Supporting Information

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SI Materials and Methods

Mice and Reagents. C57BL/6 mice were purchased from The Jackson Laboratory. All mice were housed and bred in the Center for Laboratory Animal Care of the University of Connecticut Health Center and were handled according to the National Institutes of Health federal guidelines. staphylococcal enterotoxin A (SEA) was purchased from Toxin Technology Inc. Poly(I:C) (high molecular weight) and CpG (ODN 1585) were purchased from Invivogen. SIINFEKL peptide was custom-made by Invitrogen. IL-33, IL-12, and IL-18 recombinant proteins and anti–IL-33 mAb for immunohistochemical staining were from R&D Systems. IL-2 recombinant protein was from Intergen. SIINFEKL tetramer was prepared by the National Institutes of Health tetramer core facility. Commercial sources for flow cytometry antibodies were the following: eBiosciences (Vα2, CD45.1, CD11a, CD62L, IFNγ, TNF, CD107a, and Gr-1); BD Biosciences/Pharmingen (CD4, CD8, Vβ3, and Vβ6); Biolegend (CXCR3); Invitrogen (granzyme B); and MD Bioproducts, (T1/ST2). An IFNγ ELISA kit was purchased from BD Biosciences/Pharmingen. The IL-5 ELISA kit was from eBioscience. For the purification of activated OT-I CD8 T cells, either the MACS Cell Separation System (Miltenyi Biotec) or a combination of DSB-X Biotin Protein Labeling Kit with Dynabeads FlowComp Flexi systems (Invitrogen) was used. The Milliplex MAP multiplex assay kit (Millipore) was used for cytokine analysis with help from Kyle Wright and Benjamin Rudenga (University of Connecticut Health Center).

Immunization. C57BL/6 mice were first i.p. treated with 1–50 μg poly(I:C) 3 d before immunization. One day before immunization, 0.5×10^6 $RAG^{-/-}$ OT-I cells were i.v. transferred into recipient mice. On day 0, mice received 50 μg SIINFEKL peptide i.p. and 3 d later were killed. Transfer of OT-I cells alone resulted in an average of 4.7×10^4 OT-I cells per spleen 4 d after transfer. Poly(I:C) pretreatment did not significantly affect homeostatic distribution of OT-I cells; we observed an average of 4.4×10^4 OT-I cells per spleen. In the SEA model, C57BL/6 mice received 1μg poly(I:C) i.p. 3 d before i.p. immunization with 1 μg SEA. T-cell activation was analyzed 3 d after SEA. In experiments studying the pulmonary response to SEA, the dosage of poly(I:C) was increased to achieve consistent suppressive effects. C57BL/6 mice were first i.p. treated with 200 μg poly(I:C) 3 d before intranasal (i.n.) immunization with 1 μg SEA.

Cell Processing and Flow Cytometry. To obtain lymphocytes from livers and lungs, immunized mice were first subjected to perfusion with PBS containing 75 U/mL heparin (Sigma-Aldrich). Livers were crushed through 100-μm nylon mesh cell strainers (BD Falcon) and partitioned on 35% Percoll (Sigma-Aldrich) gradient. Red blood cells (RBCs) in the liver lymphocyte preparation were lysed with Gey's solution for 5 min at room temperature before proceeding to staining or ex vivo restimulation. Lungs were excised into smaller pieces and incubated with balanced salt solution containing 1.3 mM EDTA (pH 7–7.4) for 30 min at 37 °C. Lung pieces were then digested with collagenase (type IV, *Clostridium histolyticum*, Sigma-Aldrich) for 1 h at 37 °C. The digested lung samples were crushed through cell strainers and partitioned on a 44/67% Percoll (Amersham Biosciences) gradient; lymphocytes were within the interphase cell population. Splenocytes were prepared by crushing the spleens through cell strainers followed by RBC lysis. All staining samples were analyzed using FACSCalibur Instrument or LSRII (Becton Dickinson), and data analysis was performed using FlowJo software (Tree Star).

Ex Vivo Restimulation and Intracellular Cytokine Staining. One million splenocytes or 0.5×10^6 liver lymphocytes from immunized mice were used for ex vivo restimulation in 96-well plates. Depending on the experimental models, cells were restimulated with either 5 μg/mL SIINFEKL peptide or 1 μg/mL SEA in the presence of 5 μg/mL brefeldin A in a total volume 200 μL of culture medium. Cells were incubated in 37 °C, 5% CO_2 for 5 h. At the end of 5 h, cells were fixed with 2% formaldehyde for 5 min at room temperature and permeabilized with 0.25% saponin (wt/vol) before proceeding to staining.

CD107 Staining. To detect the degranulation potential of OT-I cells, 1×10^6 total splenocytes were restimulated with 0.05–5 ng/mL SIINFEKL peptide in a total volume of 200 μL culture medium. Anti-CD107a antibody or a corresponding isotype antibody was added at the beginning of the culture at a dilution factor of 1:100. After 1 h incubation at 37 °C, 5% CO_2, cells were further stained for CD45.1 expression to identify OT-I cells. CD107a up-regulation was analyzed using flow cytometry.

Ex Vivo Culture with IL-33. One hundred thousand purified OT-I cells were cocultured with cytokines at the following concentrations: IL-33 + IL12 (1 ng/mL and 10 ng/mL, respectively), IL-33 + IL-2 (0.1 ng/mL and 1 μg/mL, respectively) in a total volume of 200 μL culture medium. In experiments where OT-I cells were activated in vivo with dual costimulation (50 μg SIINFEKL + 10 μg anti–4-1BB + 15 μg anti-OX40), better expansion contributes to higher T-cell sensitivity to IL-33. These cells were cultured at 20,000 cells per well in a 96-well plate with IL-33 (1 ng/mL), IL-12 (1 ng/mL), IL-18 (20 ng/mL), and ST2-blocking antibody (5 μg/mL). Cells were left at 37 °C, 5% CO_2 for 24 h. Supernatant was collected at the end of the 24 h for IFNγ ELISA.

OT-I Cell Purification for ex Vivo Culture with Cytokines. Single-cell suspension prepared from brachial, axillary, inguinal, and mesenteric lymph nodes of immunized mice was labeled with R-phycoerythrin (PE)-labeled anti-CD45.1 antibody. Cells were then secondarily labeled with anti-PE magnetic beads (Miltenyi Biotec) before being subjected to purification through MACS separation columns. For the purification process using Dynabeads FlowComp Fexi systems (Invitrogen), OT-I cells were labeled with DSB-X–conjugated anti-CD45.1 antibody (prepared using DSB-X Biotin Protein Labeling Kit) before purification with Dynabeads.

Bronchial-Alveolar Lavage Fluid Analysis. Two days post i.n. SEA challenge, the lungs of mice were lavaged three times using a 1-mL aliquot of PBS each time. Cells in the total bronchial-alveolar lavage (BAL) fluid were pelleted by centrifugation and subjected to flow cytometry analysis for $Gr\text{-}1^{hi}$ cells. The remaining BAL fluid was used undiluted for IFNγ ELISA or further concentrated using Amicon Ultra 0.5 mL 3K centrifugal filters (Millipore) before IL-5 ELISA. Total protein content for BAL fluid before and after the concentration step was measured with a Pierce BCA Protein Assay kit (Thermo Scientific). In the final report, cytokine level in BAL fluid was normalized to protein content in each sample.

Immunohistochemistry. C57BL/6 mice were i.n. challenged with 1 μg of SEA and killed 2 d later. Perfused lungs were inflated with 10% formalin solution, clamped for 5 min, fixed for 2 h,

and stored in 70% ethanol before paraffin embedding was performed. Lung sections were stained with methyl green (Vector Laboratories). Detection of IL-33 was done using rat monoclonal antibody against IL-33 followed by anti-rat HRP. Slides were then developed using an ABC kit (Vector Laboratories). Images were recorded using a Zeiss Axioplan 2 microscope (Carl Zeiss Microscopy) and analyzed using Axiovision 4.7.2 software.

Statistical Analysis. Unpaired, two-tailed, and equal variance Student's *t* test was performed for all data shown. Error bars indicate SE of mean; $^{*}P < 0.05$ and $^{**}P < 0.01$.

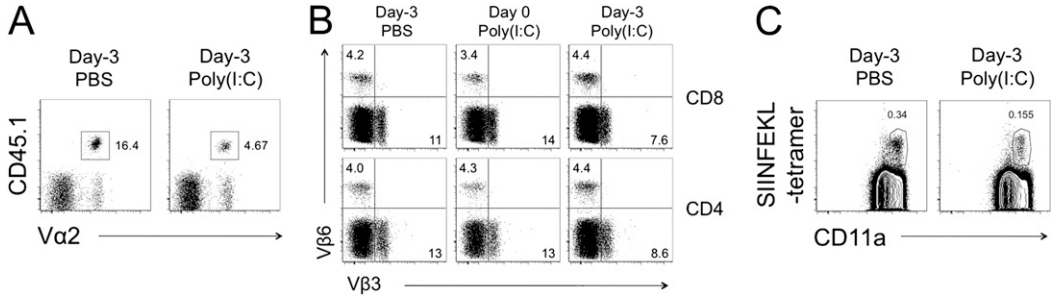

Fig. S1. Systemic pretreatment with poly(I:C) suppresses both adoptively transferred and endogenous CD8 T-cell responses. (*A*) C57BL/6 mice were injected with PBS or 1 μg poly(I:C) on day −3, with OT-I cells on day −1, and with 50 μg SIINFEKL peptide on day 0. Spleens were harvested on day 3, and the frequency of OT-I cells was determined by gating on CD8 T cells. (*B*) C57BL/6 mice were injected with PBS or 1 μg poly(I:C) on day −3 followed by 1 μg SEA on day 0 or SEA together with poly(I:C) on day 0. Spleens were harvested on day 3 post SEA immunization. The frequency of Ag-specific $V\beta3^{+}$ cells and Ag-nonspecific $V\beta6^{+}$ cells was determined by gating on CD4 or CD8 T cells. (*C*) C57BL/6 mice were injected with 100 μg poly(I:C) on day −3 and immunized with 100 μg SIINFEKL peptide, 50 μg CpG, and 5 μg anti–4-1BB agonist antibody on day 0. Spleens were harvested on day 6, and the frequency of SIINFEKL-tetramer^{+} cells was determined by gating on CD8 T cells.

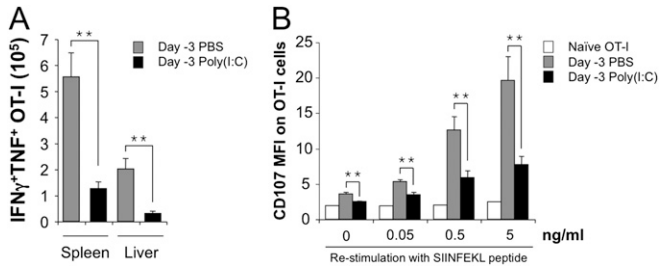

Fig. S2. Impaired antigen-dependent functionality of CD8 T cells primed in poly(I:C)-pretreated mice. C57BL/6 mice were injected with PBS or 1 μg poly(I:C) on day −3 and with OT-I cells on day −1 and immunized with SIINFEKL peptide on day 0. (*A*) Splenocytes and hepatic lymphocytes were harvested on day 3 and restimulated with SIINFEKL peptide to determine the number of effectors. (*B*) Day 3 splenocytes were restimulated with varying concentrations of SIINFEKL peptide for 1 h. CD107a staining was analyzed by gating on OT-I cells. Results were obtained from three to four experiments.

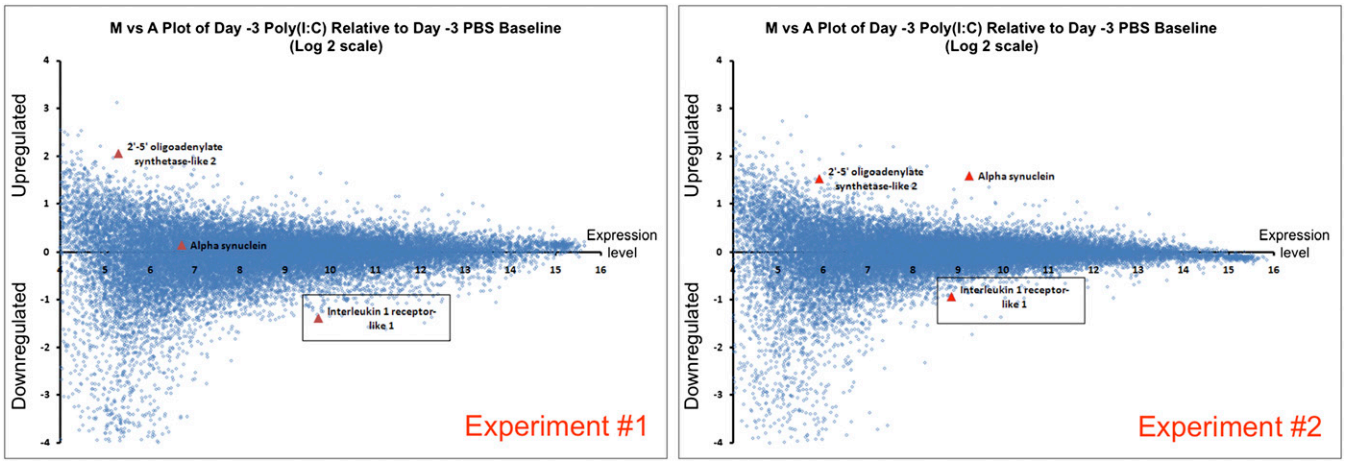

Fig. S3. ST2 (labeled as interleukin 1 receptor-like 1) is down-regulated in OT-I CD8 T cells primed in mice pretreated with poly(I:C). C57BL/6 mice were injected with PBS or 50 μg poly(I:C) on day −3, with OT-I cells on day −1, and with SIINFEKL peptide on day 0. Three days post immunization, splenic OT-I cells were purified by a three-step protocol. Total splenocytes were first passed through a nylon wool column to enrich for T cells. CD4 T cells were then depleted by MACS beads. In the last step, OT-I cells were identified by staining for the congenic marker CD45.1 and were FACS-sorted. Total RNA was extracted from OT-I cells and sent for hybridization with GeneChip Mouse Genome 430A 2.0 arrays (performed by Expression Analysis Inc.). The M versus A plots indicate changes in transcript level in OT-I CD8 T cells from poly(I:C)-treated mice compared with PBS-treated mice. Transcript signal is presented on a log2 scale on both axes. The *x* axis represents gene expression level in OTI^{PBS}. The *y* axis is the log ratio of $OTI^{Poly(I:C)}/OTI^{PBS}$. Positive value on the *y* axis depicts up-regulation of gene expression whereas a negative value depicts down-regulation. Gene expression changes that were reproducible in both experiments are noted (red triangles).

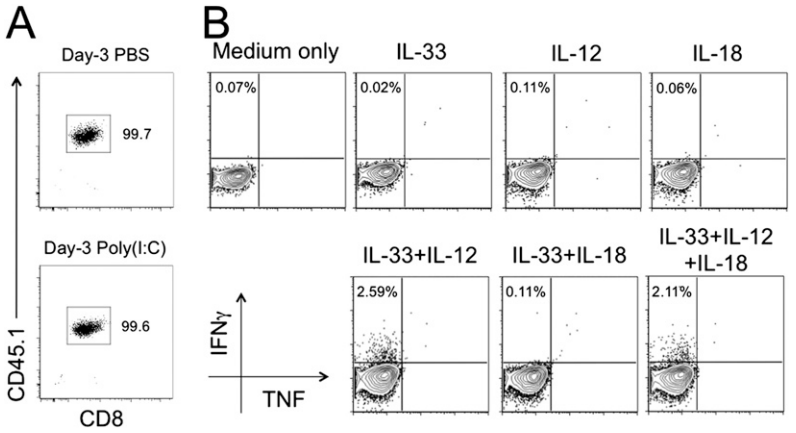

Fig. S4. IL-33 and IL-12 synergize to stimulate IFNγ production from activated CD8 T cells. OT-I CD8 T cells were transferred into C57BL/6 mice on day −1, and the recipient mice were immunized with SIINFEKL peptide on day 0. (*A*) FACS-sorting result for OT-I cells to be used for ex vivo culture with IL-33 and IL-2 in Fig. 3*B*. (*B*) OT-I cells were purified from lymph nodes by magnetic beads on day 3 and cultured with different cytokines as indicated for 24 h. Brefeldin A was added to the culture during the last 5 h, and effector cytokine production was analyzed by gating on OT-I cells.

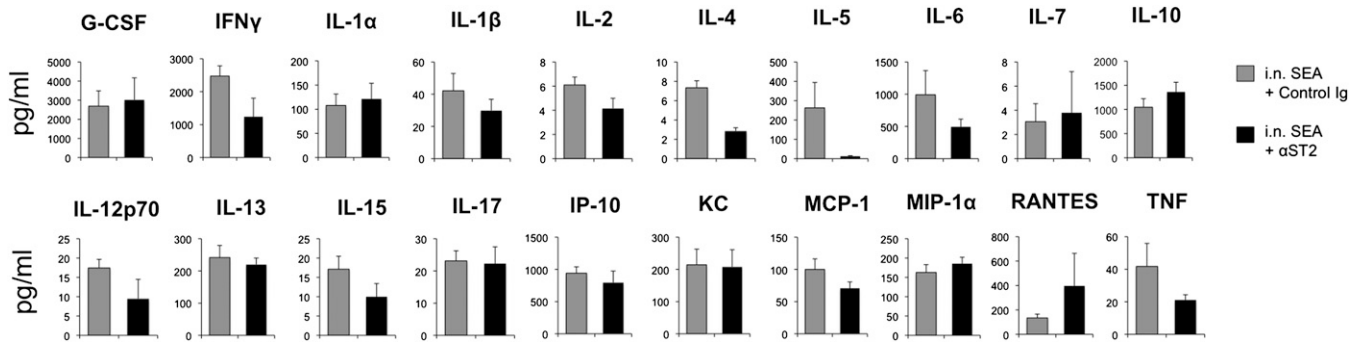

Fig. S5. Cytokine changes after ST2 blockade in the SEA-mediated lung injury experimental model. C57BL/6 mice were i.p. injected with control Ig or 500 μg of anti-ST2 on day −1 and 6 h post intranasal inoculation with 1 μg SEA. BAL fluid was collected on day 2 and subjected to cytokine analysis. *n* = 2–3 for each treatment group.