# Specificity and degeneracy: T cell recognition in CNS autoimmunity

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## Abstract

T cells play a crucial role in the pathogenesis of most autoimmune disorders. However, target antigens and pathomechanisms leading to human autoimmune diseases are still largely unknown. Cross-recognition of T cells between self and foreign antigens has been considered as a driving force in generating autoimmunity. Here, we discuss the extent of degeneracy in T cell antigen recognition and hypothesize on the role of degenerate recognition in the pathogenesis of multiple sclerosis (MS), a candidate autoimmune disease of the central nervous system (CNS).
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## 1. Introduction

T cells play a central role in the host defense against infection and cancer. Although much progress has been made in understanding antigen recognition by T cells, the process how they discriminate between self and non-self antigens has not been fully clarified. The key steps in selecting and maintaining the T cell repertoire—by removing or anergizing self-reactive but keeping protective immune cells—are determined by the interaction of each T cell with its surrounding environment (Sprent and Tough, 2001; Starr et al., 2003). Since T cells differ in the specificity of their T cell receptor (TCR) but are similar with respect to other surface molecules, the selection and maintenance process is mainly controlled by the interaction of the antigen receptor with the spectrum of encountering MHC:peptide ligands.

## 2. The extent of degeneracy in T cell antigen recognition

Until 10 years ago, antigen recognition by T cells was considered to be a highly specific process allowing interaction only with one or few defined ligands. With advances in T cell cloning technologies, TCR transgenic animal generation and peptide synthesis, it became clear, that T cell can recognize more than one ligand. This observation primed a number of studies which finally determined the extent of degeneracy in T cell antigen recognition (Kersh and Allen, 1996a; Hemmer et al., 1998b). First studies demonstrated that amino acids (aa) in MHC anchor positions could be replaced without loss of recognition as long as peptide binding to the MHC is preserved. Later it was also shown that aa in putative TCR contact positions were not always necessary for recognition by T cells. Based on these findings, Allen and co-workers claimed that a peptide is recognized as long as it contains a motif for binding to the MHC and one key residue for the TCR (Kersh and Allen, 1996b). This concept was later refined by the observation that no single residue was strictly required for recognition as long as the available residues provide enough binding energy for MHC and TCR (Hemmer et al., 1998a). Thus, peptides not sharing a single residue can productively interact with the same T cell receptor. A similar flexibility was also observed for the length of the peptide. Some CD4+ T cells need as little as four amino acids for recognition as long as they optimally fit to MHC and TCR (Hemmer et al., 2000). The concept of degenerate recognition was also strongly supported by experiments with randomized peptide libraries, which contain trillions of different peptides (Hemmer et al., 1998). Although some T cell clones do not respond to randomized peptide libraries, others respond very well. This demonstrates that T cells differ in their capacity to recognize mixtures of peptides containing each single ligand at extremely low concentration. The discrepant response suggests differences in the extent of degeneracy between individual T cells (Hemmer et al., 1998). Whereas some receptors recognize only a more limited repertoire of peptides, others are highly degenerate (Mason, 1998). Estimates based on the spectrum of possi-

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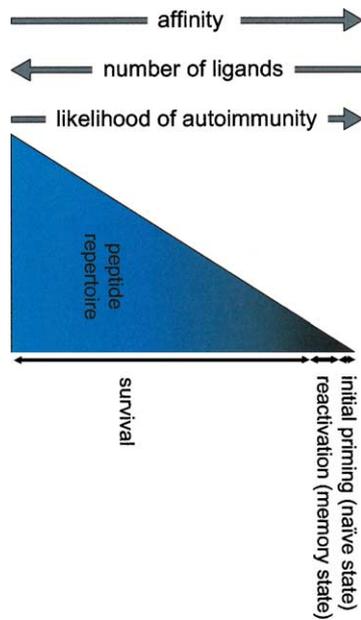
Fig. 1. Hypothetical model for ligand affinity, ligand number and likelihood of autoimmunity for a given T cell receptor. Although a T cell receptor can interact with many different peptides, the vast majority of them are recognized with low affinity. Only few ligands facilitate a high affinity interaction. According to our model, the likelihood of autoimmunity increases with the affinity of the cross-reactive antigen, thus cross-reactivity leading to autoimmunity is more likely to occur if the affinity of the mimicry peptide approaches the affinity of the initiating microbial antigen.

ble ligands and the number of T cells present in a given host suggest that one T cell can interact with as many as $10^6$ different ligands (Mason, 1998). Among those are few high affinity ligands and many of moderate to low affinity (Fig. 1).

This concept was supported by the structural resolution of the trimolecular complex consisting of TCR, MHC and peptide (Garcia et al., 1999; Hennecke and Wiley, 2001). The studies demonstrated essential flexibility in the interaction of the TCR with the MHC:peptide complex supporting the functional data. The number of interactions with the TCR varied with respect to different MHC:peptide ligands suggesting differences in the binding affinity. This affinity seemed to be the main determinant for the activation of the T cells by affecting the formation of the immunological synapse, the internalization of TCR structures, and the extent and quality of signalling (Germain and Stefanova, 1999; Lee et al., 2002). High affinity interactions of T cell receptors with the peptide/MHC complexes in the context of the appropriate costimulatory signals results in stable formation of the immunological synapse, internalization of TCRs and the transduction of full TCR signalling eventually leading to full activation of the T cell. In the lymph node environment, the T cell then undergoes maturation and expansion leading to a highly specific and efficient T cell response. In contrast, interaction with a low affinity ligand results in instable synapse formation, partial internalization and a weak/altered T cell signalling which is not sufficient to achieve full acti-

vation or even expansion of the cell. These findings led to the conclusion, that a high affinity interaction is required to mount physiological immune responses, whereas low affinity interactions may be relevant for thymic or peripheral selection and maintenance of the repertoire (Germain and Stefanova, 1999).

## 3. Autoimmune responses in MS and related disorders

Although the nature of most chronic inflammatory disorders such as multiple sclerosis is still unknown, it is believed that autoreactive immune cells play an important role in the initiation and perpetuation of disease (Steinman et al., 2002). In MS, this view is supported by data from experimental animal models and studies on autoreactive T and B cell responses in MS patients (Martin et al., 1992; Cross et al., 2001; Archelos et al., 2000). Myelin proteins are present in MS lesions and are properly processed and presented in the context of disease-associated HLA-class II molecules (Krogsgaard et al., 2000). Autoantibodies specific for a variety of CNS proteins are present in serum, cerebrospinal fluid (CSF) and brain of MS patients (Genain et al., 1999; Archelos et al., 2000). Similarly, CD4+ T cells specific for myelin antigens are found in the blood of MS patients. Studies on antigen recognition showed that CD4+ autoreactive, myelin-specific T cells from MS patients cross-react with peptides derived from bacterial or viral proteins (Hemmer et al., 1997; Wucherpfennig and Strominger, 1995). As shown by structural analyses, the same T cell receptor binds an HLA:autoantigen complex and a microbial peptide bound to another, but coexpressed HLA molecule (Lang et al., 2002). Furthermore, at least some of the autoreactive T cells recognize the microbial antigen with higher affinity than the myelin antigen (Vergelli et al., 1997; Hemmer et al., 1997). These findings led to the concept that an immune response initially activated and expanded by an infectious agent may cross-react in the activated state with autoantigens mediating CNS inflammation and destruction of the brain. The link between infection and autoimmunity via molecular mimicry has also been investigated in other inflammatory CNS diseases in particular chronic Lyme disease. Following acute infection with *Borrelia burgdorferi* (*Bb*), a chronic inflammatory disease can emerge which targets joints or CNS in the absence of bacterial infection. In this condition an autoimmune response to self antigens may offspring from the response to bacterial antigens (Martin et al., 2001). Indeed in Lyme arthritis, T cells from the synovial fluid recognized both an outer surface antigen from *Bb* and the human LFA-1 molecule (Gross et al., 1998). Similarly, *Bb*-specific T cells from the CSF of a patient with chronic neuroborreliosis cross-reacted with several self antigens, one of them being a myelin antigen (Hemmer et al., 1999).

Although these findings seem to provide a very logical explanation how autoimmune responses of the CNS could

emerge, experimental support for this concept from human studies is still limited (Benoist and Mathis, 2001). Likewise, autoreactive antibodies and T cells are not confined to MS patients but are also found in healthy donors (Martin et al., 1992; Pette et al., 1990) demonstrating that autoreactive T and B cells are part of the normal T cell repertoire and not necessarily harmful. So far it has not been possible to find compelling evidence for a difference between MS patients and controls with respect to the extent and quality of the humoral and cellular immune response to myelin antigens (Martin et al., 1992; Hemmer et al., 2002). Similarly, treatment studies based on the autoimmune concept involving oral tolerance or altered peptide ligands failed in recent MS trials (Bielekova et al., 2000). Although cross-reactivity between microbial antigens and autoantigens could explain the immune response in the inflamed tissue, we cannot rule out that different mechanisms are essentially involved. Autoreactive T cells could also be driven by other mechanisms such as epitope spreading (Vanderlugt and Miller, 2002) or reflect a counter regulatory or neuroprotective immune response (Moalem et al., 1999). Finally, it can not be ruled out that the immune response in MS is driven by a microbial antigen in the brain which upregulates the innate immune response and induces microbe-specific T and B cells (Meinl, 1999; Stohlman and Hinton, 2001).

## 4. Lessons from infectious disease models

The concept of degeneracy and molecular mimicry theoretically provides a very attractive basis to explain how autoimmunity emerges following infection. However, given the intrinsic degeneracy of the TCR it is surprising how rarely autoimmune diseases occur after infection. Up to now no animal model exists in which natural infection induces a chronic CNS autoimmune disease through molecular mimicry. T cell responses have been extensively studied in several models of acute and chronic infectious CNS disorders (Stohlman and Hinton, 2001; Haring and Perlman, 2001). In these animal models, viral infection of the CNS causes demyelinating disease. After virus replication and release of antigens from the CNS, a highly focused immune response is initiated in the periphery involving both CD4+ and CD8+ T cells. These cells accumulate in the CNS at high numbers and control virus spread. During the acute and subacute phase of disease, most T and B cells in the CNS tissue are antigen specific. Although the immune response involves a variety of clonotypes and occurs when residual microglia is activated in the CNS, virus specific T cells do not seem to cross-react with CNS antigens. Inflammation in these models is strictly dependent on viral activity and cannot be transmitted via adoptive immune transfer to a non-infected animal. However, autoimmune responses are generated in some models during the chronic phase by epitope spreading (e.g. Theiler's virus model) (Vanderlugt and Miller, 2002). These highly focused and usually not

cross-reactive responses in infectious disorders contrast the broad specificity of the TCR. Although most cross-reactive peptides will never reach the cell surface and be presented in the context of the appropriate HLA molecule, still a considerable number will be available to the T cell. This suggests to us that cross-recognition in the immune system is not only controlled on the level of availability but must also be regulated on the level of TCR binding to the MHC:peptide ligand. Since priming of T cells requires a high affinity interaction, the initiating microbial peptide will select T cells with optimal fit (Fig. 1). These T cells will undergo activation, maturation, and expansion. To induce autoimmunity, T cells should encounter a cross-reactive self-antigen in the target organ, which is able to evoke and maintain their effector function. Even if the maturation to the memory state will lower the threshold for recognition of the T cell, most low potency autoantigens will not activate the T cell in vivo although they may recognize the ligand under in vitro conditions. In particular when the ligands are present at low density, both the microbial and the autoantigen should facilitate a high affinity interaction between the TCR and the MHC:peptide ligand to achieve ongoing inflammation in the target organ. If the cross-reactive ligand has a low affinity, only high-density expression on the antigen presenting cell may compensate for the low ligand potency and achieve activation. In any case, the cross-reactive antigen may be structurally quite different from the initiating ligand as shown by cross-recognition of structurally unrelated MHC:peptide complexes (Hemmer et al., 1998a; Krogsgaard et al., 2000).

## 5. Strategies to define the role of molecular mimicry in human CNS disorders

Although many questions concerning cross-recognition can be addressed in animal models, refocusing on the human disease is required to clarify the role of molecular mimicry in these disorders. Substantial progress has been made in characterizing immune responses in human CNS diseases, in particular MS. These studies revealed some unexpected findings. Although, CD4+ T cells have been discussed as a key mediator of disease in MS based on the EAE findings and the HLA-class II association with MS, these cells are not highly prevalent in MS lesions but rather found in vascular cuffs and meninges (Gay et al., 1997). In contrast monocytes and clonotypic CD8+ T cells are found at high numbers in the CNS parenchyma (Gay et al., 1997; Babbe et al., 2000). These clonotypic CD8+ T cells are also found in the cerebrospinal fluid but not or with a much lower frequency in the peripheral blood (Babbe et al., 2000; Jacobsen et al., 2002). Similar B cells in lesions and CSF of MS patients show a restricted B cell receptor (BCR) repertoire with extensive maturation of the BCR genes (Cross et al., 2001). Both findings suggest a highly focused immune response in the diseased organ tissue involving predominantly the innate immunity, CD8+ T cells and B

cells. The low number and low extent of clonality among CD4+ T cells in the lesion raises questions about the role of these cells in the pathogenesis of MS (Babbe et al., 2000; Jacobsen et al., 2002). However, in most autoimmune models, even if CD8+ T cells initiate the disease process, CD4+ T cells are essential to amplify and escalate disease (Liblau et al., 2002). At this point, it is very difficult to determine which of the polyclonal CD4+ T cells in the MS lesion are pathogenetically relevant and contribute to the disease process. This demonstrates that concepts arising from animal models and in vitro studies of T cells may clarify the basic questions of T cell recognition and immune responses, but are not sufficient to resolve the key issues in human autoimmunity. In the future, it will be essential to focus our studies on T and B cells of whom we certainly know that they are involved in the disease pathogenesis. In contrast to randomly generated myelin-specific T cells from the blood of MS patients, we need to focus on T cells, which specifically recruit and persist in the CNS in relation to disease activity. These cells will provide a better basis to nail down specificity in CNS autoimmunity and to clarify the role of cross-reactivity. As long as we do not know, which cells are disease relevant, it will be impossible to prove the concept of mimicry for the generation of autoimmunity in MS and related disorders.

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