

Boscardin et al. <http://www.jem.org/cgi/content/full/jem.20051639/DC1>

MATERIALS AND METHODS

Peptide libraries.

Overlapping sequences from the larger proteins were determined and optimized for synthesis by using an epitope library fragment generation program, PeptGen, developed by Los Alamos National Laboratories, Los Alamos, NM, as part of the HIV Immunology Database at <http://www.hiv.lanl.gov>. The OVA library contained 107 overlapping 12–17-amino acid peptides (Table S1) divided into 10 pools, each containing 10 peptides and one pool comprised of seven peptides. For the CSP library (Table S2), 36 peptides were created comprising the NH₂ terminus (residues 57–138) and the COOH terminus (residues 289–346) portions of the CSP. Three additional peptides corresponding to the major (QGGAP)₂ and minor (QQPP)₂ repeats of the CSP and CD8 epitope (SYVPSAEQI) were also synthesized. All peptides were created using a SYMPHONY multiple peptide synthesizer (Protein Technologies) on Wang resin (Bachem) using Fmoc-protected amino acids (Anaspec; reference 1). Coupling reactions were conducted using 0.3 M HBTU/ HOBt and 0.4 M NMM in NMP (*N*-methylpyrrolidone) as the primary solvent. Simultaneous resin cleavage and side-chain deprotection were achieved by treatment with concentrated, sequencing grade trifluoroacetic acid with triisopropylsilane, water, as well as ethanedithiol in a ratio of 95:2:2:1 for 3–6 h. Rotary evaporation followed by high vacuum overnight was used to remove TFA from the resin-bound peptides. Peptides were then released in 8 M acetic acid and filtered from resin, and the acidic mixture was evaporated and redissolved in HPLC-grade water for lyophilization. All crude lyophilized products were subsequently analyzed by reverse-phase HPLC (Waters Chromatography) using a Merck Chromolith Performance C18 column. Individual peptide integrity was verified by matrix-assisted laser desorption/ionization mass spectrometry using a PerSeptive/Applied Biosystems Voyager delayed extraction spectrometer system (2).

Adoptive transfer and analysis of TCR transgenic T cell proliferation.

CD8⁺ T cells from Thy 1.1+ SYVPSAEQI-specific TCR transgenic mice were purified and CFSE was labeled as described previously (Hawiger, D., K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J.V. Ravetch, R.M. Steinman, and M.C. Nussenzweig. 2001. *J. Exp. Med.* 194:769–779). 5–10 × 10⁶ cells were transferred i.v. to naive BALB/c mice. Fusion anti-DEC-CS or control-CS antibodies were injected s.c. 24 h after T cell transfer, and cell division was measured by CFSE dilution 3 d later. Transgenic T cells were gated using Kd-SYVPSAEQI tetramers prepared as described (3) and anti-Thy 1.1 (clone OX-7) and anti-CD8 antibodies (BD Biosciences).

Immunofluorescence on *P. yoelii* sporozoites.

Sporozoites were collected by dissecting the salivary glands of infected *Anopheles stephensi* mosquitoes. Slides containing 10–20 × 10³ sporozoites were dried at room temperature and stored at –20°C until use. For staining, slides were placed at room temperature for 15–30 min, rinsed with PBS, blocked with 2% BSA (Invitrogen) in PBS for 30 min and washed twice with PBS before incubation with serial dilutions of sera for 1 h at room temperature. Parasites were visualized by incubation with a goat anti-mouse FITC-conjugated antibody (1:50 dilution; Sigma-Aldrich). Slides were examined under a fluorescence microscope (Nikon).

REFERENCES

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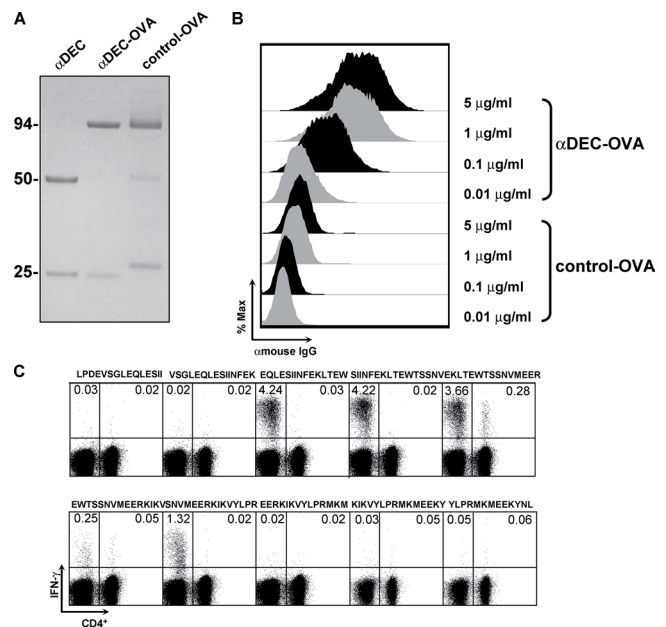


Figure S1. (A and B) The purification and binding of the antibodies anti-DEC-OVA and control-OVA to CHO cells stably transfected with DEC-205. (C) IFN- γ CD8⁺ and CD4⁺ T cell responses for individual peptides contained in pool 8.

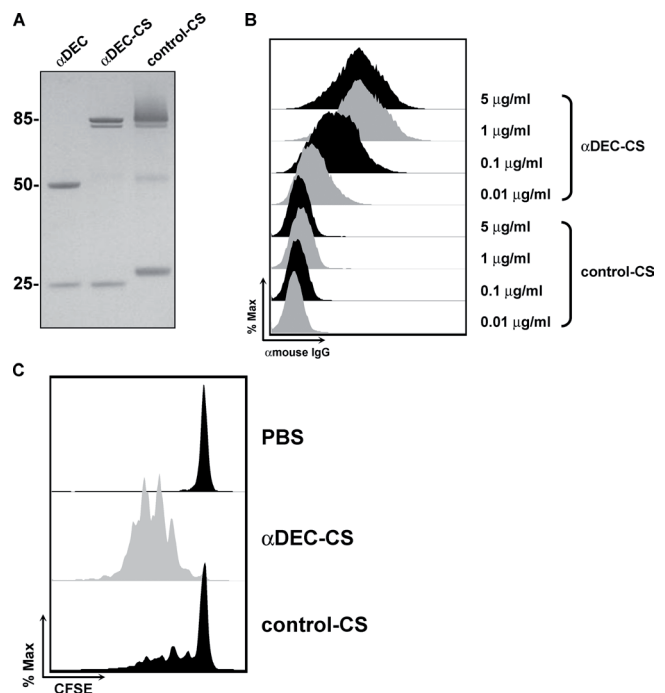


Figure S2. (A and B) The purification and binding of the antibodies anti-DEC-CS and control-CS to CHO cells stably transfected with DEC-205. (C) Transgenic CD8⁺ T cell proliferation in mice injected with anti-DEC-CS or control-CS.