Online Data Supplement

Mice. TRE-TGO mice express a protein consisting of the transmembrane domain of transferrin-receptor, aa 1-118, enhanced green-fluorescent protein (GFP), and the stimulatory epitope of ovalbumin (OVA), aa 230-359. TGO was isolated from the pEBB vector using BamHI and NotI, and inserted into the multiple cloning site of pTRE2 (Clontech, Mountain View, CA) that expresses a 42-bp tet operator sequence (tetO) upstream of a minimal CMV promoter. The newly constructed pTRE-TGO plasmid was digested with AatII and NdeI to isolate purified TRE-TGO DNA to generate the TRE-TGO transgenic mouse line. The mouse TSLP gene was disrupted by replacing the coding regions of exons 2 through 5 as follows. A TSLP gene targeting vector was constructed with a 2.1-kb short arm containing exon 1 of the TSLP gene locus and a 5.2-kb long arm fragment containing the TSLP 3' UTR genomic locus flanking a neomycin resistance cassette inserted for positive selection with G418. A thymidine kinase cassette was inserted for negative selection with FIAU. The linearized targeting vector was electroporated into GS-1 ES cells (129Sv). Targeted ES cell clones were identified by PCR screening across the short arm side of the targeting event. Southern blot analysis of digested ES cell DNA confirmed correct targeted events via homologous recombination. Selected ES cell clones were microinjected into C57BL/6 blastocysts. Male chimeras were bred with Black Swiss females to obtain mice heterozygous for the TSLP mutation (TSLP+/-). TSLP+/- mice were subsequently intercrossed to generate TSLP-/- mice (TSLP KO). TSLP KO mice were moved to a BALB/c background using Marker-Assisted Accelerated Backcrossing (MAX-BAXSM) technology at Charles River Laboratories. Mice that were identified to be 99.5% BALB/c were used to establish a

breeding colony at Charles River Laboratories. Mice for experimental use were housed at Amgen in accordance with Amgen's Internal Animal Care and Use Committee under specific pathogen-free conditions and according to federal guidelines. Further details of the procedure and analysis of the resulting phenotype will be described elsewhere (Jessup H and Comeau M, manuscript in preparation).

Intranasal treatment. Anesthetized animals were administered intranasally (i.n.) with 50 μg OVA (A7642; Sigma-Aldrich) in a total volume of 30 ml PBS. Following i.n. administration, mice were suspended in an upright position for 10 minutes to ensure complete uptake of the treatment solution.

Bronchial alveolar lavage, tissue fixation and staining. BAL was performed 24 hours after the last aerosol challenge as described before [41]. BAL cells were resuspended in PBS containing 1% BSA and counted using a hemocytometer. Differential cell counts were performed on cytospin cell preparations stained with a modified Wright-Geimsa stain (Diff-Quik^{tm} Stain Set, Siemens). After BAL extraction, lungs were excised completely from the chest cavity, inflated with 10% neutral buffered formalin (Fisher BioTech) and fixed at room temperature overnight in the same solution. Tissues were embedded in paraffin, sectioned and stained with periodic acid Schiff (PAS).

Depletion of CD4$^+$ T cells by anti-CD4 antibodies. Animals were i.p. injected on days 12 and 18 with 200 μg of anti-CD4 antibody (clone: GK1.5, UCSF Monoclonal Antibody Core) or 200 μg rIgG (Sigma) in a total volume of 200 μl PBS.

Detection of IgE, antigen-specific IgE and TSLP in serum by ELISA. High-binding 96-well ELISA plates (Costar; Corning Inc., NY) were coated with 100 ng/well anti-mouse IgE (BD Pharmingen, San Diego, CA) Ab overnight at 4°C. The plates were washed three times with PBS containing 0.5% Tween and blocked with 1% BSA-PBS for 1 h at 37°C. Plates were washed, and purified IgE (BD Pharmingen) or sera were incubated in duplicate for 2 h at 37°C. Plates were washed, and a biotinylated anti-mouse IgE was added for 2 h at 37°C. Plates were washed and incubated for 0.5 h at 37°C in the presence of streptavidin-HRP (Sigma-Aldrich). Finally, substrate (TMB) was added and incubated at RT in the dark. The reaction was stopped with 2 N H_2SO_4. OD was measured at 450 nm.

For OVA-specific IgE, plates were coated overnight with 0.2 mg of OVA/ml in PBS. Plates were washed and blocked with 1% BSA-PBS for 1 h at 37°C. Serum samples were added and plates were incubated overnight at 4°C. Plates were washed, and biotin-conjugated anti-mouse IgE was added at a 1/500 dilution and incubated for 1 h at 37°C. Plates were washed, incubated and read as described before. To detect BSA-specific IgE, plates were coated overnight with BSA and blocked with 1% fetal bovine serum.

TSLP ELISA reagents were purchased from R&D Systems. ELISA was done according to the manufacturer's instructions.

Quantitative RT-PCR. Total RNA was isolated using the GenElute Mammalian Total RNA Miniprep Kit (Sigma) and cDNA was synthesized with Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions respectively.

Quantitative PCR was performed with Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen) and 7900HT PCR System (Applied Biosystems). All data were analyzed on SDS software (Applied Biosystems) and gene expression was determined as an average of triplicate samples after drawing standard curve and normalized by GAPDH gene expression.

Oral antigen administration. Oral tolerance to OVA was induced by given ad libitum 1% OVA (grade II; Sigma-Aldrich) solution dissolved in drinking water, for 5 consecutive days.

Adoptive transfers. Donor CD4 T cells collected from secondary lymphoid tissues (axillary, brachial, cervical, mesenteric, inguinal lymph nodes, and spleen) from DO11.10/RAG-/- mice by using magnetic microbeads (Miltenyi Biotec) were labeled with 5,6-carboxyfluorescein diacetate succinimyl ester (CFSE). CD4+ T cell subsets were checked for purity (>90%) by flow cytometry before transfer. Before CFSE staining, cells were washed twice with cold PBS and incubated at 10 x 10^6 cells/ml in PBS with 2 μM CFSE at 37°C for 10 min. Cells were then washed twice with cold DMEM/10% FCS. 5x 10^5 cells/mouse were injected intravenously. Recipient mice were killed 7 days later. Single cell suspensions were prepared separately from spleen and inguinal lymph nodes. The T cells were stained by using fluorochrome-conjugated mAbs for murine CD4 (RM4-5) and DO11.10 TCR (KJ1-26) purchased from eBioscience or Biolegend.