a single T cell receptor can productively recognize a large continuum of related ligands. J Exp Med 1996;184:1259–1268
43. von Boehmer H, Kisielow P. Self-nonself discrimination by T cells. Science 1990;248:1369–1373
44. Vogt AB, Kropshofer H, Kalbacher H, et al. Ligand motifs of HLA-DRB5*0101 and DRB1*1501 molecules delineated from self-peptides. J Immunol 1994;153:1665–1673
45. Wucherpfennig KW, Sette A, Southwood S, et al. Structural requirements for binding of an immunodominant myelin basic protein peptide to DR2 isotypes and for its recognition by human T cell clones. J Exp Med 1994;179:279–290
46. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995;80:695–705
47. Vergelli M, Hemmer B, Utz U, et al. Differential activation of human autoreactive T cell clones by altered peptide ligands derived from myelin basic protein peptide (87–99). Eur J Immunol 1996;26:2624–2634
48. Vergelli M, Hemmer B, Kalbus M, et al. Modifications of peptide ligands enhancing T cell responsiveness imply large numbers of stimulatory ligands for autoreactive T cells. J Immunol 1997;158:3746–3752
49. Hemmer B, Vergelli M, Gran B, et al. Predictable TCR antigen recognition based on peptide scans leads to the identification of agonist ligands with no sequence homology. J Immunol 1998; 160:3631–3636
50. Ausubel LJ, Kwan CK, Sette A, et al. Complementary mutations in an antigenic peptide allow for crossreactivity of autoreactive T-cell clones. Proc Natl Acad Sci USA 1996;93:15317–15322
51. Houghten RA, Pinilla C, Blondelle SE, et al. Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. Nature 1991;354:84–86

52. Udaka K, Wiesmüller K-H, Kienle S, et al. Decrypting the structure of major histocompatibility complex class I-restricted cytotoxic T lymphocyte epitopes with complex peptide libraries. J Exp Med 1995;181:2097–2108
53. Hemmer B, Vergelli M, Pinilla C, et al. Probing degeneracy in T-cell recognition using combinatorial peptide libraries. Immunol Today 1998;19:163–168
54. Hemmer B, Fleckenstein B, Vergelli M, et al. Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. J Exp Med 1997;185:1651–1659
55. Mason D. A very high level of crossreactivity is an essential feature of the T-cell receptor. Immunol Today 1998;19:395–404
56. Evavold BD, Allen PM. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. Science 1991;252:1308–1310
57. Hemmer B, Stefanova I, Vergelli M, et al. Relationships among TCR ligand potency, thresholds for effector function elicitation, and the quality of early signaling events in human T cells. J Immunol 1998;160:5807–5814
58. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. Annu Rev Immunol 1996;14:1–27
59. Wekerle H, Bradl M, Linington C, et al. The shaping of the brain-specific T lymphocyte repertoire in the thymus. Immunol Rev 1996;149:231–243
60. Nossal GJ. Negative selection of lymphocytes. Cell 1994;76:229–239
61. von Boehmer H. Positive selection of lymphocytes. Cell 1994;76:219–228
62. Ashton-Rickardt PG, Bandeira A, Delaney JR, et al. Evidence for a differential avidity model of T cell selection in the thymus. Cell 1994;76:651–663
63. Ashton-Rickardt PG, Tonegawa S. A differential-avidity model for T-cell selection. Immunol Today 1994;15:362–366
64. Jameson SC, Bevan MJ. T-cell selection. Curr Opin Immunol 1998;10:214–219
65. Rudensky AY. Endogenous peptides associated with MHC class II and selection of CD4 T cells. Semin Immunol 1995;7:399–409
66. Fritz RB, Zhao M-L. Thymic expression of myelin basic protein (MBP). Activation of MBP-specific T cells by thymic cells in the absence of exogenous MBP. J Immunol 1996;157:5249–5253
67. Mor F, Boccaccio GL, Unger T. Expression of autoimmune disease-related antigens by cells of the immune system. J Neurosci Res 1998;54:254–262
68. Sospedra M, Ferrer-Francesch X, Dominguez O, et al. Transcription of a broad range of self-antigens in human thymus suggests a role for central mechanisms in tolerance toward peripheral antigens. J Immunol 1998;161:5918–5929
69. Egwuagu CE, Charukamnoetkanok P, Gery I. Thymic expression of autoantigens correlates with resistance to autoimmune disease. J Immunol 1997;159:3109–3112
70. Heath VL, Moore NC, Parnell SM, Mason DW. Intrathymic expression of genes involved in organ specific autoimmune disease. J Autoimmun 1998;11:309–318
71. Heath VL, Saoudi A, Seddon BP, et al. The role of the thymus in the control of autoimmunity. J Autoimmun 1996;9:241–246
72. Fairchild PJ, Wildgoose R, Atherton E, et al. An autoantigenic T cell epitope forms unstable complexes with class II MHC: a novel route for escape from tolerance induction. Int Immunol 1993;5:1151–1158
73. Zal T, Volkmann A, Stockinger B. Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. J Exp Med 1994;180:2089–2099
74. Bevan MJ. In thymic selection, peptide diversity gives and takes away. Immunity 1997;7:175–178
75. Ignatowicz L, Kappler J, Marrack P. The repertoire of T cells shaped by a single MHC/peptide ligand. Cell 1996;84:521–529
76. Hu Q, Bazemore Walker CR, Girao C, et al. Specific recognition of thymic self-peptides induces the positive selection of cytotoxic T lymphocytes. Immunity 1997;7:221–231
77. Kirberg J, Berns A, von Boehmer H. Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex-encoded molecules. J Exp Med 1997;186:1269–1275
78. Lohse AW, Dinkelmann M, Kimmig M, et al. Estimation of the frequency of self-reactive T cells in health and inflammatory diseases by limiting dilution analysis and single cell cloning. J Autoimmun 1996;9:667–675
79. Dutton RW, Bradley LM, Swain SL. T cell memory. Annu Rev Immunol 1998;16:201–223
80. Traugott U, Scheinberg LC, Raine CS. On the presence of Ia-positive endothelial cells and astrocytes in multiple sclerosis lesions and its relevance to antigen presentation. J Neuroimmunol 1985;8:1–14
81. De Simone R, Giampaolo A, Giometto B, et al. The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. J Neuropathol Exp Neurol 1995;54:175–187
82. Horwitz MS, Bradley LM, Harbertson J, et al. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. Nat Med 1998;4:781–785
83. Evans CF, Horwitz MS, Hobbs MV, Oldstone MBA. Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. J Exp Med 1996;184:2371–2384
84. Targoni OS, Lehmann PV. Endogenous myelin basic protein inactivates the high avidity T cell repertoire. J Exp Med 1998;187:2055–2063
85. Lee SC, Raine CS. Multiple sclerosis: oligodendrocytes in active lesions do not express class II major histocompatibility complex molecules. J Neuroimmunol 1989;25:261–266
86. Ohashi PS, Oehen S, Buerki K, et al. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991;65:305–317
87. Oldstone MBA, Nerenberg M, Southern P, et al. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 1991;65:319–331
88. Johnson RT. The virology of demyelinating diseases. Ann Neurol 1994;36(Suppl):S54–S60
89. Sobel RA, Hafler DA, Castro EE, et al. The 2H4 (CD45R) antigen is selectively decreased in multiple sclerosis lesions. J Immunol 1988;140:2210–2214
90. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 1993;11:767–804
91. Cannella B, Cross AH, Raine CS. Adhesion-related molecules in the central nervous system. Upregulation correlates with inflammatory cell influx during relapsing experimental autoimmune encephalomyelitis. Lab Invest 1991;65:23–31
92. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature 1992;358:155–157
93. Vanderlugt CJ, Miller SD. Epitope spreading. Curr Opin Immunol 1996;8:831–836