Serum was separated from the blood by centrifugation after overnight storage at 4°C.

Mammary fat pad tumor prevention model

A2L2 cells were harvested from subconfluent cultures with trypsin and EDTA as described earlier. The cells were washed once in serum-containing culture medium and were washed once in PBS. Mice were anesthetized by inhalation of isoflurane using a special apparatus developed by the veterinarians at MD Anderson Cancer Center. The fur was shaved over the lateral thorax, and a 5-mm-long incision was made to reveal mammary fat pad 2 as previously described [42]. A 0.1-ml sample containing 2.5×10^4 A2L2 cells in normal saline was injected into the fat pad. The incision was closed with a wound clip. Wound clips that had not already fallen off were removed after 7 days. The mice were then observed daily and their tumors measured in perpendicular directions with a pair of calipers. Mice with tumors 10 mm in the greatest dimension were killed according to our approved Institutional Animal Care and Use protocol. At the termination of the experiment, all mice were killed by CO_2 inhalation and all tumors were excised and weighed.

Experimental metastasis prevention model

A 0.1-ml sample containing 2.5×10^4 A2L2 cells in normal saline was injected into the tail vein of each immunized mouse. The mice were killed 30 days later, and the surface lung metastases in each animal were counted.

Tetramer analysis of immune spleen cells

A K(d) tetramer containing the peptide sequence PYVSRLLGI [43] was prepared by the National Institutes of Health Tetramer Facility at Emory University (Atlanta, GA, USA). The incorporated peptide was synthesized at the peptide synthesis facility of MD Anderson Cancer Center. We received a K(d) tetramer specific for A/PR/8/34 influenza HA containing the peptide IYSTVASSL from Linda Sherman at the Scripps Research Institute (La Jolla, CA, USA) [44]. A single-cell suspension of the spleen from a naïve mouse was prepared in R10S medium (RPMI 1640, 10% HI-FCS [Summit Biotechnology, Fort Collins, CO, USA], 1% nonessential amino acids, 100 mM MEM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 1 ml streptomycin, and 50 mM 2-mercaptoethanol) by gently swirling the spleen between frosted glass slides.

Debris was removed from the cell suspension by filtration through nylon mesh into a 10-ml centrifuge tube. The cell suspension was centrifuged for 10 min at 700 × *g* and the pellet resuspended in 5 ml ACK solution (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.01 mM NaEDTA, pH adjusted with 1 N HCl to 7.2–7.4) before being gently rocked for 5 min to lyse red blood cells. An additional 5 ml R10S medium was added to the cell suspension, and the cells were washed once with R10S medium. The naïve spleen cells were cultured with either PYVSRLLGI or IYSTVASSL (70 ng/ml) on a rocker for 2 hours at 37°C and were γ-irradiated with 20 Gray.

The mice were vaccinated three times with a 2-week interval with 10^6 infectious units (IU) VRP-*neu* or 10^6 IU VRP-HA, and the immune spleens were harvested 3 weeks after the final vaccination. The immune spleens were prepared to produce a single cell suspension in R10S medium and were co-cultured with the peptide-pulsed stimulator cells at a ratio of one stimulator to eight responders. The cell mixture was cultured for 7 days at 37°C, washed, and was resuspended in FACS buffer (PBS with 1% BSA) at a concentration of 5×10^7 cells/ml. Twenty microliters of the cell mixtures were added to 20 μl PE-conjugated Her2/*neu*-specific or HA-specific tetramers to make the final dilutions of the tetramers 1:50, 1:100, and 1:200. One microliter of PerCP-conjugated anti-mouseCD3e (PharMingen, San Diego, CA, USA) and FITC-conjugated anti-mouse CD8a (Caltag, Burlingame, CA, USA) was added to the mixture and incubated for 1 hour at 4°C in the dark. The cells were then suspended in 150 μl FACS buffer and transferred to a polystyrene round-bottomed tube. The cells were washed twice with FACS buffer and suspended in 200 μl of 1% paraformaldehyde in PBS.

List mode data were acquired with a FACScan (BD Biosciences, San Jose, CA, USA). Dead cells and monocytes were excluded from the analysis by forward scatter and side scatter gating. A total of 10,000–30,000 events were typically acquired, and compensation was optimized using unstained cells, cells stained with only PerCP-conjugated anti-mouse CD3e, cells stained with only FITC-conjugated anti-mouseCD8a and cells stained with only PE-conjugated anti-mouse CD4. The $CD3^+$ cells were gated from the total population of live cells, and the $CD8^+$ cells were gated from the $CD3^+$ cells. From the $CD3^+$ cells and the CD8 double-positive cells, the percentages of PE-tetramer-positive cells were calculated. Isotype controls for the anti-CD3e and anti-CD8a were subtracted from the acquired data. List mode files were analyzed using CELLQUEST software (BD Biosciences, San Jose, CA, USA).

Intracellular interferon-γ analysis of immune spleen cells

The procedure for this technique was obtained from PharMingen, whose reagents were used whenever possible. Spleens from VRP-*neu*-immunized and VRP-HA-immunized mice were prepared as already described for tetramer analysis. The spleen cells were stimulated with PYVSRLLGI at a concentration of 70 ng/ml at 37°C in R10S medium for 5 days as described. On the sixth day, the cells were suspended at 2×10^6/ml in R10S medium containing 10 ng/ml phorbol-12-myristate acetate (Sigma-Aldrich, St Louis, MO, USA) and 250 ng/ml ionomycin (Sigma-Aldrich). Brefeldin (1