



The initiation of the autoimmune response in multiple sclerosis

Silva Markovic-Plese^{a,*}, Clemencia Pinilla^b, Roland Martin^c

^a Department of Neurology, University of North Carolina, 6019 Neuroscience Research Building, Chapel Hill, NC 27599, USA

^b Torrey Pines Institute for Molecular Studies and Mixture Sciences, San Diego, CA, USA

^c Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes for Health, Bethesda, MD, USA

Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Most evidence supports the autoimmune pathogenesis of the disease. According to this hypothesis, the activation of autoreactive T-cells is a central event in the development of autoimmune response in MS. We examined molecular events involved in the initiation of autoimmune response in MS. Recent studies in our laboratory have reported an unexpectedly high degree of T-cell receptor (TCR) degeneracy and molecular mimicry as a frequent phenomenon that might play a role in the initiation of autoimmune response in MS. This paper provides insights into the physiologic and pathologic role of autoreactive T-cells, and characterizes structurally and functionally the specific targets for new therapies of MS.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Multiple sclerosis; Autoimmune disease; Autoreactive T-cell activation; T-cell receptor degeneracy; Costimulatory pathways

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) affecting primarily young adults in their most productive age. While its etiology remains elusive, most evidence supports an autoimmune pathogenesis. In this paper, we will review molecular events involved in the activation of autoreactive T-cells, an initial event in the development of the autoimmune disease process in MS. Recent studies suggest that autoreactive CD4+ cells undergo facilitated activation, which leads to breakdown of immunologic self-tolerance and the subsequent chronic autoimmune response. This activation is induced by two independent, yet possibly interconnected mechanisms concerning autoreactive cell specificity/degeneracy and costimulatory requirements for their activation. T-cell receptors (TCR) on autoreactive cells have a particularly high degree of flexibility/degeneracy, allowing T-cell activation in the peripheral circulation by mechanisms of molecular mimicry. Secondly, recent studies demonstrate that myelin-reactive CD4+ cells undergo activation in a costimulation-independent manner, suggesting a lower threshold for T-cell activation in the peripheral circulation

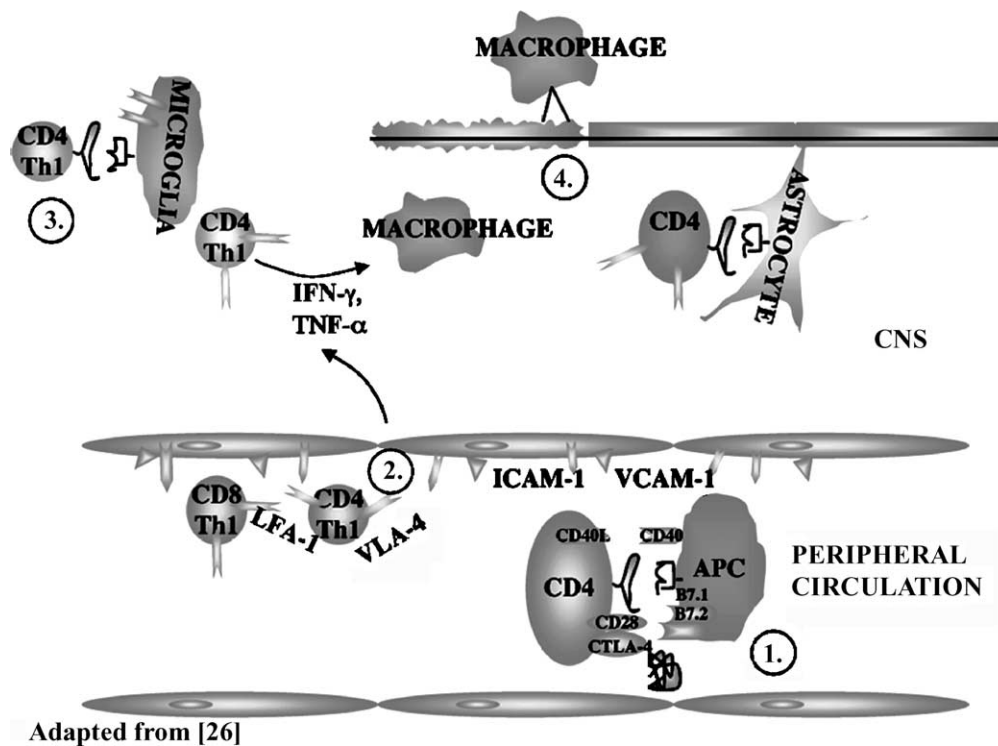
and within the CNS. Studies discussed in this article provide insights into pathogenetic role of autoreactive T-cells, and characterize, structurally and functionally, specific targets for new therapies of MS.

2. Activation of autoreactive T-cells

Current studies support the critical role of CD4+ myelin-specific cells in the initiation of autoimmune responses in MS [1]. However, myelin-reactive cells are part of the normal T-cell repertoire, and are detected at comparable frequencies in the peripheral blood of both MS patients and normal controls [2]. Thus, their presence is not sufficient to trigger pathological autoimmune response. Rather, it is the frequency of activated myelin-reactive cells that is increased in MS patients in comparison to healthy individuals [3]. Peripherally activated autoreactive CD4+ lymphocytes cross the blood brain barrier and initiate chronic inflammatory response in the CNS, as documented in the experimental autoimmune encephalomyelitis (EAE), an animal model of MS [4]. It is therefore important to understand the factors that contribute to the activation of myelin-reactive T-cells and the degree of difference in TCR specificity and costimulatory requirements between MS patients and healthy controls. Fig. 1 illustrates the current understanding of the pathogenesis of MS.

* Corresponding author. Tel.: +1-919-966-3701; fax: +1-919-843-4576.

E-mail address: markovics@neurology.unc.edu (S. Markovic-Plese).



Adapted from [26]

Fig. 1. Immunopathogenesis of MS: (1) myelin-specific T-cells are activated in the peripheral circulation; (2) activated T-cells interact with adhesion molecules on endothelial cells, and migrate through the blood brain barrier into the CNS; (3) CD4+ cells recognize myelin antigens and initiate chronic inflammatory process within the CNS. Antigens are presented by microglia and astrocytes to myelin-specific memory T-cells, and local tissue damage is perpetuated; (4) myelin is destroyed by macrophages, cytokines, oxygen and nitrogen radicals produced by local inflammation.

3. T-cell degeneracy

Over the past ten years, several groups have reported that TCR specificity is not as stringent as initially believed [5]. TCR specificity was systematically tested by serial amino acid (a.a.) substitutions in the immunodominant peptide of the myelin basic protein (MBP) 87–99, a presumed autoantigen in MS. Multiple substitutions were tolerated, indicating an unexpectedly high degree of TCR degeneracy [6]. Furthermore, it has been demonstrated that particular a.a. substitutions can increase TCR response, arguing that myelin-derived epitopes are frequently not the optimal ligands for T-cell clones. Finally, stimulatory peptides with no sequence homology were designed and their stimulatory effect predicted based on the additive effect of each a.a. substitution [7]. These results reflect a relatively independent contribution of each a.a. in the peptide and demonstrate that TCR recognition is highly degenerate, i.e. that one T-cell receptor can recognize many peptides in the context of the autologous major histocompatibility complex (MHC).

4. Physiologic and pathologic role of TCR degeneracy

TCR flexibility plays a role during the physiologic thymic selection processes, shaping of the TCR repertoire, and in

the peripheral survival of memory T-cells [8]. Studies of TCR specificity suggested that a subset of T-cells exhibit a high degree of TCR degeneracy and estimated that individual TCR can respond to up to million ligands in order to provide protective immune responsiveness [5].

In addition to the above described physiological role, TCR degeneracy raises the potential for cross-reactivity between viral/bacterial and self-antigens and for the induction of pathological autoimmune response. Molecular mimicry, whereby autoreactive T-lymphocytes are activated by cross-reactive infectious antigens, is a frequent phenomenon [9]. However, its pathogenic role in triggering clinical relapses of MS by viral/bacterial infections remains to be established at the molecular level. While cross-reactivity between foreign and self-antigens is a common occurrence, pathogenic autoimmune responses are rarely initiated. How can this be explained? Cross-recognition of foreign and self antigens is only one of the requirements for autoimmune response. This is confirmed in the MBP-specific TCR transgenic mice, which develop spontaneous EAE only in a nonsterile environment, presumably triggered by viral and bacterial infections [10]. Therefore, molecular mimicry results in the autoimmune disease only when it takes place in the context of local inflammation, presentation of released self antigens, and a sufficient number of autoreactive T-cells.

5. Peptide combinatorial libraries, a new tool in probing degeneracy of T-cell recognition

In order to dissect the specificities of autoreactive- and pathogen-specific T-cell clones, we have used peptide positional scanning synthetic combinatorial libraries (PS-SCL) composed of large numbers of individual peptides [11]. This method allows an unbiased search for stimulatory epitopes based on the stimulatory potency of systematically arranged mixtures that address each of the 20-L-amino acids at each position of a 10-mer peptide (Fig. 2).

For example, the PS-SCL-based approach was employed in chronic CNS Lyme disease to test whether immune reactivity to self-antigens contributes to the chronic phase of this infectious disease. This method provided strong evidence that the infectious organism causing Lyme disease induces a cross-reactive chronic immune response to self-antigens [12]. Currently, we are analyzing the information derived from screening with PS-SCL to identify novel ligands for clones of unknown specificity, as reviewed in [13]. PS-SCL provides a non-biased strategy that rapidly identifies new

ligands through the simultaneous testing of literally trillions candidate peptides. We are characterizing antigen specificity of the *in vivo* expanded clones isolated from the cerebrospinal fluid of patients with MS in an attempt to address an old and yet unresolved question: which antigens trigger autoimmune responses in MS?

6. Costimulatory pathways

Growing evidence indicates that, in addition to TCR specificity, functional characteristics of autoreactive T-cells determine their propensity for activation [14]. In a two-signal T-cell activation paradigm, the first signal induced by TCR engagement confers antigen specificity, while the second costimulatory signal modifies activation threshold and the functional outcome of antigen-specific activation. CD28, CTLA-4/CD80, CD86 is the most important and best-studied costimulatory pathway [15]. CD28-costimulation synergizes with TCR activation and induces production of multiple cytokines. Following activation, CD4+ cells

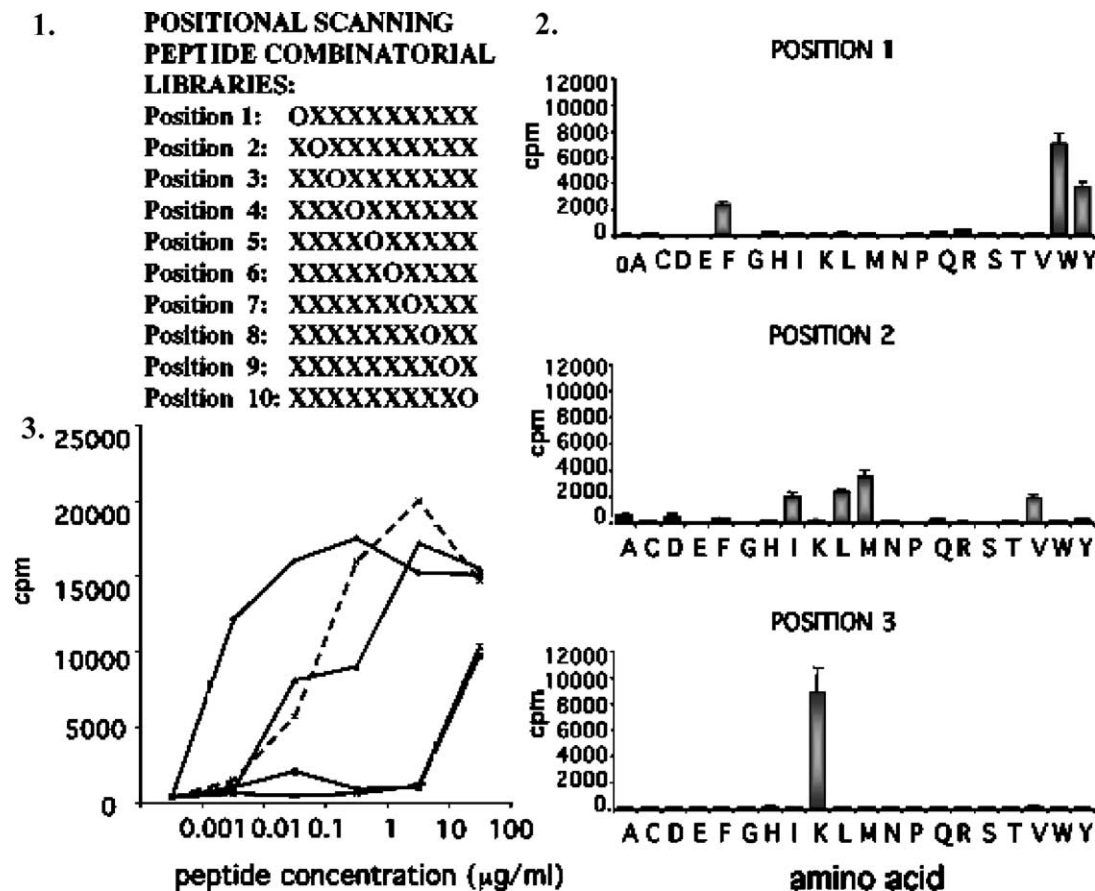


Fig. 2. Determining fine TCR specificity by combinatorial peptide libraries: (1) decapeptide positional scanning combinatorial library is composed of 10 positional libraries, and each library contains 20 sublibraries. In each positional library one position is defined by one of the 20-L-amino acids (O), while the remaining nine positions are occupied randomly by any a.a. (X); (2) T-cell clone response to positional libraries (positions 1, 2, 3, ...) is tested in a proliferation assay. The most potent a.a. in each position in a decamer peptide is defined; (3) the highest scoring self and foreign peptides identified by the PS-SCL biometrical analysis were synthesized and tested in proliferation assay to confirm their predicted stimulatory potencies.

downmodulate CD28 and express CTLA-4, a structural homologue of CD28. CTLA-4 delivers a negative signal for T-cell activation and terminates the proliferative response.

Dysregulation of costimulatory pathways in animal models of autoimmune diseases can lower T-cell activation threshold and lead to a chronic autoimmune response. Mice deficient in Cbl-b, an adaptor molecule that imposes a requirement for CD28 costimulation for T- and B-cell activation, develop spontaneous systemic autoimmune disease [16]. CD28-independent activation leads to an increased susceptibility to EAE or systemic autoimmune disease, depending on the animal's genotype [17]. The most dramatic phenotype is described in CTLA-4-deficient mice, which develop spontaneous autoimmune disease with massive lymphoproliferation, organ infiltration, and death within a month [18]. The costimulatory deficit here leads to the loss of the CTLA-4-mediated inhibitory signal which controls lymphocyte proliferation.

We have recently identified in MS patients a functionally distinct subset of CD4+ cells with no CD28 surface expression [19]. Following CD28-independent activation, CD4+ CD28– cells become fully activated, produce proinflammatory cytokines, and exhibit prolonged proliferation and increased survival. Myelin-specific cells are represented in a high frequency in this CD4+ subset. Similar CD28-costimulation-independent long-lasting CD4+ cell clonotypes were identified in other autoimmune diseases, including rheumatoid arthritis, insulin-dependent diabetes mellitus, chronic inflammatory bowel disease, and Wegener's granulomatosis [20,21]. Their antigen specificity and their role in the development of autoimmune response are presently investigated.

7. Autoantigen presentation within the CNS

Local antigen presentation is a critical requirement for the initiation and perpetuation of inflammatory responses within the CNS [22]. Although the CNS is devoid of immunocompetent antigen presenting cells (APCs), MHC class II and costimulatory molecules (CD80, CD86) are upregulated on microglia and macrophages in the setting of local inflammation, and can effectively present antigens [23]. Astrocytes, the CNS resident APCs, present antigens in a costimulation-independent manner, and stimulate only memory T-cells, which have a lower activation threshold [24].

The capacity of autoreactive T-cells to recognize many different epitopes reflects the importance of MHC/epitope density on antigen presenting cells. At a high epitope density, even weakly stimulatory epitopes can generate enough stable complexes with TCRs to trigger T-cell response [25]. Therefore, the mechanisms involved in local antigen presentation probably play an important role in the perpetuation of chronic CNS inflammatory responses.

8. Conclusions

In spite of considerable progress in immunological and imaging studies, as well as in developing therapies targeting the effector phase of the disease [26], our understanding of the initiation of autoimmune response in MS is still limited. We characterized autoreactive CD4+ cells: by examining their propensity to cross-reactive stimulation and their costimulatory requirements for activation. The results provide immunological markers of disease activity, which may translate into new targets for the selective therapy of MS. Moreover, they advance our understanding of basic mechanisms involved in T-cell activation and differentiation, and of chronicity and perpetuation of autoimmune diseases.

References

- [1] Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, et al. 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* 2000;6:1167–75.
- [2] Markovic-Plese S, Fukura H, Zhang J, al-Sabbagh A, Southwood S, Sette A, et al. T cell recognition of immunodominant and cryptic proteolipid protein epitopes in humans. *J Immunol* 1995;155:982–92.
- [3] Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner H, Hafler D. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J Exp Med* 1994;179:973–84.
- [4] Hickey W, Hsu B, Kimura H. T-lymphocyte entry into the central nervous system. *J Neurosci Res* 1991;28:254–60.
- [5] Mason D. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today* 1998;19:395–404.
- [6] Vergelli M, Hemmer B, Utz U, Vogt A, Kalbus M, Tranquill L, 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;26:2624–34.
- [7] Hemmer B, Vergelli M, Pinilla C, Houghten R, Martin R. Probing degeneracy in T-cell recognition using peptide combinatorial libraries. *Immunol Today* 1998;19:163–8.
- [8] Evavold B, Sloan-Lancaster J, Allen P. Tickling the TCR: selective T-cell functions stimulated by altered peptide ligands. *Immunol Today* 1993;14:602–9.
- [9] Martin R, Gran B, Zhao Y, Markovic-Plese S, Bielekova B, Marques A, et al. Molecular mimicry and antigen-specific T cell responses in multiple sclerosis and chronic CNS Lyme disease. *J Autoimmun* 2001;16:187–92.
- [10] Goverman J, Woods A, Larson L, Weiner L, Hood L, Zaller D. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell* 1993;72:551–60.
- [11] Zhao Y, Gran B, Pinilla C, Markovic-Plese S, Hemmer B, Tzou A, et al. Combinatorial peptide libraries and biometric score matrices permit the quantitative analysis of specific and degenerate interactions between clonotypic TCR and MHC peptide ligands. *J Immunol* 2001;167:2130–41.
- [12] Hemmer B, Gran B, Zhao Y, Marques A, Pascal J, Tzou A, et al. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. *Nat Med* 1999;5:1375–82.
- [13] Borrás E, Martin R, Judkowski V, Shukaliak J, Zhao Y, Rubio-Godoy V, et al. Findings on T cell specificity revealed by synthetic combinatorial libraries. *J Immunol Methods* 2002;267:79–97.
- [14] Anderson D, Bieganska K, Bar-Or A, Oliveira E, Carreno B, Collins M, et al. Paradoxical inhibition of T-cell function in response

- to CTLA-4 blockade heterogeneity within the human T-cell population. *Nat Med* 2000;6:211–4.
- [15] Bluestone J. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* 1995;2:555–9.
- [16] Chiang Y, Kole H, Brown K, Naramura M, Fukuhara S, Hu R, et al. Cbl-b regulates the CD28 dependence of T-cell activation. *Nature* 2000;403:216–20.
- [17] Bachmaier K, Krawczyk C, Kozieradzki I, Kong Y, Sasaki T, Oliveira-dos-Santos A, et al. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. *Nature* 2000;403:211–6.
- [18] Tivol E, Borriello F, Schweitzer A, Lynch W, Bluestone J, Sharpe A. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541–7.
- [19] Markovic-Plese S, Cortese I, Wandinger K, McFarland H, Martin R. CD4+ CD28– costimulation-independent T cells in multiple sclerosis. *J Clin Invest* 2001;108:1185–94.
- [20] Schmidt D, Goronzy J, Weyand C. CD4+ CD7– CD28– T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. *J Clin Invest* 1996;97:2027–37.
- [21] Vallejo A, Schirmer M, Weyand C, Goronzy J. Clonality and longevity of CD4+CD28null T cells are associated with defects in apoptotic pathways. *J Immunol* 2000;165:6301–7.
- [22] Dong Y, Benveniste E. Immune function of astrocytes. *Glia* 2001;36:180–90.
- [23] Markovic-Plese S, McFarland H. Immunopathogenesis of the multiple sclerosis lesion. *Curr Neurol Neurosci Rep* 2001;1:257–62.
- [24] Cornet A, Bettelli E, Oukka M, Cambouris C, Avellana-Adalid V, Kosmatopoulos K, et al. Role of astrocytes in antigen presentation and naive T-cell activation. *J Neuroimmunol* 2000;106:69–77.
- [25] Eisen H. Specificity and degeneracy in antigen recognition: yin and yang in the immune system. *Annu Rev Immunol* 2001;19:1–21.
- [26] Martin R, Sturzebecher C, McFarland H. Immunotherapy of multiple sclerosis: where are we? Where should we go? *Nature* 2001;9:785–8.