Supporting Information

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SI Methods

Antigen Presentation Assays. T cell hybridomas were maintained as previously described (1); 1.5×10^4 $CD11c^+$ purified dendritic cells from BALB/c spleen were cocultured with 5×10^4 of the HA[126-138] specific, I-A^d-restricted T cell hybridoma (TS2) (2). In some groups, 1 μM of MalE[69-84], MalE[102-115], and MalE[269-284] were added as competing peptides. IL-2 was quantified by using cytotoxic T lymphocyte line (CTLL) and (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays as previously described (3). Results appear as a mean optical density in the MTT assay from duplicate wells read at 570–650 nm on a V_{max} plate reader.

Carboxyfluorescein succinimidyl ester (CFSE) Staining and Cell Tracking. Obtain a single cell suspension of splenocytes from either HNT or DO11.10 T cell antigen receptor (TcR) transgenic mice and wash cells with PBS 2 times. Take an aliquot of cells to ascertain the percentage of $Vb8.3^+CD4^+$ or KJ $(1\text{-}26)^+CD4^+$, respectively, expressing cells by FACS analysis. Taking the total number of cells, resuspend cells at 3×10^6 cells/mL in PBS in a Falcon tube and add CFSE at 1:5,000 dilution (0.2 μM). Invert 3 or 4 times to mix in the CFSE dye and incubate 5 min at 37 °C in the waterbath. Immediately wash the suspension with media three times, and then PBS 5 times. Cells were then resuspended in PBS at 5×10^6 cells/200 μL $Vb8.3^+CD4^+$ or KJ $(1\text{-}26)^+CD4^+$, respectively, T cells for transfer via retro-orbital injection by using a 29G ½″ tuberculin syringe 24 h after the immunization. Draining lymph nodes were harvested 48 h after transfer, and cell division was assessed by FACS and analyzed by using MODFIT v.3.0.

Bone Marrow-Derived Dendritic Cell (BMDC) Preparation. Bone marrow cells were obtained by first removing the femurs from mice and stripping all of the tissue from the surface of the bone. Using scissors, cut both ends off of the femur. With a 25G ½″ syringe, insert the tip into one open end of the femur, and expel 5 mL media, removing all of the bone marrow cells, into a Petri dish containing 10 mL media. The cells were vigorously pipeted to form a single cell suspension, filtered through a 45 μM sterile filter, washed, and resuspended to 4×10^5 cells/mL; 10 mL of the suspension was plated onto Petri dishes in the presence of 20 ng/mL granulocyte-macrophage colony stimulating factor (GMCSF). At day 3, an additional 10 mL of media containing 20 ng/mL GMCSF was added to each plate. At day 6, 10 mL of media was aspirated and spun down to pellet any cells. These cells were resuspended with fresh media and added back to the original plate. On day 7, 10 ng/mL LPS (Sigma-Aldrich) was added to each plate to mature the dendritic cells. The next day, the plates were gently washed and pooled, collecting only the floating cells. Enriched BMDCs were resuspended at 5×10^5/mL in media, and pulsed with peptide at the indicated dose. Verification of enrichment for BMDC was performed by staining with class II-FITC ($39\text{-}10^{-8}$) and CD11c-PE (HL3) (BD PharMingen) and analyzed on a BD FACS Caliber.

1. Loss GE, et al. (1993) Major histocompatibility complex class II-restricted presentation of an internally synthesized antigen displays cell-type variability and segregates from the exogenous class II and endogenous class I presentation pathways. *J Exp Med* 178:73–85.
2. Scott B, et al. (1994) A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. *Immunity* 1:73–83.
3. Lazarski CL, et al. (2005) The kinetic stability of MHC class II:peptide complexes is a key parameter that dictates immunodominance. *Immunity* 23:29–40.

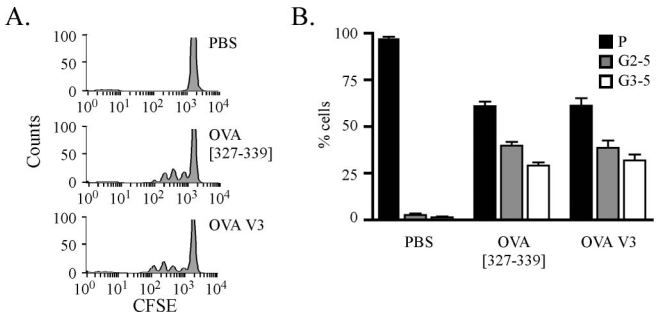

Fig. S1. The initial loading and epitope density are similar between low- and high-stability peptides. Mice were immunized in the ear pinnae with BMDC pulsed with 10 μM OVA[327-339] or 2 μM OVA V3; 24 h later, mice were injected i.v. with 5×10^6 CFSE-labeled DO11.10 TcR transgenic T cells; 48 h later, the draining lymph nodes were harvested and proliferation determined by FACS. (*A*) Representative FACS profiles of 2 independent experiments $n = 2$ per group are shown (counts were manually gated at 100 to adequately observe divisions). (*B*) The profiles of both experiments were subjected to Modfit 3.0 analyses and the average $\pm$ SEM of the percentage of cells divided is shown.

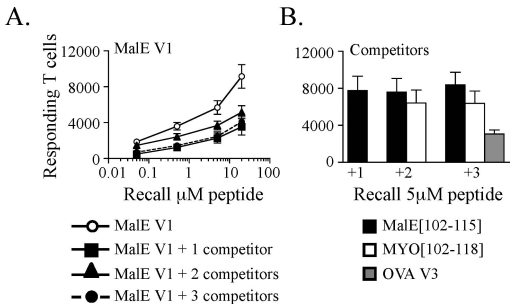

Fig. S2. Coadministration of a low-stability peptide with a single dominant peptide is sufficient to induce a loss in CD4 T cell expansion to the low-stability peptide. (*A*) BALB/c mice were immunized with 25 nmol of MalE V1 in an emulsion containing IFA/PBS and 0.6 μg/mL LPS either alone (○) or included in a mixture containing 5 nmol of the dominant peptide MalE[102-115] (■), MalE[102-115] and MYO[102-118] (▲), or MalE[102-115], MYO[102-118] and OVA V3 (●) in the hind footpad. (*B*) The total number of IL-2 secreting antigen-specific cells at day 10 was determined by using an IL-2 ELISpot assay and restimulated with increasing doses of the test peptide or 5 μM of competing dominant peptides. Draining lymph nodes from individual mice were pooled and there were 2 mice per group. The results are presented as the total number of responding T cells and are representative of 3 independent experiments ± SEM.

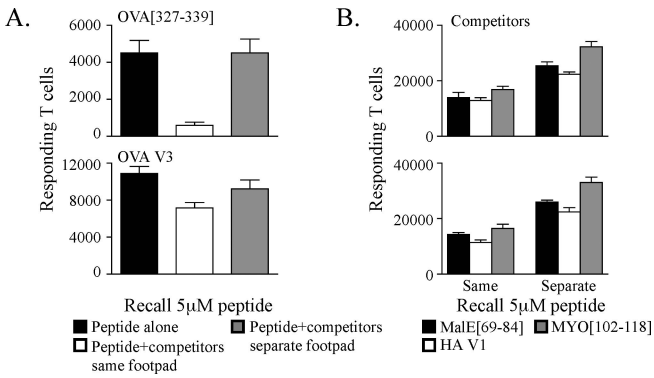

Fig. S3. Systemic suppression does not account for the failure of low-stability complexes to sustain CD4 T cell expansion. (*A*) Mice were immunized with 25 nmol of OVA[327-339] (*Upper*) or 5 nmol of OVA V3 (*Lower*) in an emulsion containing IFA/PBS and 0.6 μg/mL LPS alone (black) or as a mixture with 5 nmol of the dominant peptides MalE[69-84], HA V1 and MYO[102-118] (white). Alternatively, the test peptide was introduced in an emulsion alone in one footpad and the dominant peptides in the contralateral hind footpad (gray) and recalled to 5 μM of the test peptide. (*B*) Total number of CD4 T cells producing IL-2 with 5 μM of each competing dominant peptide. The results are representative of 4 independent experiments $\pm$ SEM.

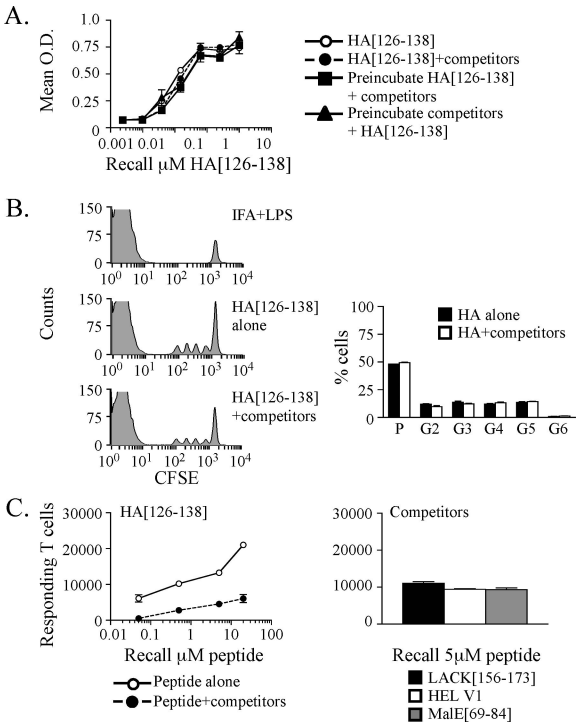

Fig. S4. Loss in T cell responses to low-stability peptides is not because of peptide competition for binding of class II molecules in vitro. (*A*) HA[126-138] was tested for its ability to stimulate TS2 hybridoma cells by using CD11c^{+} enriched splenocytes in the presence or absence of competing peptides. IL-2 production by TS2 alone (○), in the presence of 1 μM MalE[69-84], MalE[102-115], and MalE[269-284] (●), HA[126-138] preincubated with APC for 30 min before the addition of dominant peptides (■), or the dominant peptides preincubated with APC for 30 min before the addition of HA[126-138] (▲) is shown as described in *Material and Methods*. Results are presented as the mean OD from the secondary CTLL coculture, and are representative of 2 independent experiments ± SEM. (*B* and *C*) Groups of mice were immunized with 5 nmol HA[126-138] in the absence or presence of competitor peptides. (*B*) Twenty-four hours later, some of the mice were injected IV with 5 × 10^{6} CFSE-labeled HNT TcR transgenic T cells. Forty-eight hours later, the draining lymph nodes were harvested and proliferation determined by FACS (*Left*). The profiles were subjected to Modfit 3.0 analyses for the % of cells divided (*Right*). (*C*) With the remaining mice, the total number of IL-2 secreting endogenous T cells at day 10 was determined by using an ELISpot assay and restimulated with increasing doses of the test peptide (*Left*) or 5 μM of competing dominant peptides (*Right*). Draining lymph nodes from individual mice were pooled and there were 2 mice per group. The results are presented as the total number of responding T cells and are representative of 2 independent experiments ± SEM.