# Cutting Edge: Immunoregulation of Th Cells by Naturally Processed Peptide Antagonists[1]

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**Th cells recognize protein Ags as short peptides bound to MHC class II molecules. Altered peptide ligands can antagonize (inhibit) T cell responses to stimulatory peptides. Peptides generated by APC may contain peptide flanking residues (PFR), which lie outside the minimal binding epitope and can be recognized by the TCR. Our data show that PFR-dependent T cells were found to be potently antagonized by peptides that lack PFR and responded poorly to native protein or the immunogenic epitope delivered by a recombinant influenza virus. These data provide the first evidence that Ag processing generates both stimulatory and antagonist peptides from a single immunogenic epitope, an observation that may have important implications for T cell immunoregulation and autoimmunity. *The Journal of Immunology*, 1999, 162: 1–4.**

Antigenic epitopes are presented on MHC class II molecules as large nested sets of peptides that contain the same minimal MHC binding sequence but vary in the length of their $NH_2$ and COOH termini (1). Natural processing of the H-2A$^k$-restricted, immunodominant epitope of the model Ag hen egg lysozyme (HEL 52–60)[3] generates peptides that either contain or lack a C-terminal peptide flanking residue (PFR), Trp 62 (2, 3). This residue, which lies outside the minimal peptide binding epitope but within the MHC groove, is highly solvent exposed (4).

We have recently shown that T cells can directly recognize and are highly dependent on this PFR, profoundly effecting TCR-V$\beta$ usage and peptide immunogenicity (5). Numerous studies have shown that altered peptide ligands can prevent or modulate T cell function (6–8). Substitution of peptide residues can alter recognition by the TCR and generate peptides that either inhibit T cell responses to the agonist peptide (antagonist peptides) or have no effect on responses to the agonist peptide (null peptides). Given that Ag processing of HEL can simultaneously generate peptides that contain and lack PFR, can exposure of PFR-dependent T cells to this naturally processed mixture of peptides modulate their function?

## Materials and Methods

### *Production of rHEL*

Wild-type HEL and mutants were generated by recombinant PCR and cloned into pPIC-9 (Invitrogen, San Diego, CA). rHEL was made in *Pichia pastoris* yeast according to the manufacturers instructions (Invitrogen). This gave rise to a soluble, secreted product with six additional amino acids (EAEAYG) at the N terminus before the start of the native protein. T cell responses to the recombinant and native HEL (L6876; Sigma, St. Louis, MO) were indistinguishable. rHEL protein was purified from yeast broth by conventional chromatography using SP Sepharose (Pharmacia, Piscataway, NJ), CHT (Bio-Rad, Richmond, CA), and Superdex 75 (Pharmacia) run on a BioLogic Chromatography System (Bio-Rad). The purified protein gave a single band on silver-stained SDS-PAGE gels and a single species of the correct m.w. by mass spectrometry. A detailed production and purification protocol is available on request (dario.vignali@stjude.org). In addition to the wild-type rHEL (rHEL.wt), two mutants were made. rHEL.W62/63A is the complete HEL protein with the two PFR W62 and W63 replaced with alanine. rHEL.L56F.W62Y has the two TCR contact residues, L56 and W62, which are the only residues in this epitope that differ between HEL and ML replaced with the analogous ML residues phenylalanine and tyrosine, respectively.

### *Ag presentation and antagonism assays*

Ag presentation assays were performed essentially as described elsewhere (5, 9). Briefly, T cell hybridomas were stimulated with synthetic peptides (Center for Biotechnology core facility at St. Jude Children's Research Hospital, or Chiron Technologies, Emeryville, CA (Mimotopes)) at the concentrations indicated, together with LK35.2 as APC (murine B cell lymphoma; H-2A$^{kd}$). After 24 h, supernatants were removed for the estimation of IL-2 secretion against a recombinant murine IL-2 standard (Genzyme, Cambridge, MA) by culturing with the IL-2-dependent T cell line CTLL-2. Antagonism assays were set up in the same way except that the APC were first prepulsed with the agonist, washed three times, and then put into the assay with the concentration of antagonist peptide indicated.

In some experiments, the following reagents were also used: 1) Various concentrations and combinations of the rHEL proteins described above. 2) LK.HEL is the LK35.2 B cell lymphoma transfected with HEL 1–80 attached to H-2K$^k$ transmembrane and cytoplasmic tail (10). It was used instead of the LK35.2 cells as APC with or without the addition of 3 $\mu$M of rHEL.W62/63A. 3) HEL-flu is HEL 46–63 inserted into the neuraminidase stalk of influenza A virus (A/WSN/33 (H1N1)) (11). LK35.2 cells plus HEL-flu was added per well, with or without the addition of 3 $\mu$M of rHEL.W62/63A.

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[3] Abbreveations used in the paper: HEL, hen egg lysozyme; PFR, peptide flanking residues.



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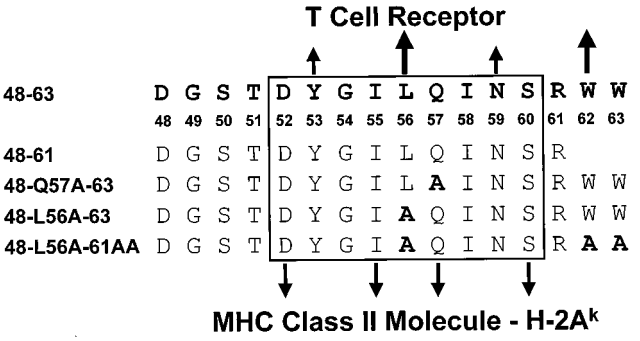


**FIGURE 1.** The immunodominant epitope of HEL for H-2A$^k$. This schematic depicts the wild-type peptides, HEL 48–63 and 48–61, and three alanine analogues that were used in this study. The box encompasses the putative minimal MHC binding epitope, with arrows indicating residues that have been suggested to bind either the MHC or TCR molecules by functional and structural studies (4, 5, 25, 26). An additional two residues at the N- and C-terminal ends of the peptide (S50, T51, R61, W62) are still within the MHC class II peptide binding groove. Although Q57 points down into the P6 pocket, substitutions at this position do not affect binding of the peptide to H-2A$^k$. The ability of 48-L56A-63 to act as a peptide antagonist is presumably due to subtle changes in the position of the immunodominant TCR contact residue L56.

### *CD3ζ tyrosine phosphorylation analysis*

CD3ζ tyrosine phosphorylation analysis was determined as previously described (12). Briefly, LK35.2 cells pulsed with 3 μM peptide were mixed with T cell hybridomas and incubated at 37°C for 5 min. The cell pellet was lysed in 1% Brij 97 (polyoxyethylene 10 oleyl ether; Sigma) at room temperature (RT) for 1 h and immunoprecipitated with a rabbit anti-CD3ζ antisera (2 μl, No. 387, gift from Larry Samelson, National Institutes of Health, Bethesda, MD) for 2 h at RT followed by 25 μl of protein A-Sepherose (Pharmacia) for 1 h at RT. Eluted proteins were resolved by SDS-PAGE and transferred onto poly(vinylidene difluoride) membrane (Schleicher & Schuell, Keene, NH). Blots were blocked with 5% BSA (Boehringer Mannheim, Indianapolis, IN) in TBS-T, and tyrosine phosphorylation was detected with biotinylated 4G10 for 60 min at RT (0.1 μg/ml; Upstate Biotechnology, Lake Placid, NY), followed by 1:12,000 dilution of streptavidin-horseradish peroxidase preformed complexes (60 min at RT) (Amersham, Arlington Heights, IL). Blots were developed using ECLplus (Amersham). To detect the original protein, blots were stripped in 100 mM of 2-ME (Bio-Rad), 2% SDS, and 62.5 mM of Tris-HCl (pH 6.7) for 30 min at 50°C; washed three times; and blocked with 5% nonfat dry milk in TBS-T at 4°C overnight. Blots were probed with the rabbit anti-CD3ζ antisera for 90 min at RT (1:200), followed by protein A-horseradish peroxidase for 60 min at RT (1:10,000) (Amersham). Blots were developed as described above.

## Results and Discussion

Unlike T cell clones, not all T cell hybridomas can be antagonized by altered peptide ligands (13, 14). We have capitalized on this observation and selected two pairs of PFR-dependent T cell hybridomas that respond identically to HEL 48–63, as determined by IL-2 production and peptide sensitivity, but differ in their susceptibility to peptide antagonism (WW241+ [TCR.Vα8Jα33/Vα16Jα33.Vβ8.3] vs WW236− [TCR.Vα8Jα22.Vβ8.3], and WW157+ [TCR.Vα4Jα23.Vβ14] vs WW221− [TCR.Vα4Jα45.Vβ8.3], where the suffix indicates their ability (+) or inability (−) to be antagonized) (5). Initially, the ability of a panel of analogue peptides to antagonize T cell responses to the agonist HEL 48–63 was tested (these included: 48-L56X-63 (where X was replaced with either N, D, Q, E, I, K, F, S, T, Y, or V), 48-L56A-61AA, and 48-Q57A-63; Fig. 1). HEL 48-Q57A-63 was identified as the best analogue peptide antagonist for the T cell hybridomas WW241+ and WW157+ (Figs. 1 and 2*A*). HEL 48-L56A-63 and 48-L56A-61AA, which bind to H-2A$^k$ with an affinity comparable to the agonist, had no effect on T cell function and are thus null peptides. In contrast, WW236− and WW221− were not antagonized by any of the peptides tested. Strikingly, WW241+ and WW157+ were strongly antagonized by HEL 48–61 in a dose-dependent manner (Fig. 2*A*). An equimolar concentration of the agonist and the 48–61 antagonist gave a 29.0% and 81.5% reduction in IL-2 response, respectively (compared with 0% and 43.1%, respectively, when 48-Q57A-63 was used as an antagonist). Thus 48–61, a naturally processed HEL peptide (2, 3), was a much more potent antagonist than the peptide analogue 48-Q57A-63. In contrast, the control T cell hybridomas, WW236− and WW221−, were not antagonized by 48–61 (Fig. 2*A*).

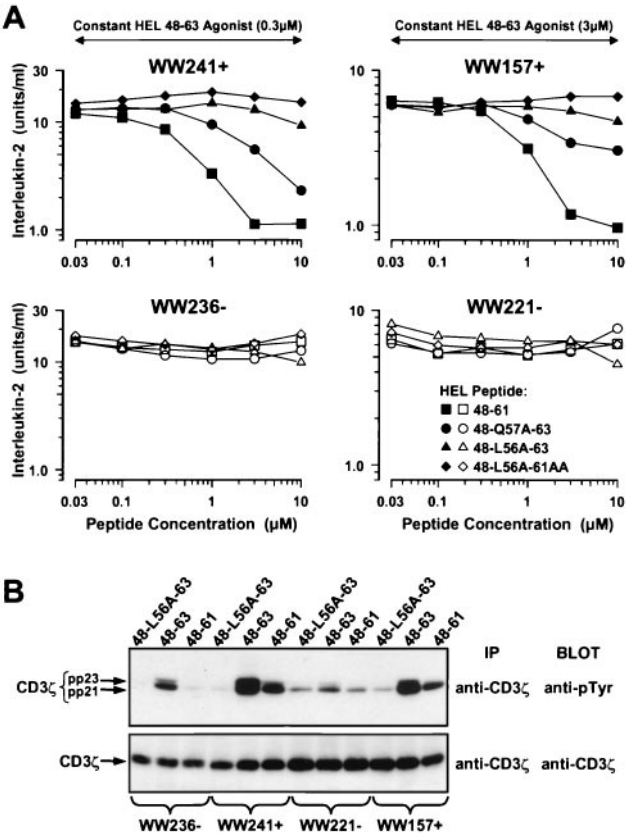


**FIGURE 2.** Conversion of peptides from agonist to antagonist by the removal of PFR. *A*, The ability of four HEL peptides to antagonize the response of four H-2A$^k$-restricted, HEL 48–62-specific, PFR-dependent T cell hybridomas to the agonist, HEL 48–63, was tested (5). The hybridomas, WW241+ and WW236−, and WW157+ and WW221−, were paired on the basis of their ability to secrete a similar amount of IL-2 upon stimulation with HEL 48–63 and respond to a comparable concentration of peptide (+ denotes ability to be antagonized; − denotes inability to be antagonized) (5). *B*, Antagonizable (+) and nonantagonizable (−) T cell hybridomas generate different patterns of CD3ζ phosphorylation. T cells were stimulated with LK35.2 cells pulsed with 3 μM agonist (48–63), antagonist (48–61), and null (48-L56A-63) peptides as indicated, and the tyrosine phosphorylation of CD3ζ determined (12).

TCR ligation generates two tyrosine phosphorylated forms of CD3ζ, a lower pp21 band and an upper pp23 band. Differential CD3ζ phosphorylation, in which only the lower band is produced, has been shown to correlate with the delivery of a partial signal and the induction of antagonism and anergy (12, 15, 16). Clear differences were observed in the CD3ζ phosphorylation pattern following stimulation of the hybridomas that were either sensitive or resistant to antagonism (Fig. 2*B*). While HEL 48-L56A-63 failed to induce CD3ζ phosphorylation, HEL 48–61 induced the

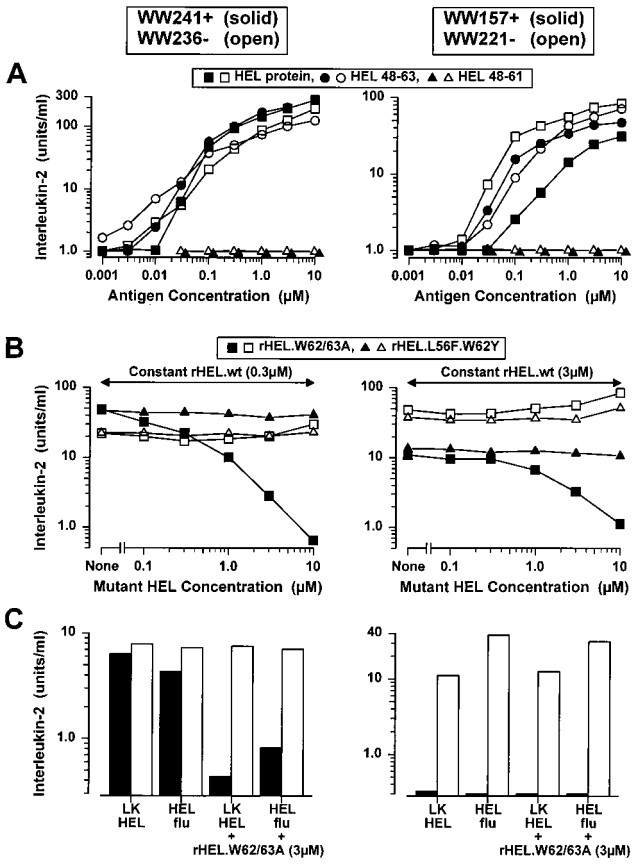


**FIGURE 3.** Ag processing generates both agonist and antagonist peptides from the same immunogenic epitope. *A*, Comparison of T cell responses to the native HEL protein, and the peptides 48–63 and 48–61. *B*, The response of T cell hybridomas to a fixed concentration of wild-type rHEL (rHEL.wt) plus an increasing concentration of mutant rHEL proteins was determined. *C*, Peptides generated from endogenously-derived proteins can also antagonize T cells. Responses to LK-HEL (HEL 1–80 attached to H-2K$^k$ transmembrane and cytoplasmic tail; Ref. 10) and HEL-flu (HEL 46–63 inserted into the neuraminidase stalk of influenza A virus (A/WSN/33 (H1N1)) (Ref. 11) with or without the addition of 3 $\mu$M of rHEL. W62/63A were compared.

lower pp21 tyrosine phosphorylated form of CD3$\zeta$, but essentially none of the upper pp23 form in the antagonizable hybridomas (WW241+ and WW157+). In contrast, HEL 48–61 did not induce any CD3$\zeta$ phosphorylation in the two nonantagonizable hybridomas (WW236− and WW221−) above that observed with the control peptide, HEL 48-L56A-63. These data indicate that there are clear biochemical differences in the way these hybridomas respond to antagonist peptides, which may underlie their functional phenotype.

Since PFR-dependent T cells were antagonized by short peptides, can naturally processed peptides generated from the native HEL protein that lacked C-terminal PFR antagonize T cell responses to agonist peptides generated from the same epitope? One of the two antagonizable T cell hybridomas (WW157+) responded more weakly to HEL than its control (WW221−) despite both producing identical IL-2 responses to HEL 48–63 (Fig. 3*A*). Could further antagonism be observed in both WW241+ and WW157+ if the ratio between naturally processed peptides containing and lacking the PFR Trp62 was altered? This was achieved by the use of rHEL produced in *P. pastoris* yeast. Three versions were generated: the wild-type protein (rHEL.wt) and two mutants. One has the PFR W62 and W63 replaced with alanine (rHEL.W62/63A), while the other has the two TCR contact residues, L56 and W62, replaced with the analogous mouse lysozyme residues, phenylalanine and tyrosine, respectively (rHEL.L56F.W62Y). Differential processing of the recombinant mutant protein rHEL.W62/63A can produce antagonist, but not agonist peptide. By increasing the concentration of rHEL.W62/63A while keeping the concentration of rHEL.wt fixed, the ratio of peptides generated that lack W62 (the antagonists) over the concentration of peptides that contain the W62 (the agonists) would increase. Our data show that both of the T cell hybridomas that were susceptible to antagonism (WW241+ and WW157+) could be significantly inhibited with modest amounts of mutant protein (Fig. 3*B*). For instance, the ratio of antagonist over agonist peptides generated from native HEL is ~1:1 (2), so addition of an equimolar concentration of mutant rHEL.W62/63A can reasonably be predicted to change this ratio to 3:1. This relatively small change gave rise to a 53.7% and 70.4% reduction in IL-2 production by WW241+ and WW157+, respectively. We reasoned that this could not be due to peptide competition for MHC binding because neither WW236− nor WW221− could be inhibited by addition of these mutant proteins, and the control mutant protein, rHEL.L56F.W62Y, which restores this epitope to the murine lysozyme sequence and generates peptides that bind to H-2A$^k$ comparably (17), had no effect on WW241+ and WW157+.

It has been suggested that the profile of peptides generated from a given epitope may vary depending on the source of the Ag, its intracellular site of degradation, or the type of APC (10, 18, 19). This was examined by analyzing T cell responses to the HEL 52–61 epitope derived from an intracellular source. LK35.2 B cells either expressing truncated HEL 1–80 tagged to the H-2K$^k$ transmembrane and cytoplasmic tail (LK-HEL) (10), or infected with a recombinant influenza virus, in which the HEL 46–63 sequence had been inserted into the neuraminidase stalk of influenza A virus (A/WSN/33 (H1N1)) by reverse genetics (HEL-flu) (11), were used. One of the T cell hybridomas, WW157+, failed to responded to both LK-HEL and HEL-flu infected LK cells while its control, WW221−, responded strongly (Fig. 3*C*). Furthermore, the response of WW241+ could be antagonized by the addition of rHEL.W62/63A. Differences in the ability of WW241+ and WW157+ to respond to HEL, LK-HEL, and HEL-flu relative to their control hybridomas is likely due to differences in the amount of 48–61 required to induce antagonism (Fig. 2*A*; molar ratio of agonist:antagonist peptides required to give 50% reduction in IL-2 secretion is 1:1.57 for WW241+ and 1:0.28 for WW157+).

Taken together, our data demonstrate that natural processing by APC can generate both agonist and antagonist peptides from the same antigenic epitope, which can modulate the response of PFR-dependent T cells. Given that over half of the T cells generated to the HEL 52–61 epitope are PFR-dependent (5), these data raise the intriguing possibility that this process could play an important role in regulating T cell responses. Indeed, previous studies have suggested that endogenous altered peptide ligands can affect peripheral T cell responses (20). Our data clearly show that HEL 48–61 was a more potent antagonist than any peptide containing substitutions at either L56 or Q57. The amino acid substitutions made to generate altered peptide ligands are often subtle, so it is striking that the complete removal of a residue would generate such a potent T cell antagonist. The alteration of PFR may therefore provide an alternative approach to producing peptide antagonists for immunotherapy.

Can normal murine or human T cells be PFR dependent? If so, can they be antagonized by peptides that lack PFR? While our

current knowledge of TCR and MHC class II structure would suggest that any TCR could recognize PFR, and that any PFR-dependent T cell could be antagonized by "short" peptides, further studies will clearly be required to confirm these assumptions. We have recently isolated a T cell clone that is partially PFR-dependent. Although it responds to low concentrations of HEL 48–63, it is only stimulated by the highest concentration of HEL 48–61. Interestingly, the response of this clone is still antagonized by substimulatory concentrations of 48–61. In addition to these studies, the generation of transgenic mice expressing PFR-dependent TCR will allow us to study this further.

Previous studies have suggested that distinct peptides may be generated if the proteins are either derived from different intracellular and extracellular sources, or processed by different cell types (10, 18, 19). Although we could not detect any significant difference in the response to exogenously- and endogenously-derived HEL peptides, a more detailed investigation of this issue is warranted. What influence might such differences have on tolerance induction and autoimmunity? With few exceptions, only MHC class II molecules present peptides containing PFR (1). It is therefore tempting to question whether this feature might be linked to the generation of autoimmune disease, the vast majority of which are MHC class II restricted. It had previously been proposed that a distinct set of peptides are presented by the thymic cortical epithelial cells that mediate positive selection (21, 22). Peptide elution studies have failed to define clear differences between MHC class II-bound peptides from the thymus vs the spleen (23). However, the recent finding that thymic cortical epithelial cells exclusively utilize the cysteine proteinase cathepsin L, while bone marrow-derived APC use cathepsin S for MHC class II-associated invariant chain degradation, suggests that the proteolytic environment in cells that mediate positive and negative selection may be different (24). If proportionally more peptides lacking PFR were generated in the thymic medulla, the site of negative selection, than in the thymic cortex, the site of positive selection, then autoreactive, PFR-dependent T cells may be inefficiently deleted and hyperstimulated in the periphery. Under normal conditions, professional APC might be expected to present a similar array of peptides as those presented by the bone marrow-derived cells that mediate negative selection in the thymus. Therefore, peptides presented from a self epitope that lack PFR may help to maintain peripheral tolerance by inducing anergy in the PFR-dependent T cells. However, if different APC that express MHC class II at the site of an autoimmune reaction generate more agonist than antagonist peptides, then this may be sufficient to break tolerance. While it remains to be determined if PFR-dependent T cells initiate autoimmunity, they may exacerbate an autoimmune response.

In summary, our data demonstrate that the natural processing of an immunogenic epitope can give rise to both agonist and antagonist peptides that can modulate T cells specific for this epitope. While this may provide an additional mechanism for controlling the response of PFR-dependent T cells, it also highlights how small changes in the ratio of agonist vs antagonist peptides generated from a protein either by different cell types or by different processing pathways could lead to deleterious responses. These data are likely to have important implications for vaccine development, peptide immunotherapy, and autoimmunity.

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## References

1. Urban, R. G., R. M. Chicz, D. A. A. Vignali, and J. L. Strominger. 1993. The dichotomy of peptide presentation by class I and class II MHC proteins. In *Naturally Processed Peptides*, 57 Ed. A. Sette, ed. Karger, Basel, p. 197.
2. Vignali, D. A. A., R. G. Urban, R. M. Chicz, and J. L. Strominger. 1993. Minute quantities of a single immunodominant epitope are presented as large nested sets by MHC class II molecules. *Eur. J. Immunol. 23:1602.*
3. Nelson, C. A., R. W. Roof, D. W. McCourt, and E. R. Unanue. 1992. Identification of the naturally processed form of hen egg white lysozyme bound to the murine major histocompatibility complex class II molecule I-$A^k$. *Proc. Natl. Acad. Sci. USA 89:7380.*
4. Fremont, D. H., D. Monnaire, C. A. Nelson, W. A. Hendrickson, and E. R. Unanue. 1998. Crystal structure of I-$A^k$ in complex with a dominant epitope of lysozyme. *Immunity 8:305.*
5. Carson, R. T., K. M. Vignali, D. L. Woodland, and D. A. A. Vignali. 1997. T cell receptor recognition of MHC class II-bound peptide flanking residues enhances immunogenicity and results in altered TCR V region usage. *Immunity 7:387.*
6. Evavold, B. D., J. Sloan-Lancaster, and P. M. Allen. 1993. Tickling the TCR: selective T-cell functions stimulated by altered peptide ligands. *Immunol. Today 14:602.*
7. Sette, A., J. Alexander, J. Ruppert, K. Snoke, A. Franco, G. Ishioka, and H. M. Grey. 1994. Antigen analogs/MHC complexes as specific T cell receptor antagonists. *Annu. Rev. Immunol. 12:413.*
8. Jameson, S. C., and M. J. Bevan. 1995. T cell receptor antagonists and partial agonists. *Immunity 2:1.*
9. Vignali, D. A. A., R. T. Carson, B. Chang, R. S. Mittler, and J. L. Strominger. 1996. The two membrane proximal domains of CD4 interact with the T cell receptor. *J. Exp. Med. 183:2097.*
10. Moreno, J., D. A. Vignali, F. Nadimi, S. Fuchs, L. Adorini, and G. J. Hammerling. 1991. Processing of an endogenous protein can generate MHC class II- restricted T cell determinants distinct from those derived from exogenous antigen. *J. Immunol. 147:3306.*
11. Walker, W. S., M. R. Catrucci, M. Y. Sangster, R. T. Carson, and Y. Kawaoka. 1997. HEL-flu: an influenza virus containing the hen egg lysozyme epitope recognized by $CD4^+$ T cells from mice transgenic for $\alpha\beta$ TCR. *J. Immunol. 159: 2563.*
12. Vignali, D. A. A. and J. L. Strominger. 1994. Amino acid residues that flank core peptide epitopes and the extracellular domains of CD4 modulate differential signaling through the T cell receptor. *J. Exp. Med. 179:1945.*
13. Racioppi, L., F. Rocnchese, L. A. Matis, and R. N. Germain. 1993. Peptide-major histocompatibility complex class II complexes with mixed agonist/antagonist properties provide evidence for ligand-related differences in T cell receptor-dependent intracellular signaling. *J. Exp. Med. 177:1047.*
14. Evavold, B. D., J. Sloan-Lancaster, and P. M. Allen. 1994. Antagonism of superantigen-stimulated helper T-cell clones and hybridomas by altered peptide ligand. *Proc. Natl. Acad. Sci. USA 91:2300.*
15. Madrenas, J., R. L. Wange, J. L. Wang, N. Isakov, L. E. Samelson, and R. N. Germain. 1995. $\zeta$ Phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. *Science 267:515.*
16. Sloan-Lancaster, J., A. S. Shaw, J. B. Rothbard, and P. M. Allen. 1994. Partial T cell signaling: altered phospho-$\zeta$ and lack of ZAP-70 recruitment in APL-induced T cell anergy. *Cell 79:913.*
17. Babbitt, B. P., G. R. Matsueda, E. Haber, E. R. Unanue, and P. M. Allen. 1986. Antigenic competition at the level of peptide-Ia binding. *Proc. Natl. Acad. Sci. USA 83:4509.*
18. Gapin, L., D. A. Bravo, A. Casrouge, J. P. Cabaniols, P. Kourilsky, and J. Kanellopoulos. 1998. Antigen presentation by dendritic cells focuses T cell responses against immunodominant peptides: studies in the hen egg-white lysozyme (HEL) model. *J. Immunol. 160:1555.*
19. Robadey, C., H. J. Wallny, and S. Demotz. 1996. Cell type-specific processing of the I-Ed-restricted hen egg lysozyme determinant 107–116. *Eur. J. Immunol. 26:1656.*
20. Vidal, K., B. L. Hsu, C. B. Williams, and P. M. Allen. 1996. Endogenous altered peptide ligands can affect peripheral T cell responses. *J. Exp. Med. 183:1311.*
21. Marrack, P. and J. Kappler. 1987. The T cell receptor. *Science 238:1073.*
22. Kourilsky, P. and J. -M. Claverie. 1989. MHC restriction, alloreactivity and thymic education: a common link. *Cell 56:327.*
23. Marrack, P., L. Ignatowicz, J. W. Kappler, J. Boymel, and J. H. Freed. 1993. Comparison of peptides bound to spleen and thymus class II. *J. Exp. Med. 178: 2173.*
24. Nakagawa, T., W. Roth, P. Wong, A. Nelson, A. Farr, J. Deussing, J. A. Villadangos, H. Ploegh, C. Peters, and A. Y. Rudensky. 1998. Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus. *Science 280:450.*
25. Allen, P. M., G. R. Matsueda, R. J. Evans, J. B. Dunbar, G. R. Marshall, and E. R. Unanue. 1987. Identification of the T-cell and Ia contact residues of a T-cell antigenic epitope. *Nature 327:713.*
26. Nelson, C. A., N. J. Viner, S. P. Young, S. J. Petzold, and E. R. Unanue. 1996. A negatively charged anchor residue promotes high affinity binding to the MHC class II molecule I-Ak. *J. Immunol. 157:755.*