Supporting Materials and Methods

Tissue Preparation. For isolation of lymphocytes from liver, lung, and kidney, mice were anesthetized and killed by total body perfusion through the left ventricle with PBS. Organs were cut into small pieces, resuspended in HBSS/2% FCS, forced through a 100 μm mesh, and centrifuged. The pellet was then resuspended in 35% Percoll (Sigma), centrifuged at 600 × *g*, and the resulting pellet was treated with 0.83% Tris-buffered ammoniumchloride to lyse residual RBCs followed by additional washes.

Antibodies. In addition to antibodies described elsewhere (7), the following antibodies and reagents were used: CD25 (7D4), CD127 (SB/14), IL-4 (11B11), IL-10 (JES5-16E3), granulocyte/macrophage colony-stimulating factor (GM-CSF) (MP1-22E9), and isotype controls (R3-34 and G235-2356) (BD Pharmingen, La Jolla, CA); CD122 (5H4) (eBioscience, San Diego); polyclonal goat anti-IL-15Rα and polyclonal goat Ig (R & D Systems). For detection of murine Ki-67, a crossreactive human Ki-67-specific antibody (B56) and MOPC 21 isotype control were used (BD Pharmingen).

Flow Cytometry. Reagents and procedures for 5-h *in vitro* restimulation and surface and intracellular antibody staining have been described in detail (7). Functional avidities were determined by calculating the peptide concentration required to elicit IFN-γ production in 50% of epitope-specific T_M (7). For surface staining of cytokine receptors, no IL-2 was added to stimulation cultures to prevent competitive binding or receptor down-modulation. Given that *in vitro* restimulation results in rapid down-regulation of CD122, cells were stained with conjugated CD122 antibody or isotype control for 1 h on ice before 5-h stimulation. Additional surface and intracellular stains were performed as described after *in vitro* culture. Surface expression of IL-15Rα was detected by using 1 μg per 10^6 cells polyclonal IL-15Rα antibody or polyclonal goat Ig control followed by a goat-specific Cy5-conjugated $F(ab')_2$ fragment (Jackson ImmunoResearch). Specificity of antibody was confirmed by using cells from IL-15Rα$^{-/-}$ mice (data not shown). To normalize expression levels of Bcl-2, Bcl-x_L or cytokine receptors, the geometric mean of fluorescence intensity (GMFI) of experimental stains was divided by the GMFI of

corresponding isotype control stains. Given that even normalized values may vary across different experiments (a function of fluorochromes used and flow cytometer settings), comparison and statistical analysis of values obtained was performed only with data from the same experiment.

***In Vivo* Proliferation Assay.** For combined detection of BrdUrd and intracellular cytokines by flow cytometry, cells were restimulated with lymphocytic choriomeningitis virus (LCMV) peptides for 5 h and stained for various cell surface antigens. After fixation and permeabilization (4% paraformaldehyde/0.1% saponin in PBS/Hanks' balanced salt solution buffer, 10 min at room temperature), cells were washed (1% FCS/0.1% saponin in PBS), incubated for 10 min on ice with 10% DMSO/1% FCS/0.1% saponin in PBS, washed, and refixed (4% paraformaldehyde/0.1% saponin in PBS/HBSS buffer, 5 min at RT). Subsequently, cells were incubated with 30 μg/100 μl per 10^6 cells DNase type II-S (Sigma) in PBS for 1 h at 37°C and combined intracellular stains for cytokines, and BrdUrd (B44, BD Biosciences) were performed according to standard protocols. It should be noted that the bone marrow contains a small subset of $CD11b^+$ $CD3^+$ cells that produce IFN-γ in the absence of peptide stimulation. Two strategies were used to circumvent this potentially confounding effect. (*i*) The fraction of $BrdUrd^+$ and $BrdUrd^-$ IFN-γ^+ T cells calculated from stimulation cultures in the absence of peptide was subtracted from the corresponding data in peptide stimulation cultures, and (*ii*) specific T cells in the bone marrow were identified by intracellular tumor necrosis factor α stains.

Production of IL-7Rα Antibody. The A7R34 hybridoma was adapted to serum-free growth in protein-free hybridoma medium (PFHM-II, GIBCO) and then scaled up to 3- to 5-liter cultures in roller bottles. After a 3–4 day culture period, the cell-free supernatants were concentrated by using an artificial dialysis kidney (Fresenius Medical Care, Lexington, MA) with a 50-Kd cutoff. The concentrated supernatants were centrifuged to remove membrane fragments and debris before being dialysed against PBS.

Statistical Analyses. Data handling, analysis, and graphic representation was performed with PRISM 3 (GraphPad, San Diego).