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Manipulating Antigenic Ligand Strength to Selectively Target Myelin-Reactive CD4+ T Cells in EAE

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Abstract

The development of antigen-specific therapies for the selective tolerization of autoreactive T cells remains the Holy Grail for the treatment of T-cell-mediated autoimmune diseases such as multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). This quest remains elusive, however, as the numerous antigen-specific strategies targeting myelin-specific T cells over the years have failed to result in clinical success. In this review, we revisit the antigen-based therapies used in the treatment of myelin-specific CD4+ T cells in the context of the functional avidity and the strength of signal of the encephalitogenic CD4+ T cell repertoire. In light of differences in activation thresholds, we propose that autoreactive T cells are not all equal, and therefore tolerance induction strategies must incorporate ligand strength in order to be successful in treating EAE and ultimately the human disease MS.

Keywords

experimental autoimmune encephalomyelitis; functional avidity; tolerance; altered peptide ligands; MHC variant peptides; SHP-1

Introduction

The central tenet of clonal selection is that only those T cell clones that receive sufficient stimulation by foreign antigenic peptide/major histocompatibility complex (pMHC) complexes are activated and expand. Because T cells compete in their responsiveness to antigen (Kedl et al. 2003), T cells with greater functional responsiveness are able to preferentially expand and predominate during an immune response (Savage et al. 1999; Fasso et al. 2000; Malherbe et al. 2004; Price et al. 2005; Zehn et al. 2009). The resulting expanded foreign antigen-specific T cell repertoire is therefore composed of a selected set of T cell clonotypes, where the highest frequency of responding T cells are those with the greatest functional responsiveness. It is unknown whether this selection of dominant T cell clonotypes also exists for T cells responding to self-antigens as this response has been presumably shaped during thymocyte maturation. In the following section, we review how ligand strength contributes to the degree of T cell activation and how this is dependent on the extent and quality of T cell signaling.

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Strength of antigenic ligand stimulation

The idea that a single amino acid change for a given epitope could selectively induce certain T cell effector functions but not others was first demonstrated in the early 1990s (Evavold and Allen 1991; De Magistris et al. 1992; Evavold et al. 1993b). These landmark studies not only demonstrated the exquisite specificity of T cells to pMHC antigen but also that T cells exhibit a similarly stunning degree of reactivity to a number of different antigenic ligands (Kersh and Allen 1996; Mason 1998; Ford and Evavold 2004b), including to nonhomologous ligands (Evavold et al. 1995; Hemmer et al. 1998). This cross-reactivity, or degeneracy, reflects the fact that T cell activation is not an on-or-off event per se but rather a continuum of events that require varying degrees of antigenic signal strength through the T cell receptor (TCR). The potential outcomes of TCR ligation by pMHC include full activation, partial activation (e.g., cytokine secretion in the absence of proliferation), anergy (persistent unresponsiveness to antigen stimulation), antagonism (inhibition of activation in the presence of agonist), or no activation. Thus, pMHC ligands are classified according to their potency based on the effector functions they can elicit. From highest to lowest potency, these are: superagonist, agonist, weak agonist, antagonist, or null peptides (Anderton and Wraith 2002). T cell functional responsiveness is therefore directly related to the strength of antigenic ligand stimulation. Here, we outline key factors that greatly influence the strength of ligand stimulation (Fig. 1) on T cell activation and how these can be manipulated to influence the functional responsiveness of T cells. Later, we discuss the roles these features play in the aberrant activation of autoreactive T cells and on developing strategies to more specifically and effectively shut down polyclonal autoreactive T cells. These findings have direct application for the treatment of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), both of which are considered CD4⁺ T-cell-mediated autoimmune diseases directed against central nervous system (CNS) myelin antigens.

TCR binding dynamics with pMHC

Although the details are far beyond the scope of this review, numerous studies have demonstrated a direct correlation between ligand potency and TCR binding kinetics (Matsui et al. 1994; Alam and Gascoigne 1998; Davis et al. 1998; Kersh et al. 1998; Rosette et al. 2001; Cole et al. 2007). These dynamics can be profoundly altered by substitution of those amino acids within the peptide that form direct contacts with the TCR. Peptides containing modifications of such TCR contacts are termed altered peptide ligands (APLs; Evavold et al. 1993a; Fig. 2). A single position in an antigenic peptide forms the primary TCR contact residue, with several other positions forming secondary TCR contacts. Alteration of the primary TCR contact is almost always completely nonstimulatory. APLs formed from modification of secondary TCR contacts can elicit the spectrum of T cell responses from superagonist to antagonist (De Magistris et al. 1992; Evavold et al. 1993a; Jameson and Bevan 1995; Sloan-Lancaster and Allen 1996). The different outcomes induced by these types of APLs is therefore a direct reflection of the strength of the interaction between the TCR and its pMHC ligand, which in turn results in differential T cell signaling and effector functions.

Affinity of peptide for MHC

MHC allelic variants present different pools of antigens due to polymorphisms in their peptide binding pockets. It is because of the unique binding motifs that certain MHC anchor residues predominate among peptides bound to a given MHC allele (Falk et al. 1991; Rudensky et al. 1992; Fremont et al. 1996). Alteration of residues at any of these positions can selectively alter the affinity of the peptide for the MHC molecule with minimal impact on TCR binding (Wraith et al. 1992), but the resulting change in pMHC half-life is crucial in setting a threshold for TCR recognition (Hemmer et al. 2000). Thus, peptides that have a low affinity for MHC tend to have lower stimulatory capacity (Reichstetter et al. 1999).

Many groups have used the term “APL” to include all peptide modifications including MHC anchor changes. However, we draw a distinction here by referring to those peptide analogs that have modified MHC binding residues as “MHC variant peptides” or MVPs (Fig. 2). Although the differences in nomenclature may seem trivial at first, these two types of peptide analogs can have profoundly different effects on polyclonal T cell responses. MVPs alter the half-life of the pMHC complex, which in turn affects the interaction of the TCR with pMHC. Thus, MVPs that increase the affinity of the antigen for MHC increase ligand strength and TCR engagement, while those that decrease the affinity of pMHC interaction decrease ligand potency. Like APLs, MVPs have varying effects on the extent of T cell activation (Ryan and Evavold 1998; Kersh et al. 2001; Ryan et al. 2004; Lazarski et al. 2005). In cases where a peptide with low affinity for MHC is able to induce a potent response, generation of an MVP with greater MHC binding stability can form a heteroclitic or superagonist response (Wraith et al. 1989). This results in a shift of the dose curve so that full activation occurs at significantly lower doses than agonistic ligands while antigen-induced cell death (AICD) typically results at doses that are agonistic for weaker ligands (Zugel et al. 1998; Wang et al. 1999). Such a possibility has important relevance in the activation of myelin-reactive CD4⁺ T cells, which will be addressed later on this review.

Amount of pMHC

It has been recently demonstrated that minimal levels of agonist pMHC, as little as a single pMHC to several hundred, are required for T cell activation (Demotz et al. 1990; Harding and Unanue 1990; Reay et al. 2000; Irvine et al. 2002; Ma et al. 2008). However, maximal activation requires significantly higher levels exceeding 5,000 pMHC (Reay et al. 2000) and far higher quantities of weak ligands are needed for T cell activation. The ability of a T cell to respond to a given amount of pMHC antigen is directly related to its functional avidity (Margulies 2001). Those T cells that are able to respond to low-dose antigen are considered to be of high functional avidity, while those responding only to high-dose antigen have a low functional avidity (Alexander-Miller et al. 1996). In addition to having different thresholds for activation, these types of T cells differ in their susceptibility to tolerance. That is, low-avidity T cells are anergized by low dose of antigen (Ryan and Evavold 1998; Korb et al. 1999; Mirshahidi et al. 2001), whereas high-avidity T cells undergo AICD following stimulation by high dose of antigen (Critchfield and Lenardo 1995; Liblau et al. 1996). Polyclonal T cell populations can therefore be skewed according to the amount of pMHC antigen available. Thus, high-functional-avidity T cells clonotypes are selected by low-dose antigen, while low-functional-avidity T cells are favored by higher doses of antigen (Rees et al. 1999).

TCR levels

The fact that extremely low levels of pMHC antigen can activate T cells begs the question as to why T cells have such high TCR levels, which number in the tens of thousands. The most likely explanation is provided by the spare receptor theory (McNeil and Evavold 2002), which states that the TCR reserve increases the flexibility of TCR recognition so as to allow activation by weak ligands (Ford and Evavold 2004b). Indeed, it has been shown that masking of the majority of surface TCRs maintains T cell reactivity to agonist peptides, but loss of small numbers results in loss of responsiveness to weak ligands (McNeil and Evavold 2002, 2003; Jones et al. 2008).

All of the above factors directly contribute to the strength of antigenic stimulation by influencing the quantitative and qualitative features of T cell signaling and ultimately the extent of activation. Thus, an increase in any of these features results in a direct increase in ligand potency. It is important to note that these factors are mutually compensatory and synergistic. For instance, increasing the dose of pMHC can augment the potency of an antigen with poor

affinity for MHC (Anderton et al. 2001). In the following sections, we explain how each of these factors contribute to TCR signaling and ultimately T cell functional responsiveness.

TCR signaling is shaped by the strength of antigenic ligand stimulation

The potency of the antigenic ligand plays an important role in determining the complete dissemination of early signaling events in a T cell (Kersh et al. 1998), but the kinetics of the propagation of the positive and negative feedback loops that comprise the net signaling outcome following TCR ligation are relatively unknown. Understanding the timing of both the positive and negative feedback loops may be an important step in understanding how low-potency ligands, such as APLs and MVPs, may utilize these feedback loops to modulate low-functional-avidity T cells. Mathematical modeling of T cell signaling proposes that these feedback loops allow for discriminating between a range of ligand affinities (Chan et al. 2004; Altan-Bonnet and Germain 2005). Indeed, one model specifically proposes a balance between a positive signal driven by the kinases Lck and Erk and a negative signal driven by the phosphatase SHP-1 to regulate T cell activation (Altan-Bonnet and Germain 2005).

T cells respond to high- or low-potency antigens by either positive or negative signaling upon TCR ligation, and the strength of the overall TCR signal is determined by the factors previously discussed. Based on this, both positive and negative feedback loops interact to regulate the overall T cell response to antigen stimulation. In one model, the positive feedback loop of a T cell can be explained by three “modules.” These three modules make up the TCR signalosome and include the src family protein tyrosine kinase module, which initiates signaling upon TCR ligation and is mediated in part by the kinase Lck; the signal-triggering module, which includes the kinase ZAP-70, the main docking and phosphorylation site for downstream signaling mediators; and the signal diversification module, which includes the many proteins of the downstream signaling cascade (Acuto et al. 2008). Negative feedback loops can interact with the three domains of the positive feedback loop to regulate signaling. Having multiple points of control in the positive feedback loop modules indicates that negative feedback loops play an integral part in T cell signaling and that they are necessary to properly regulate T cell activation.

The fact that both positive and negative feedback loops are rapidly activated upon TCR ligation may help us understand how an activation threshold is set in a T cell once we understand the kinetics of the interactions between the various feedback loops. The assorted negative feedback loops include the regulatory molecules CTLA-4 (Egen and Allison 2002), c-Cbl and Cbl-b (Naramura et al. 2002), DGK (Sanjuan et al. 2001), PTEN (Wang et al. 2000), and SHP-1 (Stefanova et al. 2003). Phosphorylation and dephosphorylation are the most rapid means of signaling in a T cell, and therefore, phosphatases are an important point of control during T cell signaling. There are many protein tyrosine phosphatases that regulate the positive signaling mediators, including the PEST family of phosphatases, SHP-1, SHP-2, and some of the DUSP family of phosphatases.

SHP-1 mediates negative signaling to weak ligand stimulation

One model of a positive and negative feedback loop involves the phosphatase SHP-1. In this model, high-potency ligands induce positive signaling events through the phosphorylation of the CD3 chains and ZAP-70 by active Lck. During the positive feedback loop, ERK is activated and phosphorylates Lck, and the recruitment of SHP-1 is inhibited (Stefanova et al. 2003). In contrast, ligands with low potency trigger an active negative feedback loop involving SHP-1. In this scenario, weak ligands cause activated Lck to tyrosine phosphorylate SHP-1, which in turn dephosphorylates Lck (as well as other src family substrates), resulting in downregulation of the T cell response (Stefanova et al. 2003; Acuto et al. 2008). During T cell interaction with antigen, the phosphatase SHP-1 and the tyrosine kinase cascades are activated concurrently,

and the outcome of positive kinase signals and negative phosphatase signals is thereby influenced by the strength of antigenic signal.

SHP-1 therefore represents the importance of phosphatases as checkpoints in T cell signaling following weak ligand stimulation. For instance, a deficiency in SHP-1 results in a reduced activation threshold of T cells and increases the overall T cell response (Carter et al. 1999; Johnson et al. 1999). Further, SHP-1 is necessary for TCR antagonists to mediate their suppressive effect on TCR signaling (Kilgore et al. 2003), and reduction of SHP-1 lowers the threshold for activation by antagonists (Stefanova et al. 2003). In addition, MVP-induced anergy also requires SHP-1, suggesting a major role for SHP-1 in regulating low-avidity TCR interactions (Wasserman et al. 2008).

Although SHP-1 has proximal influences on TCR triggering, other phosphatases are also important for regulating the extent of a T cell response. For example, the overexpression of the microRNA miR-181a can augment the sensitivity of TCR ligation by increasing the level of active Lck, while also decreasing the level of the phosphatases PTPN22, SHP-2, DUSP5, and DUSP6 (Li et al. 2007). Similar to the effects of SHP-1 reduction, these miR-181a-mediated effects on signaling allow antagonists to activate T cells (Li et al. 2007). This suggests that multiple negative signaling loops may play into the major positive signaling cascade to regulate T cell activation to weak ligands. The ability for SHP-1 to regulate a T cell's response to variant peptides highlights the importance of negative feedback loops in maintaining tolerance. Later in this review, we discuss the importance of SHP-1 in the regulation of autoreactive T cells.

Escape from tolerance: low functional avidity of myelin-specific T cells

The fact that autoimmune disease arises begs the question: why do the various mechanisms of central and peripheral tolerance fail to prevent the activation of all autoreactive T cell clones? Here, we review the various mechanisms of tolerance and how this relates to the survival and activation of autoreactive T cells in the induction of EAE.

All T cells are required to have a minimal responsiveness for self-antigen in order to survive thymic development (i.e., positive selection). Conversely, those developing thymocytes with high avidity for self are negatively selected so as to limit the possibility of autoimmunity, a process that is clearly imperfect since low-avidity self-reactive T cells can escape thymic deletion (Ridgway et al. 1999; Bouneaud et al. 2000; Sandberg et al. 2000; Zehn and Bevan 2006; Kawamura et al. 2008). In the context of EAE, it has been shown that lack of thymic expression or low-affinity binding of myelin antigens allows the survival of potentially encephalitogenic T cells (Liu et al. 1995; Targoni and Lehmann 1998; Harrington et al. 1998; Anderson et al. 2000; Kawamura et al. 2008). These studies therefore highlight not only the importance of central tolerance in the elimination of T cells with a high functional avidity for self-antigen but also in reducing the frequency of the potentially autoreactive T cell repertoire.

In a manner similar to positive selection in the thymus, all mature T cells must also interact with self-antigens in the periphery in order to survive (Ernst et al. 1999; Muranski et al. 2000; Stefanova et al. 2002). Such an interaction is believed to be of weak affinity but sufficient to provide a low-level TCR-dependent signal vital for T cell survival (Stefanova et al. 2003; Davis et al. 2007). Importantly, the spare receptor theory of TCR reserve helps to explain how T cells require interactions with self-pMHC in the periphery for survival since the excess of TCRs would allow for the accumulation of these low-level signals.

The numerous mechanisms of peripheral tolerance, including ignorance, apoptosis (e.g., AICD), anergy/exhaustion, cytokine skewing, and Treg activation, prevent the activation of

those mature T cells with a higher functional avidity for self-antigen (Kuchroo et al. 2002; Walker and Abbas 2002; Anderton 2006). Thus, it is this unique low functional avidity that allows autoreactive T cells to escape the various mechanisms of tolerance. This has been substantiated in various self-reactive T cell models, in which autoreactive T cells with a sufficiently low functional avidity (but still high enough to be activated) escape peripheral tolerance and induce autoimmune disease (de Visser et al. 2000; Gronski et al. 2004; Mallone et al. 2005; Zehn and Bevan 2006). In the context of EAE, it has been demonstrated that the weak binding of myelin basic protein (MBP)_{Ac1-11} to IA^u allows the activation of higher-avidity MBP-specific CD4⁺ T cells and the induction of EAE (Anderton et al. 2001). Thus, in light of the deletion or inactivation of those T cells with high avidity for self by central and peripheral tolerance, the surviving mature T cell repertoire is postulated to be composed of T cells with a high affinity for foreign antigen (i.e., nonselecting ligands) and a lower affinity for cross-reactive self-antigens (i.e., selecting ligands; Hemmer et al. 1997).

Low structural affinity of myelin-specific CD4⁺ T cells

Numerous studies have demonstrated that MHC II tetramer binding is directly related to the affinity of the TCR for pMHC (Crawford et al. 1998; Savage et al. 1999; Rees et al. 1999; Fasso et al. 2000; Reichstetter et al. 2000; Anderton et al. 2001; Garcia et al. 2001; Gebe et al. 2003; Falta et al. 2005) as well as the overall TCR levels (Crawford et al. 1998; Stone et al. 2001; Mallone et al. 2005). Therefore, CD4⁺ T cells with a high structural affinity for the MHC II tetramer exhibit a higher mean fluorescence intensity than those with a lower affinity (Crawford et al. 1998; Reichstetter et al. 2000; Anderton and Wraith 2002; Vollers and Stern 2008; Wooldridge et al. 2009). Moreover, CD4⁺ T cells with a sufficiently low affinity for antigen are undetectable by tetramer staining. Highlighting the low TCR affinity of autoreactive T cells is the inability to detect a large frequency of myelin-reactive CD4⁺ T cells in various EAE models (Radu et al. 2000; Reddy et al. 2003; Bischof et al. 2004; our unpublished results) as well as other autoimmune disease models (Gebe et al. 2003; Falta et al. 2005; Mallone et al. 2005).

We therefore believe that a fundamental tenet of auto-reactive CD4⁺ T cell populations is a broad range of functional avidities for self-antigen, which is overall lower than those for foreign antigen and which is highly influenced by an array of TCR structural affinities. This therefore precludes the identification of all self-reactive CD4⁺ T cells by conventional MHC class II tetramer staining. Based on this concept, tetramer analysis can be used as a tool to assess the overall avidity of polyclonal autoreactive CD4⁺ T cell populations by analyzing the frequency of tetramer-positive (high avidity) and tetramer-negative T cells (low avidity), an idea that has been proposed by several other groups (Anderton and Wraith 2002; Reijonen et al. 2002). However, tetramers are nonetheless limited in assessing the actual frequency of autoreactive T cells, since it is likely that in many situations at least a portion of the tetramer-negative repertoire is not antigen specific. In EAE, the tetramer-based frequency of myelin-specific CD4⁺ T cells has been estimated to be less than 10% of CNS-infiltrating CD4⁺ T cells (Bischof et al. 2004; Korn et al. 2007; Sabatino et al. 2008; Matsushita et al. 2008). We contend that is likely an underestimation of the genuine frequency of myelin-specific CD4⁺ T cells due to the low structural affinity of autoreactive T cells. Due to this limitation of tetramers, other techniques need to be developed in order to more precisely measure the frequency and avidity of autoreactive CD4⁺ T cells.

SHP-1 in tolerance of autoreactive T cells

The importance of maintaining appropriate levels of negative signaling mediators is well-exemplified by SHP-1 deficiency models, including the *motheaten* (*me*) or *motheaten viable* (*me^v*) mutations. Both *me* and *me^v* mice develop severe autoimmunity early in life that leads to premature a death (Green and Shultz 1975; Shultz et al. 1984). The significance of SHP-1

in autoimmunity is due to effects on both central and peripheral tolerance. TCR responsiveness is enhanced in thymocytes expressing a dominant negative form of SHP-1 (Zhang et al. 1999b), and *motheaten* mice show an increase in both positive and negative selection (Zhang et al. 1999b). In mature T cells, the proliferative response as well as IL-2 production is increased in *motheaten* T cells (Lorenz et al. 1996) and show an increased sensitivity for TCR-induced apoptosis (Zhang et al. 1999a). As mentioned previously, SHP-1 is necessary for both antagonism and anergy induced by mutant peptide ligands in various models (Kilgore et al. 2003; Stefanova et al. 2003; Wasserman et al. 2008).

This demonstrates that SHP-1 acts a crucial checkpoint by preventing the inappropriate activation of T cells in response to low antigenic signals, such as those that might occur in low-avidity autoreactive T cells. Highlighting the relevance of SHP-1 in MS is the demonstration that MS patients have shown lower levels of SHP-1 in peripheral blood mononuclear cells (PBMCs) as compared to healthy controls (Christophi et al. 2008).

Targeting myelin-reactive CD4+ T cells based on their low functional avidity

We contend that the functional avidity of a given T cell is directly correlated with its susceptibility to tolerance induction. Thus, although the low functional avidity of autoreactive T cells allows their escape from tolerance, the same characteristic allows a unique opportunity to selectively target these T cells. Namely, the factors that affect TCR ligand potency outlined above can be manipulated to specifically induce tolerizing or downmodulating effects on low-avidity autoreactive T cells, some of which has been reviewed elsewhere (Anderton 2001). In the next section, we review how these features can be altered to more specifically target autoreactive CD4+ T cells in EAE.

Targeting TCR/pMHC interactions through APL treatments in EAE

Very soon after the discovery of the peptide analog-mediated inhibition of T cell function, APLs were applied in an effort to downmodulate myelin-specific CD4+ T cells in EAE. Prevention or amelioration of EAE by myelin APLs has been shown in a number of models (Wraith et al. 1992; Karin et al. 1994; Franco et al. 1994; Kuchroo et al. 1994; Nicholson et al. 1995; Brocke et al. 1996; Gaur et al. 1997; Anderton et al. 1998, 1999; Ben-Nun et al. 2006; Table 1). Despite this promise, these APL studies presented a number of problems. Firstly, although APLs could inhibit certain subsets of myelin T cell clones, they often activated other myelin-reactive T cells and therefore proved to be either non-protective (Wraith et al. 1992; Karin et al. 1994; Brocke et al. 1996) or were encephalitogenic themselves (Anderton et al. 1998; Ford and Evavold 2003). Thus, the diversity of the autoreactive T cell repertoire is something that cannot be overlooked and highlights the limitations of TCR transgenic models of EAE. Secondly, several of these APLs proved highly potent in inducing Th2 cytokine switching (Nicholson et al. 1995; Brocke et al. 1996), which can predispose animals with EAE towards hypersensitivity and anaphylaxis (Genain et al. 1996; Pedotti et al. 2001). The problems encountered in myelin antigen APL studies in EAE revealed themselves in an alarming way in two phase II clinical trials to treat MS. Both trials had to be halted midway for exacerbation of neurological symptoms (Bielekova et al. 2000) or hypersensitivity reactions (Kappos et al. 2000).

Targeting myelin antigen/MHC stability by MVP treatments in EAE

A popular and effective way to tolerize autoreactive T cells is to deliver a stronger signal via superagonists generated by increasing the affinity of myelin antigen for MHC. In the MBP_{Ac1-11}/IA^u model, both the 4A and 4Y MVPs function as superagonists by increasing the peptide affinity for IA^u and protect against EAE (Wraith et al. 1989; Smilek et al. 1991; Samson and Smilek 1995). Moreover, two MBP₇₂₋₈₅ MVPs both protect against EAE in Lewis rats

(Wauben et al. 1992). Other studies have shown that the MBP_{Ac1-11} MVPs cause the apoptosis, CD5 upregulation, or cytokine skewing of high-affinity MBP-specific CD4⁺ T cells (Pearson et al. 1997; Anderton et al. 2001; Ryan et al. 2005). In SJL mice, a proteolipid protein (PLP)₁₃₉₋₁₅₁ super-agonist causes the apoptosis of PLP-specific CD4⁺ T cells (Nicholson et al. 1998). In a cross-reactive TCR transgenic model, a potent non-self ligand caused reduced responsiveness to myelin antigen (Munder et al. 2002). Conversely, immunization with an MBP_{Ac1-11} subagonist generated high-functional-avidity myelin-reactive CD4⁺ T cells, which were poorly encephalitogenic (McCue et al. 2004), suggesting possible tolerance by AICD.

An alternative way to use MVPs to target myelin-reactive T cells is to destabilize the pMHC interaction. We have demonstrated that weakly binding MVPs for myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ (termed 45D) and PLP₁₃₉₋₁₅₁ (termed 145D) can anergize polyclonal myelin-specific CD4⁺ T cells and significantly attenuate EAE in B6 and SJL mice, respectively (Ford and Evavold 2003, 2004a; Margot et al. 2005; Table 2). As proof of safety, these MVPs did not skew towards a Th2 profile and did not cause anaphylaxis even after repeated treatment (Ford and Evavold 2003; Margot et al. 2005). Of interest, treatment of MOG₃₅₋₅₅-specific CD4⁺ T cells with the MVP 45D maintained the secretion of the Th1 cytokine interferon (IFN)- γ . Surprisingly, deficiency of IFN- γ or IFN- γ R reverses the tolerizing effects of 45D, suggesting that IFN- γ signaling is somehow involved in regulating the responsiveness of MOG-specific CD4⁺ T cells to weak ligands (Sabatino et al. 2008). This suggests that cytokine deficiencies may have important consequences on myelin-specific T cell functional responsiveness and can affect the therapeutic efficacy of weak ligands.

These results demonstrate that altering pMHC interactions is an effective way to skew the avidity of the myelin-reactive T cell repertoire. By increasing the stability of the pMHC complex to generate superagonists, T cell functional avidity can be increased so that T cells become susceptible to AICD. Conversely, the pMHC interaction can be destabilized to reduce functional avidity and induce anergy. Because of the heterogeneity of functional avidities in polyclonal auto-reactive T cell populations, employing both of these strategies will likely be necessary to effectively downregulate all pathogenic self-reactive T cells.

Altering pMHC and TCR density in EAE

As previously described, pMHC dosing can be used to skew polyclonal T cell populations towards higher or lower functional avidity. The effectiveness of oral tolerance in treating EAE, which has long been demonstrated, has been achieved by using both low and high doses of myelin antigen. High-dose treatment causes the deletion of myelin-specific T cells by AICD (Critchfield et al. 1994; Whitacre et al. 1996). In contrast, low-dose myelin antigen has been shown to convert myelin-reactive CD4⁺ T cells into a regulatory phenotype by bystander suppression (Chen et al. 1996; Whitacre et al. 1996). A potential downside to this is the possible suppression of nonautoreactive T cells and inhibition of immune responses to foreign antigen. Although myelin-specific T cells from MS patients can be skewed to higher or lower functional avidities by restimulation with variable doses of MBP (Mazzanti et al. 2000), these therapies have not yet been successfully applied to MS treatment.

Although somewhat unconventional, an alternative way to lower the responsiveness of autoreactive T cells is through reductions in surface TCR levels. In the P14 TCR transgenic model, reductions in TCR prevented reactivity to the low-affinity self-antigen DBM, but responsiveness to the cognate gp₃₃₋₄₁ antigen was preserved (Jones et al. 2008). In EAE, one group showed that treatment with a pan anti- $\alpha\beta$ TCR antibody resulted in T cell hyporesponsiveness and amelioration of EAE (Matsumoto et al. 1994). Recently, our lab successively applied clone-specific TCR reduction in the treatment of EAE. Using the 2D2 MOG₃₅₋₅₅-specific TCR transgenic model, reductions in the levels of surface TCRs using anti-V α 3.2 antibody resulted in loss of functional responsiveness and decreased encephalitogenicity

(Wasserman and Evavold 2008). However, an obvious drawback to this strategy is the inability to specifically target autoreactive T cells because of the broadness of TCR blockade.

Targeting of SHP-1 for the treatment of EAE

Due to its role in regulating the responsiveness of T cells to weak ligand stimulation, a number of studies have investigated the role of SHP-1 in modulating myelin-specific CD4⁺ T cell responses in EAE. To test this, *me*^{+/-} or *me*^{v+/-} mice have been utilized, as they have about one half the amount of functional SHP-1 of wild-type mice but seem to be phenotypically normal (Kozlowski et al. 1993). The reduction in SHP-1 in *me*^{+/-} or *me*^{v+/-} mice enhances the T cell response to the low-affinity ligand MBP_{Ac1-11} and exacerbates EAE in this model (Deng et al. 2002). As mentioned previously, SHP-1 is also necessary for induction of anergy using low-affinity MVPs. Results from our lab recently demonstrated that SHP-1 is required for the induction of anergy by the tolerizing MVP 45D in MOG₃₅₋₅₅-immunized B6 mice. Reduction of SHP-1 in this model allows the induction of EAE by 45D (Wasserman et al. 2008), which is poorly encephalitogenic in wild-type mice (Ford and Evavold 2003, 2004a). The increased encephalitogenicity of 45D by loss of SHP-1 is remarkably similar to the phenotype observed in IFN- γ -deficient mice immunized with this weak ligand (Sabatino et al. 2008). Although no mechanism is known at this point, it is intriguing to speculate that IFN- γ and SHP-1 signaling might somehow converge to regulate the responsiveness of myelin-specific CD4⁺ T cells.

As previously mentioned, SHP-1 deficiency has been observed in PBMCs from MS patients (Christophi et al. 2008). Interestingly, IFN- β , a mainstay of MS treatment, has been recently shown to increase the level and activity of SHP-1 in MS patients (Christophi et al. 2009). In support of this, phosphorylation of STAT1, which mediates interferon signaling and is elevated in MS patients, has been reported to be linked to SHP-1 levels (Feng et al. 2002). These findings therefore support further study of the role of SHP-1 in controlling low-functional-avidity T cell responses, with direct applicability to the treatment of EAE and MS.

Conclusion

The mechanisms of central and peripheral tolerance effectively eliminate or functionally suppress all T cell clonotypes with high and no functional avidity for self-antigens, including myelin-derived antigens. Thus, low functional avidity is a characteristic of essentially all autoreactive T cells, including the myelin-specific CD4⁺ T cell response of EAE. This is corroborated by the low structural affinity of CNS-infiltrating CD4⁺ T cells in EAE using MHC II tetramer analysis. Based on the various factors that modulate T cell functional avidity, numerous studies have sought to target the low functional avidity of the encephalitogenic CD4⁺ T cell response. This has been done in a variety of ways, including altering TCR/pMHC dynamics (e.g., APLs), altering the stability of the pMHC complex (e.g., MVPs), altering pMHC dose, reducing TCR levels, and reducing the levels of the phosphatase SHP-1. Although all of these studies have proved the potential efficacy of these strategies, it is not known whether any of these individual therapies have been able to effectively downmodulate all myelin-reactive CD4⁺ T cells, while also leaving the nonautoreactive T cell repertoire intact.

An important complexity to the antigen-specific targeting of autoreactive T cells is the polyclonal nature of the autoimmune response. In most models of EAE, myelin-reactive T cells are a heterogeneous population with a diverse TCR repertoire (Kuchroo et al. 1994; Mendel et al. 1996; Anderton et al. 2001). This has important consequences on the fine specificity of the various encephalitogenic CD4⁺ T cell clonotypes as well as their functional responsiveness. We contend that within this lower-functional-avidity self-specific compartment, the myelin-reactive T cell repertoire is comprised of clones of varying avidities, accompanied by varying degrees of susceptibility to tolerance (Fig. 3). That is, certain clonotypes have higher functional

avidities and are more likely to be successfully tolerized by increasing antigenic ligand potency. This would include such strategies as the generation of superagonists to induce AICD. Conversely, other clonotypes possess lower functional avidities and would therefore be more susceptible to tolerance by decreasing the strength of antigenic stimulation. This can be accomplished, for example, by destabilization of pMHC binding to induce anergy. In addition, greater attention should be paid to the TCR affinities of the responding myelin-specific CD4⁺ T cell population, which may provide guidance in determining whether antigen-based therapies should increase or decrease T cell functional avidity. In light of the limitations of tetramers in detecting low-avidity T cells, other techniques should be developed to more accurately quantify the frequency and avidity of those T cells mediating CNS attack against myelin antigens.

Finally, a greater understanding of how T cell functional avidity relates to positive and negative signaling may provide a deeper understanding of antigen-specific therapies in EAE. Because of the exquisite sensitivity of T cell recognition of cognate ligands, the advantages of peptide-specific therapy cannot be fully utilized, and the limitations cannot be fully appreciated without greater knowledge of how these therapies work in the context of polyclonal populations of autoreactive T cells with an array of functional avidities to myelin antigens. The role for phosphatases, such as SHP-1, in maintaining control of low-avidity T cells, coupled with its role in dampening T cell responses upon encountering weak ligands, implicate the phosphatase as a plausible therapeutic target for intervention in autoimmunity. Thus, altering the levels of phosphatases such as SHP-1, either directly or through manipulation of antigenic ligand strength, will hopefully lead to the development of antigen-specific therapies for T-cell-mediated autoimmune diseases, such as MS.

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Abbreviations

AICD	Antigen-induced cell death
APL	Altered peptide ligand
EAE	Experimental autoimmune encephalomyelitis
MHC	Major histocompatibility complex
MVP	MHC variant peptide
MS	Multiple sclerosis
MBP	Myelin basic protein
MOG	Myelin oligodendrocyte glycoprotein
PLP	Proteolipid protein
TCR	T cell receptor

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Fig. 1.

Strategies of altering TCR antigenic ligand strength of stimulation. The strength of TCR antigenic ligands can be altered by the modifying the following factors: (1) TCR/pMHC dynamics, (2) peptide/MHC stability, (3) amount of pMHC presented by antigen-presenting cells (APCs), and (4) TCR levels on the T cell. These factors can be modified respectively by: (1) altered peptide ligands (APLs), (2) MHC variant peptides (MVPs), (3) pMHC antigen dosing, and (4) TCR masking. Ultimately, these factors directly contribute to the balance of positive and negative signaling feedback loops and the functional responsiveness of the T cell

Fig. 2.

Alteration of TCR or MHC contact residues distinguishes APLs and MVPs. Displayed are hypothetical TCR and MHC contact positions (*P*), which are based on common but not universal peptide motifs. For instance, P5 is often the primary TCR contact. Alteration of any of the TCR contact residues generates altered peptide ligands or APLs. MHC contacts are typically formed by P1, P4, P6, and P9. Substitution of any of these residues generates MHC variant peptides or MVPs

Fig. 3.

Model for targeting low functional avidity myelin-reactive T cells. The mechanisms of central and peripheral tolerance ensure the inactivation or elimination of low and high functional avidity autoreactive T cells. However, this process is incomplete and allows the survival of myelin-reactive CD4⁺ T cells. Aberrant activation of these T cells results in CNS autoimmune attack and EAE. In our model, these potentially encephalitogenic T cells possess low but variable functional avidities for myelin antigens, which in turn endows susceptibilities to different mechanisms of tolerance. Certain clonotypes possess higher (but overall still intermediate relative to non-self) functional avidities and are more susceptible to tolerance by overactivation (e.g., AICD). Lower avidity clones, on the other hand, could be better targeted by reducing the level of stimulation to induce nonresponsiveness (e.g., anergy or ignorance). Therefore, antigen-specific therapies should focus on altering the strength of activation, either by lowering or increasing it, in order to allow the autoreactive T cell repertoire to become susceptible to tolerance.

Table 1

Summary of APL-based studies in treating EAE

EAE model	APL	MHC II	Outcome	Ref
MBP ₈₇₋₉₉	91A, 95A, 96A	RT.D ¹	91A protective; 95A and 96A not protective	(Karin et al. 1994)
	96A ^a	IA ^s	Protective against certain clones	(Brocke et al. 1996)
	91A ^b , 97A ^c	IA ^s	Protective, nonencephalitogenic	(Gaur et al. 1997)
PLP ₁₃₉₋₁₅₁	Y144, L144	IA ^s	Each partially protective	(Franco et al. 1994)
	L144/R147	IA ^s	Protective	(Kuchroo et al. 1994)
	Q144	IA ^s	Protective	(Nicholson et al. 1995)
MBP _{Ac1-11}	6G, 6T, 6V	IA ^{u/s}	Encephalitogenic	(Anderton et al. 1998)
	3D, 3I, 3K	IA ^u	Protective	(Anderton et al. 1999)
	3F, 3H, 3M, 3Y	IA ^u or IA ^{u/s}	Nonencephalitogenic	(Wraith et al. 1992)
MOG ₃₅₋₅₅	41A, 44A	IA ^b	Protective	(Ben-Nun et al. 2006)
	47A	IA ^b	Not protective and encephalitogenic	(Ford and Evavold 2003)

Listed are the different types of myelin antigen-based APLs that have been used in treating EAE. The name of the APL, the MHC II restriction haplotype (denoted by the superscript), and outcome of the studies are given. Encephalitogenicity refers to the ability of the APL to induce EAE, whereas protection refers to ability to treat EAE induced by wild-type myelin antigen

^a 96A is classified by its authors as APLs, but whether this is a TCR contact or MHC contact is not clear. 96A is a partial agonist for certain clones of MBP₈₇₋₉₉-reactive CD4⁺ T cells

^b 91A is classified by its authors as APLs, but whether this is a TCR contact or MHC contact is not clear. 91A appears to function as an antagonist

^c 97A is classified by its authors as APLs, but whether this is a TCR contact or MHC contact is not clear. 97A appears to function as a superagonist

Table 2

Summary of MVP-based studies in treating EAE

EAE Model	MVP	MHC II	Outcome	Ref
MBP _{Ac1-11}	4A	IA ^u or IA ^{uXS}	Protective, nonencephalitogenic	(Wraith et al. 1989; Smilek et al. 1991; Anderton et al. 2001)
	4Y	IA ^u or IA ^{uXS}	Protective, nonencephalitogenic	(Samson and Smilek 1995; Anderton et al. 2001)
MBP ₇₂₋₈₅	1028	RT1.B ^L	Protective	(Wauben et al. 1992)
MOG ₃₅₋₅₅	45D	IA ^b	Protective, nonencephalitogenic	(Ford and Evavold 2003; Ford and Evavold 2004a, b)
PLP ₁₃₉₋₁₅₁	145D	IA ^s	Protective	(Margot et al. 2005)

Shown are the various studies using myelin antigen-based MVPs to treat EAE. The name of the MVP, the MHC II restriction haplotype (denoted by the superscript), and outcome of the studies are given. Encephalitogenicity refers to the ability of the MVP to induce EAE, whereas protection refers to the ability to treat EAE induced by wild-type myelin antigen