Published in final edited form as:

Cell Immunol. 2010 ; 261(1): 64–68. doi:10.1016/j.cellimm.2009.11.002.

A unique unresponsive CD4+ T cell phenotype post TCR antagonism

Lindsay J. Edwards and Brian D. Evavold

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, USA.

Abstract

The functional outcomes of the T cell's interaction with the peptide:MHC complex can be dramatically altered by the introduction of a single amino acid substitution. Previous studies have described the varied effects of these altered peptide ligands (APL) on T cell responses. These outcomes of T cell interaction with an APL include the induction of clonal unresponsiveness (anergy) and inhibition of T cell responses (antagonism). The phenotype of peptide-induced anergy, i.e. low proliferation and low IL-2 production, has been extensively described, and a number of groups have demonstrated antagonism. However, the response of T cells to an agonist ligand after encountering an antagonistic stimulus has not been previously characterized. Here, we show that T cells post-antagonism fail to proliferate but produce large quantities of IL-2 upon stimulation with their wild type ligand. This unique phenotype is not due to differences in IL-2 receptor expression or rates of apoptosis, and cannot be overcome by the addition of recombinant IL-2. The response of CD4 T cells to agonist stimulation after encountering an antagonist is a novel phenotype, and is distinct from previously described forms of anergy.

Keywords

CD4 T cells; Cytokines; Immune evasion

Introduction

A T cell response is elicited following interaction of the T cell receptor (TCR) with its cognate ligand. The outcome of this interaction can be affected by introducing changes in the immunogenic peptide sequence [1,2]. Peptides in which amino acid substitutions have been made at a TCR contact residue are known as altered peptide ligands (APL)[1]. APL have an affinity for MHC similar to that of the wild type peptide and are classified based on the potency of the T cell response they elicit. Agonist peptides stimulate T cells to levels similar to that of the wild type peptide, while weak agonists stimulate suboptimal proliferative and cytokine responses. Partial agonists stimulate only a subset of effector functions [1]. This class of APL has been used to induce anergy, a state of unresponsiveness defined by a lack of proliferation

© 2009 Elsevier Inc. All rights reserved.

Corresponding Author: Brian D. Evavold, PhD Department of Microbiology and Immunology Emory University School of Medicine 1510 Clifton Rd Atlanta, GA 30322 Phone: 404-727-3393 Fax: 404-727-3659 evavold@microbio.emory.edu.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and IL-2 production in response to an immunogenic stimulus [1]. An anergic phenotype can also be induced by lack of costimulation [3-5], a low dose agonist ligand [6], or unstable peptides containing substitutions at their MHC anchor residues [7,8].

In addition to partial agonists, which have been shown to induce T cell anergy, another category of APL that dampens the T cell response is antagonists. Antagonist ligands have been defined *in vitro* by their inability to stimulate T cell responses when presented alone and their ability to inhibit the T cell response to wild type peptide when both the antagonist and agonist are present [2,9,10]. Epitopes behaving as antagonists have been identified in a number of infections, including HIV [11,12], Hepatitis B and C [13,14] and malaria [15]. Additionally, antagonist ligands may play a role in maintaining peripheral tolerance [16].

Although the phenomenon of antagonism has been extensively described in a number of systems, the exact mechanism by which antagonism occurs remains unclear. Studies utilizing dual-receptor expressing T cells suggest that the mechanism is one of active inhibition, rather than a passive effect of competition [17-20]. Further studies have indicated that antagonism impacts the signaling cascades normally activated upon T cell stimulation, activating a negative feedback loop involving the phosphatase SHP-1 [21-23].

The response of a T cell under antagonist conditions is similar to the response of anergic cells to wild type stimulation. In both these conditions, T cells fail to proliferate and secrete little or no IL-2. To define the fate of these cells following rechallenge with agonist ligand has not been considered. We examine the phenotype of previously antagonized T cells in response to wild type stimulation. We have found that the phenotypes of anergy and antagonism differ dramatically when cells are rechallenged with wild type ligand post-antagonism. Both groups fail to proliferate upon stimulation with wild type peptide, but we demonstrate that previously antagonized cells make significant amounts of cytokines including IL-2. This distinct difference in cytokine production is a stark and important contrast between the phenotypes of antagonism and anergy. Thus, we describe a unique response pattern of T cells, a phenotype distinct from previously described forms of anergy.

Materials and Methods

Mice

3.L2 TCR transgenic mice were used at 6-12 weeks of age. The 3.L2 TCR is specific for Hb64-76 presented by I-E^k [24]. Mice were bred and housed in the Emory University Department of Animal Resources facility according to federal guidelines.

Peptides

The wild type peptide, Hb64-76 (GKKVITAFNEGLK) and the antagonist 72I (GKKVITAFIEGLK) were purchased from Invitrogen (Carlsbad, CA). 72I is a single amino acid substitution, Asn (N)→Ile (I) at position 72.

Antagonism

Antagonism was performed as previously described by Sette and colleagues [2]. Briefly, for antagonist conditions, cells were pre-pulsed with 1μM Hb64-76 for 2h at 37°C, washed twice in HBSS, then cultured with 10μM 72I or the indicated concentration of peptide for proliferation assays.

Cells and Reagents

3.L2 spleen cells (2×10⁶/well) were cultured *ex vivo* with 1μM Hb64-76, pre-pulse only (1μM Hb64-76 for 2h, at 37°C) or under antagonist conditions (1μM Hb64-76 pre-pulse,

10 μ M 72I continuously) with 10 pg/mL IL-2 for 7 days in 24-well plates. Live cells were purified over a Ficol gradient (Mediatech) and were restimulated with irradiated syngeneic splenocytes (2000 rad) and the indicated concentrations of peptide. Culture media consisted of RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine, .01M Hepes buffer, 100 μ g/mL gentamicin (Mediatech, Herndon, VA) and 5 $\times$ 10⁻⁵ M 2-mercaptoethanol (Sigma, St. Louis, MO).

Proliferation Assay

Naïve 3.L2 splenocytes (3 $\times$ 10⁵/well) or cultured T cells (5 $\times$ 10⁴/well) and irradiated syngeneic splenocytes (2000 rad, 5 $\times$ 10⁵/well) were cultured in 96-well plates with the indicated concentration of peptide at 37°C. Where indicated, recombinant IL-2 (BD Pharmingen, San Diego, CA) was added at 3.5ng/well. After 48h in culture, 0.4 μ Ci/well of [³H] thymidine was added. After an additional 18h, cells were harvested on a FilterMate harvester (PerkinElmer Life and Analytical Sciences, Wellesly, MA) and analyzed on a Matrix 96 Direct Beta Counter (PerkinElmer).

Cytokine ELISA

After one week in culture under the indicated conditions, 3.L2 T cells (1 $\times$ 10⁶/well) were stimulated with irradiated syngeneic splenocytes (2000 rad, 5 $\times$ 10⁶/well) and the indicated concentration of peptide for 24h (IL-2) or 48h (IFN- γ). Supernatants were incubated in triplicate on microtiter plates that had been coated with 50 μ l of purified anti-IL-2 (5 μ g/mL, clone JES6-1A12, BD Pharmingen) or anti-IFN- γ (5 μ g/mL, clone R4-6A2, BD Pharmingen) overnight at 4°C. Recombinant IL-2 or IFN- γ (BD Pharmingen) was used as a standard. Captured cytokines were detected using biotinylated anti-IL-2 (JES6-5H4, BD Pharmingen; 100 μ g/mL, 100 μ l/well) or biotinylated anti-IFN- γ (clone XMG1.2, BD Pharmingen; 100 μ g/mL, 100 μ l/well) followed by alkaline phosphatase-conjugated avidin (Sigma, St. Louis, MO) and p-nitrophenylphosphate (pNPP) substrate (BioRad, Hercules, CA). Colorimetric change was measured at 405 nm on a Microplate Autoreader (Biotek Instruments, Winooski, VT).

Flow Cytometry

3.L2 T cells were stained with anti-CD25 (IL-2R α)-FITC (clone PC61), anti-V β 8.3-PE (clone 1B3.3) and anti-CD4-APC (clone RM4-5; all BD Pharmingen) at 24h and 48h after restimulation. Alternatively, cells were stained with PE conjugated CD122 (clone TM-b1) or CD132 (clone 4G3). For intracellular cytokine staining, cells were stimulated for 6h, then fixed and permeabilized with Caltag Fix and Perm cell permeabilization kit per the manufacturers instructions (Caltag, San Diego, CA). Permeabilized cells were stained with anti-CD4-PerCP and anti-IL-2-APC (clone JES6-5H4) or anti-IL-4-APC (clone 11B11; all BD Pharmingen). Annexin V and 7-AAD staining was performed according to manufacturer's instructions (BD Pharmingen). Flow cytometry was performed on a BD FACSCalibur (Franklin Lakes, NJ) and data were analyzed using FlowJo Software (Tree Star, San Carlos, CA). Data are gated on V β 8.3+ or CD4+ cells as indicated.

Statistical analysis

Data were analyzed using GraphPad Prism. Statistical significance was determined by t test or ANOVA, as indicated in the figure legends.

Results

Characterization of Antagonist Peptide

The response of 3.L2 T cells to both Hb64-76 and the APL 72I has been previously described [24,25]. As expected, the antagonist peptide alone induced minimal proliferation of 3.L2 T

cells, whereas stimulation with the wild type peptide resulted in a dose dependent proliferative response (Fig. 1A, $p=0.0184$). Additionally, cells pre-pulsed with the wild type peptide were effectively antagonized (>95%) by addition of 72I (Fig. 1B). Thus the APL 72I acts as an antagonist, as it fails to stimulate proliferation when presented alone, and inhibits the T cell response to wild type peptide in a dose-dependent manner when both wild type and 72I are presented.

Phenotype Post-antagonism

Although T cell antagonism has been extensively described [10,17,19,26-28], the response of these antagonized cells to subsequent stimulation with wild type ligand has not yet been described. Previous studies have shown that under antagonist conditions, T cells exhibit a dose dependent decrease in proliferation to wild type stimulus [10,17-19,26-28], altered cytokine production [1,29], and altered signaling [10,12,18]. In order to assess the effects of T cell antagonism on subsequent T cell function, we have characterized the proliferative and cytokine responses of previously antagonized T cells to rechallenge with wild type ligand.

After 7d in culture with either wild type peptide, pre-pulse alone or under antagonist conditions, cells were restimulated with wild type peptide. Cells cultured on wild type peptide show characteristic, dose-dependent proliferation upon restimulation with wild type peptide. However, cells cultured on the antagonist condition show significantly blunted proliferation relative to wild type cultured cells (Fig. 2A, $p<0.05$). Antagonized cells secrete near normal or slightly elevated levels of the effector cytokine IFN- γ after 48h of wild type stimulation (Fig. 2B, $p=0.3878$).

The failure of pre-pulse only and antagonized cells to proliferate in response to wild type stimulation suggests a lack of IL-2, as is the case in classical anergy. As expected, cells cultured on pre-pulse alone produce little IL-2 after 24h of stimulation (Fig. 3, prepulse vs. antagonist, $p<0.001$). The lack of IL-2 production in the pre-pulse only condition coupled with the hypoproliferative response defines these cells as anergic. This is consistent with previous data indicating that anergy can be induced by a low dose of agonist ligand [6]. Antagonized cells, however, secrete significantly greater amounts of IL-2 relative to wild type cultured cells after 24h of restimulation with wild type peptide (Fig. 3, Hb64-75 vs. antagonist $p<0.01$). This increased production of IL-2 contrasts sharply with the lack of proliferation exhibited by the antagonized T cells. This phenotype is clearly distinct from previously described forms of anergy, in which IL-2 production is blunted or entirely absent.

IL-2 and Antagonism

The differences in IL-2 production in these groups do not appear to be due to cell death, as no significant differences in Annexin V or 7-AAD staining were observed at 48h. (Fig. 4A). Additionally, these cells have not been skewed to a Th2 phenotype, as they are not producing IL-4 as measured by cytokine capture ELISA or intracellular cytokine staining (data not shown). The disconnect between IL-2 production and proliferation that is observed in antagonized cells upon restimulation with wild type peptide suggests a disruption of the IL-2 signaling pathway. To address the mechanism of this unique phenotype, IL-2R levels were determined by flow cytometry. These studies indicated that expression of CD25, the high affinity IL-2R, is similar in both antagonized and wild type cultured cells across a range of doses at both 24h and 48h after restimulation (Fig. 4B, $p=0.4112$). Expression of CD122 and CD132 is also similar (data not shown), which implies that the disruption in the IL-2 pathway is not at the level of receptor expression.

Additionally, the anergic phenotype of cells cultured on pre-pulse alone can be overcome by the addition of exogenous IL-2 (Fig. 5; Hb64-76 vs. prepulse $p>0.05$), as has been described

in other cases of low dose anergy [6]. However, proliferation of antagonized cells cannot be rescued in this way (Fig. 5, Hb64-76 vs. antagonist $p < 0.05$). These data further support the idea that a blockage in the IL-2 signaling pathway is responsible for the phenotype of hypoproliferation yet enhanced IL-2 production we have observed post-antagonism.

Discussion

In this study we have found that after stimulation under antagonist conditions, T cells respond to wild type antigen by producing high levels of IL-2 while also exhibiting a marked lack of proliferation. This lack of proliferation is similar in both anergized and antagonized cells, although these two groups of cells exhibit significant differences in their ability to produce and respond to IL-2. These findings indicate that antagonism is a distinctly different phenomenon than known forms of anergy.

Previous studies have described anergy induced by lack of costimulation [3-5,30], low dose of agonist peptide [6], APL [1] and peptides containing substitutions at their MHC anchor residues [7,8]. Lack of proliferation and IL-2 production are key characteristics of all of the forms of anergy. Our data show that antagonized cells exhibit a phenotype of hypoproliferation and normal IFN- γ production that is similar to anergic cells. In most forms of anergy, including that induced by low doses of wild type peptide, proliferation can be rescued by the addition of exogenous IL-2 [1,3]. However, the lack of proliferation in antagonized cells is not reversible by the addition of exogenous IL-2. The failure of IL-2 to rescue the hypoproliferative phenotype post-antagonism is similar to APL induced anergy, suggesting that these phenotypes may share some mechanistic features. In most forms of anergy, for example the pre-pulse only condition, cells are unable to produce IL-2, but they remain able to respond to it [6]. In contrast, antagonized cells secrete large quantities of IL-2, but have been rendered unresponsive to this growth factor. Furthermore, our data demonstrate that the presence of an antagonist is sufficient to at least partially overcome low dose induced anergy, suggesting that this phenotype is mechanistically distinct from low dose induced anergy.

The failure of antagonized cells to respond to IL-2 from both endogenous and exogenous sources is not due to differences in apoptosis or decreased expression of IL-2R, as we have found that all three chains of the high affinity IL-2R are expressed at similar levels in both antagonized and wild type cultured cells. These data suggest an intracellular disruption of the IL-2 signaling pathway as the mechanism mediating the phenotype post-antagonism.

Several pathogens have been shown to generate escape epitopes that function as T cell antagonists, including HIV [11,12], Hepatitis B and C [13,14] and malaria [15]. In the context of one of these infections, the phenotype of T cells that encounter their cognate ligand after interacting with an antagonist mutant epitope could dramatically impact the course of the infection. The data presented herein would suggest that these T cells would produce large quantities of IL-2, perhaps expanding or activating regulatory T cells [31,32], and thereby potentiating the efficacy of the pathogen's escape strategy. Alternatively, this cytokine production could result in non-specific bystander activation, which could also result in subsequent failure to clear the infection and/or immunopathology. The phenotype that we have observed post-antagonism underscores the role of T cell epitope mutation in the escape of chronic infections from control by the immune system.

T cell unresponsiveness can result from many different pathways, as evidenced by the diverse mechanisms of anergy that have been previously described. In T cells that are anergic, the proliferative and IL-2 responses are significantly reduced. Similarly, T cell antagonism also results in a refractory state in which proliferation is blocked. Unlike classical anergy, however, antagonized cells retain their ability to secrete the growth factor IL-2, despite their inability to

respond to it. This inability to respond is similar to APL induced anergy, as well as MHC variant peptide induced anergy, which has been shown to be mediated by the negative regulatory phosphatase SHP-1 [33]. SHP-1 has also been shown to play a role in the early stages of antagonism [21,25]. Furthermore, at least one report has demonstrated association of SHP-1 with the IL-2 receptor complex [34]. Taken together, these data suggest that this phosphatase may play an important role in regulating the phenotype that we have described. In this way, the phenotype post-antagonism may be mechanistically similar to anergy induced by altered peptide ligands, as neither of these states can be overcome by IL-2.

The data presented herein demonstrate that the response of T cells post-antagonism is distinct from previously described forms of anergy, as antagonized cells respond to wild type stimulation by producing large quantities of IL-2, whereas anergized cells produce very little or no IL-2. The increased production of IL-2 by antagonized T cells in response to wild type stimulus contrasts sharply with the failure of these cells to proliferate. This cytokine hyperproduction coupled with the inability to proliferate represents a unique phenotype in T cell biology.

Acknowledgments

This work was supported by NIH R01 awards AI056017 and NS062358. The authors wish to thank the members of the Evavold Lab for critical reading of the manuscript.

References

1. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu Rev Immunol* 1996;14:1–27. [PubMed: 8717505]
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;68:625–634. [PubMed: 1739971]
3. Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 1987;165:302–19. [PubMed: 3029267]
4. Jenkins MK, Chen C, Jung G, Mueller DL, Schwartz RH. Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J Immunol* 1990;144:16–22. [PubMed: 2153162]
5. Quill H, Schwartz RH. Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferation nonresponsiveness. *J Immunol* 1987;138:3704–3712. [PubMed: 3035012]
6. Korb LC, Mirshahidi S, Ramyar K, Akha AAS, Sadegh-Nasseri S. Induction of T Cell Anergy by Low Numbers of Agonist Ligands. *J Immunol* 1999;163:6401–6409. [PubMed: 10352253]
7. 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]
8. 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]
9. Jameson SC, Bevan MJ. T cell receptor antagonists and partial agonists. *Immunity* 1995;2:1–11. [PubMed: 7600296]
10. LaFace DM, Couture C, Anderson K, Shih G, Alexander J, Sette A, Mustelin T, Altman A, Grey HM. Differential T cell signaling induced by antagonist peptide-MHC complexes and the associated phenotypic responses. *J Immunol* 1997;158:2057–2064. [PubMed: 9036949]
11. Norris PJ, Stone JD, Anikeeva N, Heitman JW, Wilson IC, Hirschhorn DF, Clark MJ, Moffett HF, Cameron TO, Sykulev Y, Stern LJ, Walker BD. Antagonism of HIV-specific CD4+ T cells by C-terminal truncation of a minimum epitope. *Mol Immunol* 2006;43:1349–57. [PubMed: 16216327]

12. 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 U S A* 1998;95:4527–32. [PubMed: 9539771]
13. Burroughs NJ, Rand DA. Dynamics of T-cell antagonism: enhanced viral diversity and survival. *Proc Biol Sci* 1998;265:529–35. [PubMed: 9569671]
14. 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]
15. 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]
16. Haque SJ, Harbor P, Tabrizi M, Yi T, Williams BR. Protein-tyrosine phosphatase Shp-1 is a negative regulator of IL-4- and IL-13-dependent signal transduction. *J Biol Chem* 1998;273:33893–6. [PubMed: 9852037]
17. 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]
18. 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]
19. 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]
20. 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]
21. 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]
22. Wylie DC, Das J, Chakraborty AK. Sensitivity of T cells to antigen and antagonism emerges from differential regulation of the same molecular signaling module. *Proc Natl Acad Sci U S A* 2007;104:5533–8. [PubMed: 17360359]
23. Lipniacki T, Hat B, Faeder JR, Hlavacek WS. Stochastic effects and bistability in T cell receptor signaling. *J Theor Biol* 2008;254:110–22. [PubMed: 18556025]
24. Kersh GJ, Donermeyer DL, Frederick KE, White JM, Hsu BL, Allen PM. TCR transgenic mice in which usage of transgenic alpha- and beta-chains is highly dependent on the level of selecting ligand. *J Immunol* 1998;161:585–93. [PubMed: 9670931]
25. 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]
26. Alexander J, Ruppert J, Snoke K, Sette A. TCR antagonism and T cell tolerance can be independently induced in a DR-restricted, hemagglutinin-specific T cell clone. *Int Immunol* 1994;6:363–367. [PubMed: 8186187]
27. Bachmann MF, Speiser DE, Zakarian A, Ohashi PS. Inhibition of TCR triggering by a spectrum of altered peptide ligands suggests the mechanism for TCR antagonism. *Eur J Immunol* 1998;28:3110–9. [PubMed: 9808179]
28. Gebe JA, Masewicz SA, Kochik SA, Reijonen H, Nepom GT. Inhibition of altered peptide ligand-mediated antagonism of human GAD65-responsive CD4+ T cells by non-antagonizable T cells. *Eur J Immunol* 2004;34:3337–45. [PubMed: 15549775]
29. Takato-Kaji R, Totsuka M, Ise W, Nishikawa M, Hachimura S, Kaminogawa S. T-cell receptor antagonist modifies cytokine secretion profile of naive CD4+ T cells and their differentiation into type-1 and type-2 helper T cells. *Immunol Lett* 2005;96:39–45. [PubMed: 15585306]
30. Jenkins MK, Taylor PS, Norton SD, Urdahl KB. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J Immunol* 1991;147:2461–6. [PubMed: 1717561]
31. Furtado GC, de Lafaille M. A. Curotto, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J Exp Med* 2002;196:851–7. [PubMed: 12235217]

32. Yagi H, Nomura T, Nakamura K, Yamazaki S, Kitawaki T, Hori S, Maeda M, Onodera M, Uchiyama T, Fujii S, Sakaguchi S. Crucial role of FOXP3 in the development and function of human CD25⁺CD4⁺ regulatory T cells. *Int Immunol* 2004;16:1643–56. [PubMed: 15466453]
33. 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]
34. 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 U S A* 1998;95:3845–50. [PubMed: 9520455]

Figure 1.

72I acts as a TCR antagonist. A. Splenocytes were cultured with indicated concentrations of wild type peptide (Hb64-76) or antagonist peptide (72I) for 48h. Cells were cultured an additional 18h with [3 H] thymidine and proliferation was measured by [3 H] thymidine incorporation. $p=0.0184$ by t test. B. Splenocytes were cultured on the indicated doses of antagonist peptide (72I) following a 2h pre-pulse with 1μM wild type peptide. The dashed line indicates proliferation caused by the pre-pulse alone. Proliferation was assayed as in part A. Assays were performed in duplicate and data are representative of 5 independent experiments.

Figure 2.

Antagonism causes blunted proliferation but normal IFN- γ production in response to wild type peptide. A. After 7d in culture with either wild type peptide ($1\mu\text{M}$) or under antagonist conditions ($1\mu\text{M}$ wild type prepulse plus continuous culture with $10\mu\text{M}$ antagonist peptide), and proliferation was assayed. Cells were stimulated with irradiated syngeneic splenocytes as antigen presenting cells and the indicated concentrations of wild type peptide (Hb64-76) for 48h. Cells were cultured an additional 18h with [^3H] thymidine and proliferation was measured by [^3H] thymidine incorporation. * indicates $p < 0.05$ by t test. Assays were performed in duplicate. Data are representative of 4 independent experiments. B. Cells were cultured and restimulated as described in A. IFN- γ production was assayed by cytokine capture ELISA 48h

after restimulation. Differences are not statistically significant by t test ($p=0.3878$). Assays were performed in triplicate and data are representative of 3 independent experiments.

Figure 3.

Antagonized cells exhibit altered IL-2 production. Cells were cultured 7d with either wild type peptide (1 μ M) or under antagonist conditions (1 μ M wild type prepulse plus continuous culture with 10 μ M antagonist peptide) and then restimulated for 24h with irradiated syngeneic splenocytes and the indicated doses of wild type peptide. IL-2 production was assayed by cytokine capture ELISA. Data were analyzed by ANOVA with Bonferroni's Multiple Comparison Test. * p<0.01, ** p<0.001, ns=not significant. Assays were performed in triplicate and data are representative of 3 independent experiments.

Figure 4.

Antagonism does not induce apoptosis or alter IL-2R expression. A. After 7d in culture on either wild type peptide (1 μ M) or under antagonist conditions (1 μ M wild type prepulse plus continuous culture with 10 μ M antagonist peptide), cells were restimulated with irradiated syngeneic splenocytes and 10 μ M wild type peptide. At 48h, cells were stained for Annexin V and 7-AAD. B. After 7d in culture on either wild type peptide (1 μ M) or under antagonist conditions (1 μ M wild type pre-pulse plus continuous culture with 10 μ M antagonist peptide), cells were restimulated with irradiated syngeneic splenocytes and 10 μ M wild type peptide. Cells were stained with anti-CD25-FITC at 24h after restimulation and analyzed by flow cytometry. All data were gated on V β 8.3+ cells. Differences are not significant by t test ($p=0.4112$). Data are representative of 3 independent experiments.

Figure 5.

Exogenous IL-2 restores proliferation of pre-pulsed but not antagonized cells. Cells were cultured for 7d on either wild type peptide (1 μM), under antagonist conditions (1 μM wild type prepulse plus continuous culture with 10 μM antagonist peptide) or pre-pulsed with 1 μM wild type and then washed. Cells were restimulated with irradiated syngeneic splenocytes and indicated amounts of wild type peptide with or without the addition of recombinant IL-2 for 48h. Cells were cultured an additional 18h with [³H] thymidine and proliferation was measured by [³H] thymidine incorporation. Differences between Hb64-76 and prepulse only were not significant. Differences between Hb64-76 and antagonist $p < 0.05$. Data were analyzed by ANOVA with Dunnett's Multiple Comparison Test. Assays were performed in duplicate and data are representative of 3 independent experiments.