Published in final edited form as:

Immunol Res. 2011 May ; 50(1): 39–48. doi:10.1007/s12026-011-8204-3.

T cell recognition of weak ligands: roles of signaling, receptor number, and affinity

Lindsay J. Edwards and Brian D. Evavold

Department of Microbiology and Immunology, Emory University, 1510 Clifton Road, Atlanta, GA 30322, USA

Brian D. Evavold: bevavol@emory.edu

Abstract

T cell recognition of antigen is a crucial aspect of the adaptive immune response. One of the most common means of pathogen immune evasion is mutation of T cell epitopes. T cell recognition of such ligands can result in a variety of outcomes including activation, apoptosis and anergy. The ability of a given T cell to respond to a specific peptide–MHC ligand is regulated by a number of factors, including the affinity, on- and off-rates and half-life of the TCR–peptide–MHC interaction. Interaction of T cells with low-potency ligands results in unique signaling patterns and requires engagement with a larger number of T cell receptors than agonist ligands. This review will address these aspects of T cell interaction with weak ligands and the ways in which these ligands have been utilized therapeutically.

Keywords

T cell; Altered peptide ligands; Intracellular signaling; Viral escape; Autoimmunity

T cells were once thought to be highly specific for a single antigen. Over time, as our knowledge of the intricacies of T cell activation has increased, we have come to realize that T cell activation is not a digital phenomenon. That is to say, a T cell is not either activated or inactivated at any given time. On the contrary, even at rest, a T cell is a complex mix of positive and negative signaling events. These same signaling events are activated or inactivated to varying degrees upon a T cell's encounter with antigen, and the balance between positive and negative signaling events determines the outcome of the interaction.

Stimulation with an agonist peptide rapidly activates both positive and negative signaling events. However, due to the nature of the interaction, the positive signaling events that are initiated are sufficient to either inhibit the negative signals or overbalance them to such an extent that the result is full activation of the T cell, as evidenced by proliferation and the development of effector functions such as cytokine production. As our laboratory and others have demonstrated, T cells can recognize a broad spectrum of ligands, encompassing stimuli that result in activation of only a subset of effector functions (partial agonists) or even inhibition of the T cell's ability to respond to agonist stimulation (antagonists) [1–3].

T cell recognition of both strong and weak ligands is an important aspect of T cell biology. Variants of agonist ligands occur frequently during viral infection, providing impetus for the continued study of the biology of T cell responses to a broad spectrum of pMHC ligands.

Studies of signaling in response to weak ligands have demonstrated that T cell recognition of ligands that vary in potency is quantitatively and qualitatively different and not attributable to simple dose effects ([4, 5], our unpublished data). Furthermore, the kinetic parameters of a given interaction between the trimolecular TCR–peptide–MHC complex correlate with the stimulatory capacity of the ligand [6, 7]. The ability of T cells to respond to ligands that vary in potency affects the response to pathogens that are able to mutate their T cell epitopes and may also be harnessed for therapeutic use in several different ways. Each of these themes will be explored further in this review.

Escape mutants: naturally occurring variant peptides

Pathogens employ a number of different mechanisms to evade the immune system. Among the most common of these approaches is mutation of T cell epitopes. The stochastic nature of the mutations generated by pathogens can result in a broad spectrum of ligand stimulatory capacity, mimicking all of the classes of altered peptide ligands (weak and partial agonists, antagonists) initially described using synthetic peptides and model systems. Of course, the mutation itself may be detrimental to the fitness of the pathogen. However, the impact of such a mutation on the T cell response can have an equally dramatic effect on the maintenance or clearance of the infection.

Mutated T cell epitopes have been most widely studied in viruses, although escape epitopes have also been identified in bacteria and parasites [8–12]. Viruses, particularly those with an RNA genome, are more likely than bacteria or parasites to develop mutations in T cell epitopes due to the error-prone nature of their genomic replication [13]. Mutations leading to the generation of an antagonist epitope have been described for several viruses and parasites [9, 10, 14–18]. Antagonists are defined as altered peptide ligands that induce little to no T cell activation when given alone, but are able to inhibit the T cell's ability to respond to agonist stimulation with both agonist and antagonist present [2]. The phenomenon of antagonism was initially described *in vitro*; however, the generation of an escape mutant during an ongoing viral infection could also present a situation in which the agonist ligand and the mutant are encountered by a single T cell. Inhibition of the T cell response due to the generation of antagonist ligands has been described in human immunodeficiency virus [10, 19, 20], as well as hepatitis B virus, [9], human T lymphotropic virus [14], *Plasmodium falciparum* [18], lymphocytic choriomeningitis virus [16, 21], and hepatitis C virus [15, 17, 22].

A related phenomenon that is distinct from classical antagonism but likewise mediated by variant peptides has been termed “immune interference”. This means of immune evasion was initially described for a variant epitope from *Plasmodium falciparum* [23]. Peptides that mediate this interference effectively block initial T cell priming events, thereby altering the effective repertoire in the infected individual. Another means by which escape mutants can alter the T cell repertoire is by selectively expanding cross-reactive T cell populations [24]. Similarly, a subsequent study utilizing a model antigen and an antagonist variant demonstrated that the presence of the antagonist ligand at the time of initial priming resulted in a failure of the T cells to develop normal effector functions, and the cells were ultimately eliminated via apoptosis [25]. These studies emphasize that the presence of variant ligands at the time of infection (i.e., concurrent infection with multiple strains or subspecies) can be just as devastating to the immune response as the mutation of T cell epitopes during ongoing infection, and this situation likely plays an important role in the subsequent development of T cell memory.

The outcomes of T cell encounter with an epitope mutant vary dramatically depending on the T cell activation state, context of antigen encounter and nature of the antigenic ligand

(i.e., affinity of the epitope for MHC and TCR). One frequently observed result of T cell encounter with a variant ligand is a shift in effector phenotype. For example, after encounter with a variant epitope from *Plasmodium falciparum*, CD4 T cells were shown to shift from IFN γ production to IL-10 secretion [23]. In some systems, escape mutants have been demonstrated to induce anergy in the responding T cells [26], apoptosis [23, 27], or a failure of T cell activation [21].

These responses to mutant epitopes are driven by active negative signaling events or significant alterations in the kinetics of normally activating pathways. For example, we have demonstrated that in vitro stimulation of T cells with a viral escape mutant epitope from lymphocytic choriomeningitis virus resulted in abrogated positive signaling events (Erk1/2 phosphorylation) and augmented negative signaling events (SHP-1 phosphatase activity) [28]. The induction of altered signaling pathways by variant ligands will be discussed in greater detail later in this review.

It is becoming increasingly clear that weak ligands play a significant role in shaping the immune response. As identification of antigenic epitopes is ongoing, it is impossible to know just how many variant peptides may exist in nature. However, continually increasing our understanding of the impact that variants have on the T cell response may allow us to harness this aspect of the immune response to develop more effective vaccine strategies, as well as treat infections, particularly chronic infections that are often resistant to common therapies. Furthermore, understanding the aspects of T cell binding to pMHC that determine the outcome of the interaction as well as the downstream consequences of these interactions will be crucial to overcoming the effects of epitope escape mutations during infection.

Binding kinetics of antigen recognition

The affinity of the interactions between TCR and peptide–MHC as well as between the antigenic peptide and MHC have long been recognized as playing a crucial role in the outcome of T cell stimulation. Initial measures of the kinetic parameters governing the TCR–peptide–MHC interaction primarily utilized surface plasmon resonance. This technique measures binding interactions with one purified protein immobilized on a solid surface and the interacting partner in the fluid phase. This setup allows for the molecules to interact in three dimensions (3D) and provides data allowing for the extrapolation of kinetic parameters such as on-rate, off-rate, half-life, and affinity. The measures of TCR–peptide–MHC interaction derived in 3D correlate to some extent with ligand potency, although discrepancies were commonly observed for antagonist ligands [7, 29, 30].

In a typical cell–cell interaction as would occur in vivo, membrane-bound receptor–ligand pairs interact in only two dimensions (2D), which is to say each of the proteins is anchored in the cell membrane. This type of interaction is significantly more constrained than the 3D interactions that can be observed by surface plasmon resonance. In order to measure binding interactions in a more physiologically relevant manner, several novel techniques have been developed in recent years that are capable of assessing 2D binding events. One of these methods is based on single-molecule Förster resonance energy transfer (FRET), while the other is a mechanically based assay that visualizes binding via membrane stretching [6, 31]. The single-molecule FRET approach described by Davis and colleagues utilizes pMHC anchored to a planar lipid bilayer and intact T cells. The peptide is labeled with a fluorophore, and the T cell receptor is tagged by a fluorophore-labeled antibody fragment. By bringing these two labels into contact via TCR interaction with pMHC, one fluorophore served as a donor and the second as an acceptor to provide a FRET readout that allowed for calculation of various binding parameters (Fig. 1a).

The mechanical micropipette adhesion frequency assay utilizes an intact T cell and measures its binding to a red blood cell (RBC) that serves as a surrogate antigen-presenting cell [7, 32–34]. In this assay, the RBC coated with pMHC monomers is used as a biosensor to detect binding events. In order to determine adhesion frequency, a T cell is aspirated onto one micropipette and a pMHC-coated RBC is aspirated onto a second micropipette. The two cells are brought into contact for a defined period of time, and the T cell is retracted to terminate the contact. If binding occurs, the soft RBC membrane elongates (Fig. 1b). By repeating this contact multiple times for each cell pair and for a number of different cells pairs and interaction times, the kinetic parameters of the binding interaction can be determined, including on-rate, off-rate, half-life of interaction as well as affinity. We have demonstrated that a number of these kinetic parameters strongly correlate with biological function (i.e., ligand potency), suggesting that 2D measurements are a more faithful representation of receptor ligand interactions as they occur physiologically than the 3D measurements that have been used in the past [7].

One major advantage of the 2D approaches is that it is not necessary to express and purify the individual proteins of interest. This aspect makes the assessment of polyclonal populations difficult and time-consuming, if not impossible in three-dimensional analysis. However, the micropipette assay is readily adaptable to polyclonal T cells, as we have recently published [33]. The utility of the micropipette assay in determining the binding characteristics of polyclonal T cell populations may allow for more extensive profiling of autoreactive populations and their responsiveness to various therapeutic interventions as well as characterization of responses following vaccination or during chronic infection.

Both the FRET and micropipette 2D approaches demonstrated that in both CD4 [6] and CD8 [7] T cells, binding kinetics varied somewhat from what had been previously described using the 3D system, even when the same pMHC and TCRs were utilized. Two-dimensional analysis of TCR interaction with cognate pMHC resulted in rapid association and rapid dissociation and accordingly short half-lives of interactions. This is in stark contrast to the kinetics observed in the 3D system, which displayed rapid on-rates, but slow dissociation rates and thus longer interaction times [7, 29, 30]. The 2D interaction kinetics suggest a model in which T cells rapidly sample antigen, with strong stimuli resulting in short dwell time and thus perhaps engaging a greater number of TCRs. In contrast, weaker ligands bound with slower off-rates and longer half-lives, suggesting that interaction with a weak ligand may ultimately limit the number of T cell receptors that encounter antigen. These differences in binding kinetics likely affect signaling downstream of the TCR as well, providing one means of translating the interaction with antigen into effector outcomes.

Signaling in response to weak ligands: quantitatively and qualitatively unique

The simplest explanation for the differences in T cell activation observed with weak ligands is that there is a dose effect. If this were the case, then a low dose of a highly stimulatory peptide should induce signaling events similar to a high dose of a weak ligand. However, this is not the case. Studies assessing signaling events downstream of T cell stimulation with weak ligands have repeatedly demonstrated that unique signaling patterns result from weak stimuli.

In the seminal study assessing signaling in response to altered peptide ligands, Allen and colleagues demonstrated that stimulation of T cell clones with an anergy-inducing variant peptide resulted in a unique pattern of zeta chain phosphorylation [4]. Similarly, in a different system, altered zeta chain phosphorylation was demonstrated upon stimulation with variant peptides representing a partial agonist and an antagonist [5]. This unique pattern of

zeta chain phosphorylation resulted in either a failure of ZAP-70 to associate with the T cell receptor complex or a lack of phosphorylation and activation of this kinase [4, 5]. Importantly, this effect was not mimicked by stimulating cells with even a 100- to 1,000-fold lower doses of wild-type peptide [4, 5], emphasizing that signaling in response to weak ligands is not explained by a simple dose effect but may in fact represent an alternative pathway of cellular activation.

The altered phosphorylation patterns observed in these early studies of T cell responses to weak ligands could potentially be explained by several different mechanisms. The simplest explanation for decreased phosphorylation events is a decrease in kinase activity. Alternatively, the signal delivered by a weak ligand could activate one or more phosphatases, which would rapidly remove the activating phosphorylation events. Given the complex nature of the signaling events downstream of the TCR, it is likely that both of these mechanisms are playing a role. As yet, no comprehensive studies have assessed both the positive and negative signaling events that occur upon stimulation of a T cell with a weak ligand.

In addition to the altered phosphorylation pattern of zeta and the lack of ZAP-70 activity observed on stimulation with weak ligands, a number of other positive signaling mediators are also absent. For example, MAPK activation is transient on stimulation of a CD4+ T cell with a partial agonist [35]. Similarly, in CD8+ T cells, stimulation with a weak agonist resulted in no detectable calcium flux or phosphorylation of Erk1/2, p38, or Jnk. However, cells stimulated with the weak ligand did accumulate phosphorylated c-Jun to the same level as agonist stimulated cells by 24 h of stimulation, suggesting that even undetectable levels of early positive signaling events may somehow accumulate in distal mediators of T cell activation, thus driving the limited positive responses to weak or partial agonist ligands ([30], our unpublished data).

A number of unique signaling events have been described in the setting of antagonism. Analysis of cells stimulated in this unique manner where both agonist and antagonist ligands are present suggests that the phenotype of antagonism is mediated by an active negative signal. Activation of Vav, SLP-76, LAT, and Erk1/2 was not observed by traditional biochemical methods on stimulation under antagonist conditions, although some minor phosphorylation events were observed at the T cell–APC interface using confocal microscopy [36]. Calcium flux, however, is not required for the induction of antagonism [37], and this reduced or absent calcium flux may play a role in the blockade of proliferation and cytokine production [38]. Additionally, antagonism resulted in altered LFA-1/ICAM-1 interactions [38] and failure to form mature T cell–APC conjugates [39]. Other studies observed phosphorylation of Vav and SLP-76 upon antagonism, as well as stable T cell–APC conjugate formation [40, 41], suggesting that some aspects of antagonism may be dependent upon the specific system or conditions.

Furthermore, we have demonstrated that extended culture of transgenic T cells under antagonist conditions can permanently alter the phenotype of the cells, in a manner similar to what has been described for anergy [42]. Unlike the anergic phenotype, cells that have been antagonized and subsequently restimulated with agonist are capable of producing IL-2. In fact, these antagonized cells secrete more IL-2 than cells cultured on agonist, but are unable to proliferate, despite expressing the high-affinity IL-2 receptor at similar levels as agonist cultured cells. Similar to anergy induced by APLs, the non-proliferative phenotype cannot be rescued by the provision of exogenous IL-2. This unique phenotype emphasizes once more that the encounter of T cells with variant ligands can have a profound effect on both the early signaling events as well as the resulting effector functions of the cells.

Antagonism of T cells with altered peptide ligands has also been associated with the induction of negative signaling mediators, specifically activation of the Src homology 2 domain containing protein tyrosine phosphatase (SHP-1) [43, 44]. SHP-1 is a broadly expressed phosphatase that is generally thought to function as a negative regulator of cellular activation. In hematopoietic cells, SHP-1 has been shown to associate with a number of signaling intermediates including Lck, ZAP-70, various Jaks and Stats, the p85 subunit of PI3 K, CDK2, and the IL-2 receptor complex [45–54]. SHP-1 is thought to be activated by phosphorylation of its C-terminal tail by kinases including Lck, making it perhaps the most TCR proximal negative regulator [46, 55, 56]. In addition to its role in T cell antagonism, we have also demonstrated that SHP-1 activity is required for anergy induction by an MHC variant peptide [57] and is upregulated in response to a viral escape mutant epitope [28]. These data suggest that the negative signals driven by T cell interaction with weak ligands may be largely regulated by SHP-1.

Recently, overexpression of the microRNA miR-181a has been demonstrated to abolish the inhibitory effect of an antagonist peptide, but was insufficient to convert the response to the level of the agonist [58]. This microRNA was shown to decrease expression of a number of inhibitory mediators in T cells, including several phosphatases. However, SHP-1 expression was not affected. These data suggest a model in which the negative signals delivered by an antagonist peptide engage multiple pathways, perhaps with SHP-1 as the most TCR proximal regulator and other phosphatases controlling downstream signaling events, although the details of these pathways remain to be clearly determined.

Role of receptor number in recognition of weak ligands

Several studies have utilized T cells expressing two distinct TCRs in an attempt to determine whether T cell antagonism is a passive process mediated by competition for ligand binding or an active process mediated by the activation of a negative signal. By using T cells expressing two receptors of different antigen specificity and/or MHC restriction, it is possible to deliver an agonist stimulus via one TCR and antagonist stimulus via the second TCR. Studies of this nature, while well conceived, gave mixed results. The initial studies of this setup failed to observe “cross-antagonism” in CD8+ dual TCR transgenic cells [59, 60]. That is to say, when the dual-receptor T cells were stimulated with an agonist for one receptor and an antagonist for the second receptor, no inhibition of cytotoxicity was observed. Subsequent studies by our laboratory and others, however, demonstrated potent cross-antagonism of proliferation and cytokine production in CD4+ dual-receptor T cells [43, 61]. It has been suggested that this apparent disconnect between CD4+ and CD8+ T cell antagonism is due at least in part to the assays utilized to measure antagonism. Cross-antagonism was consistently observed when the readout was proliferation or cytokine production, but not when the readout was cytotoxicity [43, 59–61]. These data suggest that the regulatory mechanisms governing various effector outcomes differ, rendering proliferation more susceptible to inhibition by antagonist ligands than cytotoxicity. These differences could be driven by different signaling requirements or differences in kinetics of both input and output signals, as proliferation is necessarily a longer process than degranulation-mediated cytotoxicity.

One caveat to the dual TCR system is that these T cells tend to preferentially express one TCR over the other. That is, one of the TCRs typically represents 70% or more of the TCR on the cell surface. Although this issue is never directly addressed in the early studies assessing antagonism in dual TCR cells, it is evident from assessing the fluorescence intensity of the staining for the TCR that one beta chain is more highly expressed. Considering this, we sought to determine what role TCR level may play in susceptibility to antagonism. By using antibodies to block specific TCRs, we demonstrated that antagonists

as well as weak ligands require a significantly greater number of TCRs on the cell surface to exert their effects, when compared with agonist [62–64]. Full proliferation could be stimulated with agonist peptide with as many as 95% of the TCRs blocked [63–65]. This is in good accord with other studies demonstrating activation in response to a very few pMHC ligands, which is essentially the reciprocal experiment [66–68]. However, blockade of only 50% of the available TCR was sufficient to dampen responses to weak agonists, as well as the ability of an antagonist to inhibit the T cell response to agonist [63].

These studies assessed TCR transgenic cells (AND, P14, and OTI) that have a very high affinity for their respective ligands. It has been postulated that autoreactive T cells are of low affinity for antigen due to both central and peripheral tolerance mechanisms [69–71]. Given this idea, we sought to determine whether blocking the TCR of a self-reactive T cell would result in the inability of those T cells to respond to their “agonist” ligand. In accordance with the idea that autoreactive cells respond to their agonist ligand as if it were a weak agonist due to the low affinity of the TCR, we demonstrated that a 5% decrease in the number of TCR on the surface increased the EC50 by threefold, and a 75% reduction in TCR shifted the EC50 by more than 350-fold [64].

Together, these data are reminiscent of the pharmacological idea of a spare receptor theory. The spare receptor theory postulates that a given ligand (i.e., pMHC in the case of T cell activation) can exert maximum biological effect while only engaging a small number of the available receptors (Fig. 2). This, of course, begs the question of the purpose of having such an excess of receptors. In the case of T cells, it seems likely that the importance of this receptor reserve is twofold. First, a high number of receptors may allow for the recognition of self-peptides and/or MHC that is required for survival signals as well as homeostatic proliferation [65, 72, 73]. Secondly, excess receptors allow for the recognition of a broad spectrum of ligands [62–64]. This is a key aspect of T cell protection from pathogens, as the number of T cell specificities in the body is limited. A system employing spare receptors allows for each T cell to potentially recognize a greater number of ligands through cross-reactivity. This cross-reactivity likely increases the risk of autoimmune disease, in addition to increasing the chance that the T cell repertoire will be able to respond to a pathogenic challenge. Similar to this, a recent study described a system in which infection with a virus was sufficient to induce autoimmune disease [74]. In this system, mice transgenic for a TCR recognizing a myelin basic protein epitope were activated via a second, endogenous receptor specific for a viral epitope. This activation was sufficient to allow the cells to mediate tissue damage. The delicate balance between autoimmunity and immunity to pathogens lies at the heart of the T cell response. Peripheral tolerance mechanisms are typically able to keep autoimmunity at bay, allowing for the maximum available repertoire to respond to foreign antigens, although escape from tolerance can and does occur.

Therapeutic potential of variant peptides

Given the pivotal nature of the TCR–peptide–MHC interaction in determining the subsequent T cell response, a number of attempts have been made to utilize variant peptides therapeutically. Variant peptide therapies have been described in animal models of multiple sclerosis [75–77], myasthenia gravis [78, 79], allergy [80, 81], type 1 diabetes [82, 83], arthritis [84–86], and psoriasis [87]. These efforts have generally been very successful in animal models of autoimmune disease, but have often been less successful in clinical trials [88–90].

Initial studies of peptide therapies in experimental autoimmune encephalomyelitis (EAE), a rodent model of multiple sclerosis, utilized altered peptide ligands with one or more amino acid substitutions in an attempt to ameliorate disease. This approach, which frequently

resulted in immune deviation from a Th1 to a Th2 phenotype, was effective in EAE induced by several different antigenic epitopes, including proteolipid protein [76] and myelin basic protein [75, 77]. An altered version of the myelin basic protein epitope was subsequently utilized in clinical trials as a means to treat multiple sclerosis [88, 89]. The phase I trial was ultimately too small and too short-lived to assess efficacy and was halted due to adverse reactions, including exacerbation of disease and some hypersensitivity responses [88]. A subsequent phase II trial demonstrated no significant differences in frequency or number of relapses, although a decrease in volume and number of lesions following treatment and a shift to a Th2 phenotype in responding T cells were observed [89, 91]. The Th2 phenotype of cells from these patients was maintained long term (2–4.5 years) [92]. The phase II trial was also halted due to adverse reactions, particularly hypersensitivity responses in approximately 9% of patients. This adverse reaction most frequently manifested as itching or rash and seemed to correlate with the number of injections the patient had received [89]. Interestingly, injection of some unaltered self-peptides or altered peptide ligands resulted in hypersensitivity in the form of anaphylaxis in several mouse models of autoimmunity (type 1 diabetes and EAE) [93–96], suggesting that hypersensitivity may be a typical rather than an atypical response to peptide therapies (wild type or altered) during ongoing autoimmune disease. Although subsequent studies have successfully engineered peptide therapies that did not cause anaphylaxis in mice [96, 97], clinical trials utilizing altered peptide ligands to treat multiple sclerosis have not been revived.

An additional problem with therapeutic use of altered peptide ligands, which typically contain amino acid substitutions at the TCR contact residues, is the outgrowth of T cell clones specific for the APL itself [88]. This type of response could at best impede therapeutic efficacy and at worst exacerbate ongoing autoimmune disease. In order to circumvent this problem, we have utilized an approach in which amino acid substitutions are made at MHC anchor residues rather than TCR contacts (Fig. 3). In this way, the peptide–MHC interaction can be destabilized, resulting in the decreased ability of the T cells to be activated. In fact, we have demonstrated that in addition to inducing anergy in autoreactive T cells, MHC variant peptides can prevent the induction of EAE when used in a vaccination strategy as well as decrease disease severity when given in soluble form during ongoing EAE [96, 98]. Outgrowth of variant specific populations has not been observed, likely because the face of the peptide recognized by the T cells is not dramatically altered by the substitutions that have been introduced. Furthermore, no evidence of anaphylaxis was observed upon treatment with MHC variant peptides, even though altered peptide ligands in the same system did cause rapid death due to an anaphylactic reaction [96]. Perhaps these and other improvements on peptide therapies will lead to new clinical trials, with the hope of treating diseases for which current therapies are of limited efficacy.

Summary

The ability of T cells to recognize and respond to a broad spectrum of ligands is a critical aspect of T cell biology. Variant ligands are frequently generated by pathogens as a means to escape the T cell response and therefore present as a unique target to boost immune responses against chronic pathogens. The use of variant ligands has provided novel insights into signaling pathways downstream of the TCR that may be applicable to the regulation of T cell responses. Related studies have demonstrated that the number of T cell receptors expressed on the surface also plays a crucial role in the T cell response to antigen, particularly self-antigens and weak ligands. The insights into T cell biology that have been generated by exploration of variant peptides have shown promising results in the treatment of mouse models of several autoimmune diseases. Hopefully, these insights will be parlayed into novel therapeutics for the treatment of autoimmunity and chronic infections, two classes of disease that are notoriously difficult to effectively treat.

Acknowledgments

The authors would like to thank the members of the Evavold lab for their critical reading of the manuscript. Funding was provided by the National Institutes of Health grant numbers NS 062358 and NS 071518 and the National Multiple Sclerosis Society grant numbers RG4047 and RG 4482.

References

1. Evavold BD, Allen PM. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science*. 1991; 252:1308–10. [PubMed: 1833816]
2. De Magistris MT, Alexander J, Coggeshall M, Altman A, Gaeta FCA, Grey HM, Sette A. Antigen analog-major histocompatibility complexes act as antagonists of the T cell receptor. *Cell*. 1992; 68:625–34. [PubMed: 1739971]
3. Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature*. 1993; 363:156–9. [PubMed: 8483498]
4. Sloan-Lancaster J, Shaw AS, Rothbard JB, Allen PM. Partial T cell signaling: altered phospho-zeta and lack of zap70 recruitment in APL-induced T cell anergy. *Cell*. 1994; 79:913–22. [PubMed: 8001128]
5. Madrenas J, Wange RL, Wang JL, Isakov N, Samelson LE, Germain RN. Zeta phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. *Science*. 1995; 267:515–8. [PubMed: 7824949]
6. Huppa J, Axmann BM, Mörtelmaier MA, Lillemeier BF, Newell EW, Brameshuber M, Klein LO, Schütz GJ, Davis MM. TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. *Nature*. 2010; 463:963–7. [PubMed: 20164930]
7. Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N, Evavold BD, Zhu C. The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. *Nature*. 2010; 464:932–6. [PubMed: 20357766]
8. Bertoletti A, Costanzo A, Chisari FV, Levrero M, Artini M, Sette A, Penna A, Giuberti T, Fiaccadori F, Ferrari C. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. *J Exp Med*. 1994; 180:933–43. [PubMed: 7520476]
9. Bertoletti A, Sette A, Chisari FV, Penna A, Levrero M, De Carli M, Fiaccadori F, Ferrari C. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. *Nature*. 1994; 369:407–10. [PubMed: 8196768]
10. Klenerman P, Rowland-Jones S, McAdam S, Edwards J, Daenke S, Laloo D, Koppe B, Rosenberg W, Boyd D, Edwards A, Giangrande P, Phillips RE, McMichael AJ. Cytotoxic T-cell activity antagonized by naturally occurring HIV-1 Gag variants. *Nature*. 1994; 369:403–10. [PubMed: 7515165]
11. Johannis TM, Ertelt JM, Lai JC, Rowe JH, Avant RA, Way SS. Naturally occurring altered peptide ligands control Salmonella-specific CD4+ T cell proliferation, IFN-gamma production, and protective potency. *J Immunol*. 2010; 184:869–76. [PubMed: 20026741]
12. Gilbert SC, Plebanski M, Gupta S, Morris J, Cox M, Aidoo M, Kwiatkowski D, Greenwood BM, Whittle HC, Hill AV. Association of malaria parasite population structure, HLA, and immunological antagonism. *Science*. 1998; 279:1173–7. [PubMed: 9469800]
13. Domingo E, Holland JJ. RNA virus mutations and fitness for survival. *Annu Rev Microbiol*. 1997; 51:151–78. [PubMed: 9343347]
14. Kubota R, Soldan SS, Martin R, Jacobson S. An altered peptide ligand antagonizes antigen-specific T cells of patients with human T lymphotropic virus type I-associated neurological disease. *J Immunol*. 2000; 164:5192–8. [PubMed: 10799878]
15. Frasca L, Del Porto P, Tuosto L, Marinari B, Scotta C, Carbonari M, Nicosia A, Piccolella E. Hypervariable region 1 variants act as TCR antagonists for hepatitis C virus-specific CD4+ T cells. *J Immunol*. 1999; 163:650–8. [PubMed: 10395654]
16. Ciurea A, Hunziker L, Martinic MM, Oxenius A, Hengartner H, Zinkernagel RM. CD4+ T-cell-epitope escape mutant virus selected in vivo. *Nat Med*. 2001; 7:795–800. [PubMed: 11433343]

17. Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghayeb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science*. 2003; 302:659–62. [PubMed: 14576438]
18. Lee EA, Flanagan KL, Minigo G, Reece WH, Bailey R, Pinder M, Hill AV, Plebanski M. Dimorphic *Plasmodium falciparum* merozoite surface protein-1 epitopes turn off memory T cells and interfere with T cell priming. *Eur J Immunol*. 2006; 36:1168–78. [PubMed: 16619284]
19. Sewell AK, Harcourt GC, Goulder PJ, Price DA, Phillips RE. Antagonism of cytotoxic T lymphocyte-mediated lysis by natural HIV-1 altered peptide ligands requires simultaneous presentation of agonist and antagonist peptides. *Eur J Immunol*. 1997; 27:2323–9. [PubMed: 9341776]
20. Purbhoo MA, Sewell AK, Klenerman P, Goulder PJ, Hilyard KL, Bell JI, Jakobsen BK, Phillips RE. Copresentation of natural HIV-1 agonist and antagonist ligands fails to induce the T cell receptor signaling cascade. *Proc Natl Acad Sci USA*. 1998; 95:4527–32. [PubMed: 9539771]
21. Puglielli MT, Zajac AJ, van der Most RG, Dzuris JL, Sette A, Altman JD, Ahmed R. In vivo selection of a lymphocytic choriomeningitis virus variant that affects recognition of the GP33–43 epitope by H-2Db but not H-2Kb. *J Virol*. 2001; 75:5099–107. [PubMed: 11333891]
22. Wang S, Buchli R, Schiller J, Gao J, VanGundy RS, Hildebrand WH, Eckels DD. Natural epitope variants of the hepatitis C virus impair cytotoxic T lymphocyte activity. *World J Gastroenterol*. 2010; 16:1953–69. [PubMed: 20419832]
23. Plebanski M, Lee EA, Hannan CM, Flanagan KL, Gilbert SC, Gravenor MB, Hill AV. Altered peptide ligands narrow the repertoire of cellular immune responses by interfering with T-cell priming. *Nat Med*. 1999; 5:565–71. [PubMed: 10229235]
24. Haanen JB, Wolkers MC, Kruisbeek AM, Schumacher TN. Selective expansion of cross-reactive CD8(+) memory T cells by viral variants. *J Exp Med*. 1999; 190:1319–28. [PubMed: 10544203]
25. Haribhai D. Functional reprogramming of the primary immune response by t cell receptor antagonism. *J Exp Med*. 2004; 200:1371–82. [PubMed: 15557350]
26. Bouhdoud L, Villain P, Merzouki A, Arella M, Couture C. T-cell receptor-mediated anergy of a human immunodeficiency virus (HIV) gp120-specific CD4(+) cytotoxic T-cell clone, induced by a natural HIV type 1 variant peptide. *J Virol*. 2000; 74:2121–30. [PubMed: 10666241]
27. Ream RM, Sun J, Braciale TJ. Stimulation of naive CD8+ T cells by a variant viral epitope induces activation and enhanced apoptosis. *J Immunol*. 2010; 184:2401–9. [PubMed: 20139280]
28. Schnell FJ, Alberts-Grill N, Evavold BD. CD8+ T cell responses to a viral escape mutant epitope: active suppression via altered SHP-1 activity. *J Immunol*. 2009; 182:1829–35. [PubMed: 19201834]
29. Alam SM, Travers PJ, Wung JL, Nasholds W, Redpath S, Jameson SC, Gascoigne NR. T-cell-receptor affinity and thymocyte positive selection. *Nature*. 1996; 381:616–20. [PubMed: 8637599]
30. Rosette C, Werlen G, Daniels MA, Holman PO, Alam SM, Travers PJ, Gascoigne NR, Palmer E, Jameson SC. The impact of duration versus extent of TCR occupancy on T cell activation: a revision of the kinetic proofreading model. *Immunity*. 2001; 15:59–70. [PubMed: 11485738]
31. Chesla SE, Selvaraj P, Zhu C. Measuring two-dimensional receptor-ligand binding kinetics by micropipette. *Biophys J*. 1998; 75:1553–72. [PubMed: 9726957]
32. Huang J, Edwards LJ, Evavold BD, Zhu C. Kinetics of MHC-CD8 interaction at the T cell membrane. *J Immunol*. 2007; 179:7653–62. [PubMed: 18025211]
33. Sabatino JJ, Huang J, Zhu C, Evavold BD. High prevalence of low affinity peptide-MHC II tetramer-negative effectors during polyclonal CD4+ T cell responses. *J Exp Med*. 2011; 208:81–90. [PubMed: 21220453]
34. Jiang N, Huang J, Edwards LJ, Liu B, Zhang Y, Beal CD, Evavold BD, Zhu C. Two-stage cooperative T cell receptor-peptide major histocompatibility complex-CD8 trimolecular interactions amplify antigen discrimination. *Immunity*. 2011; 34:13–23. [PubMed: 21256056]
35. Montixi C, Langlet C, Bernard AM, Thimonier J, Dubois C, Wurbel MA, Chauvin JP, Pierres M, He HT. Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. *EMBO J*. 1998; 17:5334–48. [PubMed: 9736612]

36. Smyth LA, Ardouin L, Williams O, Norton T, Tybulewicz V, Kioussis D. Inefficient clustering of tyrosine-phosphorylated proteins at the immunological synapse in response to an antagonist peptide. *Eur J Immunol.* 2002; 32:3386–94. [PubMed: 12432569]
37. Wulfig C, Rabinowitz JD, Beeson C, Sjaastad MD, McConnell HM, Davis MM. Kinetics and extent of T cell activation as measured with the calcium signal. *J Exp Med.* 1997; 185:1815–25. [PubMed: 9151707]
38. Sumen C. T cell receptor antagonism interferes with MHC clustering and integrin patterning during immunological synapse formation. *J Cell Biol.* 2004; 166:579–90. [PubMed: 15314068]
39. Ehrlich LI, Ebert PJ, Krummel MF, Weiss A, Davis MM. Dynamics of p56lck translocation to the T cell immunological synapse following agonist and antagonist stimulation. *Immunity.* 2002; 17:809–22. [PubMed: 12479826]
40. Huang J, Sugie K, La Face DM, Altman A, Grey HM. TCR antagonist peptides induce formation of APC-T cell conjugates and activate a Rac signaling pathway. *Eur J Immunol.* 2000; 30:50–8. [PubMed: 10602026]
41. Huang J, Tilly D, Altman A, Sugie K, Grey HM. T-cell receptor antagonists induce Vav phosphorylation by selective activation of Fyn kinase. *Proc Natl Acad Sci USA.* 2000; 97:10923–9. [PubMed: 11005864]
42. Edwards LJ, Evavold BD. A unique unresponsive CD4+ T cell phenotype post TCR antagonism. *Cell Immunol.* 2010; 261:64–8. [PubMed: 20031121]
43. Dittel BN, Stefanova I, Germain RN, Janeway CA Jr. Cross-antagonism of a T cell clone expressing two distinct T cell receptors. *Immunity.* 1999; 11:289–98. [PubMed: 10514007]
44. Kilgore NE, Carter JD, Lorenz U, Evavold BD. Cutting edge: dependence of TCR antagonism on Src homology 2 domain-containing protein tyrosine phosphatase activity. *J Immunol.* 2003; 170:4891–5. [PubMed: 12734331]
45. Somani AK, Bignon JS, Mills GB, Siminovitch KA, Branch DR. Src kinase activity is regulated by the SHP-1 protein-tyrosine phosphatase. *J Biol Chem.* 1997; 272:21113–9. [PubMed: 9261115]
46. Stefanova I, Hemmer B, Vergelli M, Martin R, Biddison WE, Germain RN. TCR ligand discrimination is enforced by competing ERK positive and SHP-1 negative feedback pathways. *Nat Immunol.* 2003; 4:248–54. [PubMed: 12577055]
47. Plas DR, Johnson R, Pingel JT, Matthews RJ, Dalton M, Roy G, Chan AC, Thomas ML. Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science.* 1996; 272:1173–6. [PubMed: 8638162]
48. Brockdorff J, Williams S, Couture C, Mustelin T. Dephosphorylation of ZAP-70 and inhibition of T cell activation by activated SHP1. *Eur J Immunol.* 1999; 29:2539–50. [PubMed: 10458769]
49. Jiao H, Berrada K, Yang W, Tabrizi M, Platanias LC, Yi T. Direct association with and dephosphorylation of Jak2 kinase by the SH2-domain-containing protein tyrosine phosphatase SHP-1. *Mol Cell Biol.* 1996; 16:6985–92. [PubMed: 8943354]
50. Klingmuller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell.* 1995; 80:729–38. [PubMed: 7889566]
51. Cuevas B, Lu Y, Watt S, Kumar R, Zhang J, Siminovitch KA, Mills GB. SHP-1 regulates Lck-induced phosphatidylinositol 3-kinase phosphorylation and activity. *J Biol Chem.* 1999; 274:27583–9. [PubMed: 10488096]
52. Caron D, Savard PE, Doillon CJ, Olivier M, Shink E, Lussier JG, Faure RL. Protein tyrosine phosphatase inhibition induces anti-tumor activity: evidence of Cdk2/p27 kip1 and Cdk2/SHP-1 complex formation in human ovarian cancer cells. *Cancer Lett.* 2008; 262:265–75. [PubMed: 18234419]
53. Simoneau M, Boulanger J, Coulombe G, Renaud MA, Duchesne C, Rivard N. Activation of Cdk2 stimulates proteasome-dependent truncation of tyrosine phosphatase SHP-1 in human proliferating intestinal epithelial cells. *J Biol Chem.* 2008; 283:25544–56. [PubMed: 18617527]
54. Migone TS, Cacalano NA, Taylor N, Yi T, Waldmann TA, Johnston JA. Recruitment of SH2-containing protein tyrosine phosphatase SHP-1 to the interleukin 2 receptor; loss of SHP-1 expression in human T-lymphotropic virus type I-transformed T cells. *Proc Natl Acad Sci USA.* 1998; 95:3845–50. [PubMed: 9520455]

55. Zhang Z, Shen K, Lu W, Cole PA. The role of C-terminal tyrosine phosphorylation in the regulation of SHP-1 explored via expressed protein ligation. *J Biol Chem.* 2003; 278:4668–74. [PubMed: 12468540]
56. Liu Y, Kruhlak MJ, Hao JJ, Shaw S. Rapid T cell receptor-mediated SHP-1 S591 phosphorylation regulates SHP-1 cellular localization and phosphatase activity. *J Leukoc Biol.* 2007; 82:742–51. [PubMed: 17575265]
57. Wasserman HA, Beal CD, Zhang Y, Jiang N, Zhu C, Evavold BD. MHC variant peptide-mediated anergy of encephalitogenic T cells requires SHP-1. *J Immunol.* 2008; 181:6843–9. [PubMed: 18981103]
58. Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P, Klein LO, Davis MM, Chen CZ. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell.* 2007; 129:147–61. [PubMed: 17382377]
59. Daniels MA, Schober SL, Hogquist KA, Jameson SC. Cutting edge: a test of the dominant negative signal model for TCR antagonism. *J Immunol.* 1999; 162:3761–4. [PubMed: 10201890]
60. Yang W, Grey HM. Study of the mechanism of TCR antagonism using dual-TCR-expressing T Cells. *J Immunol.* 2003; 170:4532–8. [PubMed: 12707330]
61. Robertson JM, Evavold BD. Cutting edge: dueling TCRs: peptide antagonism of CD4+ T cells with dual antigen specificities. *J Immunol.* 1999; 163:1750–4. [PubMed: 10438905]
62. McNeil LK, Evavold BD. TCR reserve: a novel principle of CD4 T cell activation by weak ligands. *J Immunol.* 2003; 170:1224–30. [PubMed: 12538680]
63. Jones DS, Reichardt P, Ford ML, Edwards LJ, Evavold BD. TCR antagonism by peptide requires high TCR expression. *J Immunol.* 2008; 181:1760–6. [PubMed: 18641313]
64. Wasserman HA, Evavold BD. Induction of anergy by antibody blockade of TCR in myelin oligodendrocyte glycoprotein-specific cells. *J Immunol.* 2008; 180:7259–64. [PubMed: 18490725]
65. Labrecque N, Whitfield LS, Obst R, Waltzinger C, Benoist C, Mathis D. How much TCR does a T cell need? *Immunity.* 2001; 15:71–82. [PubMed: 11485739]
66. Harding CV, Unanue ER. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature.* 1990; 346:574–6. [PubMed: 2115981]
67. Demotz S, Grey HM, Sette A. The minimal number of class II MHC-antigen complexes needed for T cell activation. *Science.* 1990; 249:1028–30. [PubMed: 2118680]
68. Kimachi K, Croft M, Grey HM. The minimal number of antigen-major histocompatibility complex class II complexes required for activation of naive and primed T cells. *Eur J Immunol.* 1997; 27:3310–7. [PubMed: 9464819]
69. Liu GY, Fairchild PJ, Smith RM, Prowle JR, Kioussis D, Wraith DC. Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity.* 1995; 3:407–15. [PubMed: 7584132]
70. Bouneaud C, Kourilsky P, Bousso P. Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. *Immunity.* 2000; 13:829–40. [PubMed: 11163198]
71. Zehn D, Bevan MJ. T cells with low avidity for a tissue-restricted antigen routinely evade central and peripheral tolerance and cause autoimmunity. *Immunity.* 2006; 25:261–70. [PubMed: 16879996]
72. Tanchot C, Lemonnier FA, Perarnau B, Freitas AA, Rocha B. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science.* 1997; 276:2057–62. [PubMed: 9197272]
73. Ernst B, Lee DS, Chang JM, Sprent J, Surh CD. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity.* 1999; 11:173–81. [PubMed: 10485652]
74. Ji Q, Perchellet A, Gorman JM. Viral infection triggers central nervous system autoimmunity via activation of CD8+ T cells expressing dual TCRs. *Nat Immunol.* 2010; 11:628–34. [PubMed: 20526343]
75. Karin N, Mitchell DJ, Brocke S, Ling N, Steinman L. Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor

- antagonism and reduction of interferon gamma and tumor necrosis factor alpha production. *J Exp Med.* 1994; 180:2227–37. [PubMed: 7525850]
76. Nicholson L, Nicholson B, Greer JM, Sobel RA, Lees MB, Kuchroo VK. An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. *Immunity.* 1995; 3:397–405. [PubMed: 7584131]
 77. Brocke S, Gijbels K, Allegretta M, Ferber I, Piercy C, Blankenstein T, Martin R, Utz U, Karin N, Mitchell D, Veromaa T, Waisman A, Gaur A, Conlon P, Fairchild PJ, Wraith DC, O'Garra A, Fathman CG, Steinman L. Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. *Nature.* 1996; 379:343–6. [PubMed: 8552189]
 78. Kirshner SL, Zisman E, Fridkin M, Sela M, Mozes E. Altered peptide ligands of a myasthenogenic epitope as modulators of specific T-cell responses. *Scand J Immunol.* 1996; 44:512–21. [PubMed: 8947604]
 79. Faber-Elmann A, Paas-Rozner M, Sela M, Mozes E. Altered peptide ligands act as partial agonists by inhibiting phospholipase C activity induced by myasthenogenic T cell epitopes. *Proc Natl Acad Sci USA.* 1998; 95:14320–5. [PubMed: 9826698]
 80. Ikagawa S, Matsushita S, Chen YZ, Ishikawa T, Nishimura Y. Single amino acid substitutions on a Japanese cedar pollen allergen (Cry j 1)-derived peptide induced alterations in human T cell responses and T cell receptor antagonism. *J Allergy Clin Immunol.* 1996; 97:53–64. [PubMed: 8568138]
 81. Kinnunen T, Jutila K, Kwok WW, Rytönen-Nissinen M, Immonen A, Saarelainen S, Narvanen A, Taivainen A, Virtanen T. Potential of an altered peptide ligand of lipocalin allergen Bos d 2 for peptide immunotherapy. *J Allergy Clin Immunol.* 2007; 119:965–72. [PubMed: 17335888]
 82. Geluk A, van Meijgaarden KE, Roep BO, Ottenhoff TH. Altered peptide ligands of islet autoantigen Imogen 38 inhibit antigen specific T cell reactivity in human type-1 diabetes. *J Autoimmun.* 1998; 11:353–61. [PubMed: 9776713]
 83. Kim HJ, Antel JP, Duquette P, Alleva DG, Conlon PJ, Bar-Or A. Persistence of immune responses to altered and native myelin antigens in patients with multiple sclerosis treated with altered peptide ligand. *Clin Immunol.* 2002; 104:105–14. [PubMed: 12165271]
 84. Prakken BJ, Roord S, van Kooten PJ, Wagenaar JP, van Eden W, Albani S, Wauben MH. Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analog of an arthritis-related heat-shock protein 60 T cell epitope. *Arthritis Rheum.* 2002; 46:1937–46. [PubMed: 12124879]
 85. Ohnishi Y, Tsutsumi A, Matsumoto I, Goto D, Ito S, Kuwana M, Uemura Y, Nishimura Y, Sumida T. Altered peptide ligands control type II collagen-reactive T cells from rheumatoid arthritis patients. *Mod Rheumatol.* 2006; 16:226–8. [PubMed: 16906372]
 86. Boots AM, Hubers H, Kouwijzer M, den Hoed-vanZandbrink L, Westrek-Esselink BM, van Doorn C, Stenger R, Bos ES, van Lierop MJ, Verheijden GF, Timmers CM, van Staveren CJ. Identification of an altered peptide ligand based on the endogenously presented, rheumatoid arthritis-associated, human cartilage glycoprotein-39(263–275) epitope: an MHC anchor variant peptide for immune modulation. *Arthritis Res Ther.* 2007; 9:R71. [PubMed: 17645792]
 87. Yu Z, Maoui M, Zhao ZJ, Li Y, Shen SH. SHP-1 dephosphorylates 3BP2 and potentially downregulates 3BP2-mediated T cell antigen receptor signaling. *Febs J.* 2006; 273:2195–205. [PubMed: 16649996]
 88. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, Gran B, Eaton J, Antel J, Frank JA, McFarland HF, Martin R. 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. [PubMed: 11017150]
 89. Kappos L, Comi G, Panitch H, Oger J, Antel J, Conlon P, Steinman L. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The altered peptide ligand in relapsing MS study group. *Nat Med.* 2000; 6:1176–82. [PubMed: 11017151]
 90. Walter M, Philotheou A, Bonnici F, Ziegler AG, Jimenez R. No effect of the altered peptide ligand NBI-6024 on beta-cell residual function and insulin needs in new-onset type 1 diabetes. *Diabetes Care.* 2009; 32:2036–40. [PubMed: 19690081]

91. Crowe PD, Qin Y, Conlon PJ, Antel JP. NBI-5788, an altered MBP83–99 peptide, induces a T-helper 2-like immune response in multiple sclerosis patients. *Ann Neurol*. 2000; 48:758–65. [PubMed: 11079539]
92. Kim HJ, Antel JP, Duquette P, Alleva DG, Conlon PJ, Bar-Or A. Persistence of immune responses to altered and native myelin antigens in patients with multiple sclerosis treated with altered peptide ligand. *Clin Immunol*. 2002; 104:105–14. [PubMed: 12165271]
93. Pedotti R, Mitchell D, Wedemeyer J, Karpuz M, Chabas D, Hattab EM, Tsai M, Galli SJ, Steinman L. An unexpected version of horror autotoxicus: anaphylactic shock to a self-peptide. *Nat Immunol*. 2001; 2:216–22. [PubMed: 11224520]
94. Pedotti R, DeVoss JJ, Youssef S, Mitchell D, Wedemeyer J, Madanat R, Garren H, Fontoura P, Tsai M, Galli SJ, Sobel RA, Steinman L. Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination. *Proc Natl Acad Sci USA*. 2003; 100:1867–72. [PubMed: 12576552]
95. Liu E, Moriyama H, Abiru N, Miao D, Yu L, Taylor RM, Finkelman FD, Eisenbarth GS. Anti-peptide autoantibodies and fatal anaphylaxis in NOD mice in response to insulin self-peptides B: 9–23 and B:13–23. *J Clin Invest*. 2002; 110:1021–7. [PubMed: 12370280]
96. Margot CD, Ford ML, Evavold BD. Amelioration of established experimental autoimmune encephalomyelitis by an MHC anchor-substituted variant of proteolipid protein 139–151. *J Immunol*. 2005; 174:3352–8. [PubMed: 15749867]
97. Leech MD, Chung CY, Culshaw A, Anderton SM. Peptide-based immunotherapy of experimental autoimmune encephalomyelitis without anaphylaxis. *Eur J Immunol*. 2007; 37:3576–81. [PubMed: 18000952]
98. Ford ML, Evavold BD. Regulation of polyclonal T cell responses by an MHC anchor-substituted variant of myelin oligodendrocyte glycoprotein 35–55. *J Immunol*. 2003; 171:1247–54. [PubMed: 12874212]

Fig. 1.

a FRET-based two-dimensional binding assay. In this assay, T cells labeled with a fluorescent anti-TCR antibody were allowed to act with a planar membrane setup expressing MHC with fluorescently labeled peptide, ICAM1 and CD80. If no binding occurred, no fluorescent signal was observed from the acceptor fluorophore. If binding occurred, the two fluorescent molecules were juxtaposed closely enough to allow for the detection of the FRET signal. **b** Micropipette-based adhesion frequency assay. In this assay, red blood cells are coated with biotin, followed by streptavidin, followed by biotinylated peptide–MHC monomers. The pMHC-coated red blood cell is aspirated onto one micropipette while a T cell is aspirated onto a second micropipette. The cells are brought into contact and binding is observed upon retraction of the T cell by distension of the soft red blood cell membrane

Fig. 2.

At physiological levels of TCR expression, T cells are able to recognize a broad spectrum of ligands. When T cells express approximately 50% of normal TCR numbers, responses to weak ligands are diminished and antagonism is abolished, but responses to agonist stimulation is unaffected. At 5% of normal TCR numbers, responses to low-potency ligands are not observed; however, responses to agonist stimulation are unaffected

Fig. 3.

The stimulatory capacity of an antigenic peptide is determined by its interactions with both the MHC and the TCR. Altering the amino acids that interact with either aspect can dramatically alter the ability of a T cell to recognize the ligand. Peptides containing substitutions at TCR contacts have been termed altered peptide ligands (APL), while peptides containing substitutions at MHC anchor residues are known as MHC variant peptides (MVP)