Fallon et al., http://www.jem.org/cgi/content/full/jem.20051615/DC1

SUPPLEMENTAL RESULTS

Generation of *il25*$^{-/-}$ mice to study IL-25 function

Although transgenic mice overexpressing IL-25 have implicated this cytokine in the development of type 2 responses, the biological significance of this cytokine has remained undefined. To investigate the in vivo roles of IL-25, we have generated *il25*$^{-/-}$ mice. The targeting vector comprised a 5′ arm of homology and a 3′ arm of homology positioned on either side of the neomycin resistance cassette (Fig. S1 A) and was designed to remove a region from exon 2 of the *il25* gene encoding 64 amino acids of the mature protein. Genotyping of wild-type (*il25*$^{+/+}$), heterozygous (*il25*$^{+/-}$), and homozygous null (*il25*$^{-/-}$) mice is shown in Fig. 1 SB. *il25*$^{-/-}$ mice were healthy and displayed no overt phenotypic abnormalities. Analysis of the *il25*$^{-/-}$ mice failed to detect *il25* RNA transcripts from activated mast cells (Fig. S1 C) or activated lymphocytes differentiated under Th2-polarizing conditions (not depicted), using reverse transcriptase–polymerase chain reaction assays.

SUPPLEMENTAL MATERIALS AND METHODS

Targeted disruption of the mouse *il25* gene in embryonic stem (ES) cells

The replacement vector was constructed to insert the neomycin resistance gene into exon 2 of the *il25* gene, deleting the nucleotides encoding amino acids 22–86 (64 amino acids from the 169 amino acid coding sequence). The targeting vector consisted of 2.3 kb of the *il25* gene providing the 5′ arm of homology (generated using PCR primers 5′-CCGGTTGGCTGCCAGGCAAGTAGAG-3′ and 5′-CTCCTGGATCCGCAAGCTGACGGTGTGGGTTCCCAC-GATC-3′ from which a BamHI fragment was isolated for subcloning) and 4.6 kb comprising the 3′ homology arm (generated using PCR primers 5′-TTATATTCTAGAGGCTAATACATAGGTCAAATGCAACAATG-3′ and 5′-ATAAATCTAG-ATCAACCAGGAGGCAGAGGGGAGACCAGG-3′). The targeting vector was linearized and electroporated into E14.1 ES cells (43). EcoRI-digested genomic DNA from isolated clones was screened by hybridization using a flanking probe. The probe was made by PCR using the primers: 5′-CCGGTTGGCTGCCAGGCAAGTAGAG-3′ and 5′-GTTCCAGCTTCCT-GTTCCCCAGC-3′. The targeted ES cell clone was microinjected into 3.5-d C57BL/6 blastocysts to generate chimeras. These mice were mated with C57BL/6 mice and transmitted the ES cell genotype through the germline. Mice homozygous for the disrupted *il25* gene were obtained by interbreeding the heterozygotes. Genotyping was performed by PCR using primers 2207 (5′-TTGGCAATGATCGTGGGAACC-3′) and 2208 (5′-CATTTGACCTATGTATTAGCC-3′) that give a product of 479 bp for the wild-type allele and 1,238 bp for the targeted allele. The *il25* gene-targeted and wild-type animals

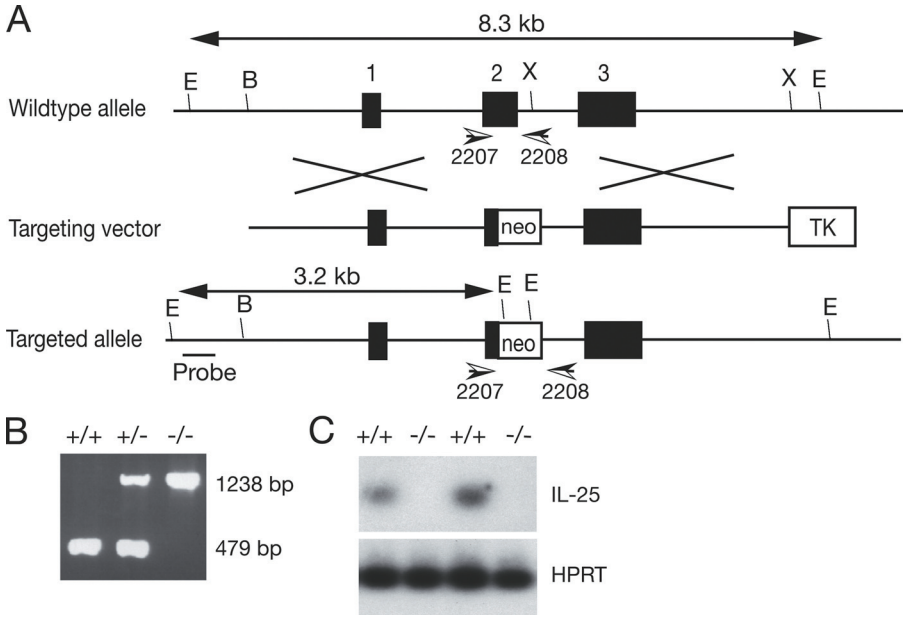

Figure S1. Inactivation of the *il25* gene by homologous recombination. (A) Structure of the *il25* locus, the targeting vector, and the predicted homologous recombination event are shown. Neo, neomycin cassette; TK, thymidine kinase cassette; B, BamHI; E, EcoRI; and X, XbaI. (B) PCR detection of the wild-type and targeted *il25* alleles. (C) RT-PCR analysis of *il25* expression. Mast cells were cultured with PMA and ionomycin for 1 h, RNA was prepared, and RT-PCR was performed.

www.jem.org/cgi/doi/10.1084/jem.20051615

used in the experiments reported were maintained on 129 x C57BL/6 (F_2) backgrounds in a specific pathogen-free environment.

Mast cells and RNA preparation

Bone marrow was aseptically flushed from femora and tibias of mice. The cell suspension was cultured at 4×10^5 cells/ml in the presence of 5 ng/ml of IL-3 and 10% WEHI-3B conditioned supernatant for 3–4 wk, with media changes every 7 d. The resultant cell populations were ~95% c-kit$^+$ and Gr-1$^+$ when analyzed by flow cytometry (BD Biosciences). After washing, cells were resuspended at 10^6 cells/ml and stimulated for 1 h with the polyclonal activators PMA at 50 ng/ml and/or calcium ionophore A23187 at 500 ng/ml. Total RNA was prepared using RNAzolB. RT-PCR primers for *il25* were 5′-GTCAGCTT-GCGGATCCAGGAG-3′ and 5′-AGTCCCTGTCCAACTCATAGC-3′. The internal oligonucleotide used for hybridization was 5′-GCTGAAGTGGAGCTCTGCATCTGTGTC-3′. *hprt* primers and conditions were as described previously (McKenzie, G.J., C.L. Emson, S.E. Bell, S. Anderson, P. Fallon, G. Zurawski, R. Murray, and A.N.J. McKenzie. 1998. *Immunity*. 9:423–432.).

In vitro Th1 and Th2 cell development is normal in cells from *il25*$^{-/-}$ mice

FACS analysis of cell suspensions prepared from mesenteric lymph node, spleen, bone marrow, and thymus, from naive *il25*$^{-/-}$ and wild-type mice, demonstrated no apparent differences in the expression of the following cell surface markers: CD4, CD8, T cell receptor, CD5, CD25, CD45, IgM_{a+b}, CD23 (not depicted); expression of CD11b was normal in mesenteric lymph node, spleen, and thymus. We observed a small increase in the proportion of CD11b positive cells in bone marrow of the naive *il25*$^{-/-}$ mice (not depicted). Furthermore, when lymph node cells were cultured in vitro under conditions that promote differentiation of Th1 or Th2 cells, no significant differences were observed in the generation of these lineages in the *il25*$^{-/-}$ or wild-type mice (Fig. S3). These data indicate that the absence of IL-25 does not impair the ability of T cells to differentiate into Th1 or Th2 cells.

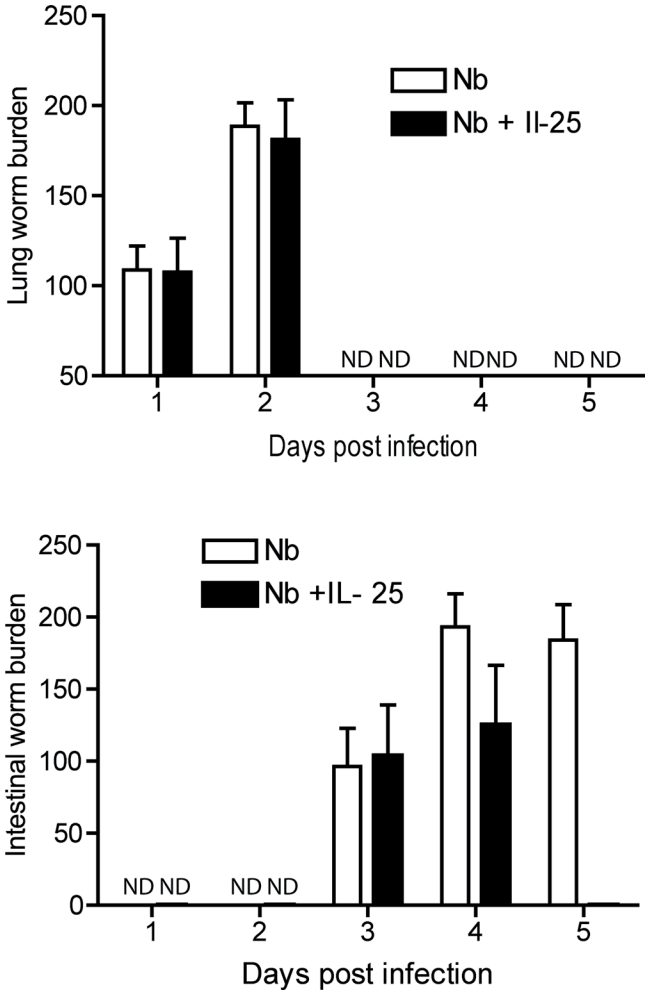

Figure S2. Worm counts in lungs and intestines of *N. brasiliensis*–infected wild-type mice after treatment with rIL-25 or control saline. *N. brasiliensis* **larvae were initially able to migrate normally to the lungs and intestines of infected mice after IL-25 treatment; however, the parasites were expelled more rapidly from the intestines than in control animals. Data represent the mean ± SEM from seven individual mice. Data are representative of two repeat experiments.**

In vitro T helper cell differentiation assays

Mesenteric lymph node cells were cultured on anti-CD3 antibody-coated plates (1 μg/ml of clone 2C11; Becton Dickinson) in the presence of exogenous cytokines or anti-cytokine antibody as indicated. 10 ng/ml IL-2 (R&D Systems) was added to all cultures. Th2 cell differentiation was promoted in the presence of 100 ng/ml IL-4 (R&D Systems) and anti-IFNγ antibody (10 μg/ml of clone XMG1.2; Becton Dick-

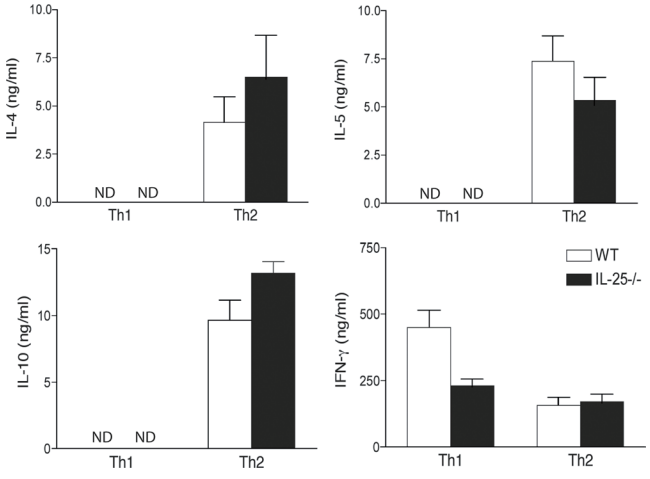

Figure S3. Cytokine production after in vitro T helper cell differentiation. Data represent the mean ± SD from five individual mice. ND, not detected. Data are representative of two repeat experiments.

inson), whereas Th1 differentiation was promoted by anti–IL-4 antibody at 10 μg/ml (clone 11B11; DNAX Research Institute) and 1 ng/ml IL-12 (Genzyme). Cells were cultured for 5 d, washed, and resuspended at 10^6 cells/ml for 24 h in the presence of plate-bound anti-CD3. Supernatants were analyzed by cytokine ELISA.

Production of recombinant IL-25

A PCR product consisting of mouse IL25 Val17-Ala 169 with a COOH-terminal 6 His tag was made, sequenced, and cloned into pET 28a (Novagen). The construct was transformed into the *Escherichia coli* expression host BL21 DE3. Cell pellets were solubilized in 100 mM NaH_2PO_4, 10 mM Tris, 6 M guanadine, pH 7, at 5 ml/gram wet weight (1 h stirring at room temperature) and centrifuged at 10,000 *g* for 30 min 1 ml of 50% NiNTA agarose slurry (QIAGEN) was added per 4 ml of lysate and mixed on an end-over-end mixer for 1 h at room temperature. The lysate/slurry mix was loaded into an empty column and washed sequentially with 100 mM NaH_2PO_4, 10 mM Tris, 300 mMNaCl, 5 mM β-mercaptoethanol, 20 mM imidazole, pH 7, containing 8, 4, and 2 M urea. rIL25-His was eluted with 100 mM NaH_2PO_4, 10 mM Tris, 300 mM NaCl, 5 mM β-mercaptoethanol, 500 mM imidazole, 2 M urea, pH 7. The purified, denatured rIL25-His was diluted to 50 μg/ml in folding buffer (100 mM NaH_2PO_4, 10 mM Tris, 300 mM NaCl, 50 mM glycine, 2 mM reduced glutathione, 0.2 mM oxidized glutathione, pH 7) containing 2 M urea and dialyzed sequentially against folding buffer containing 1 M urea, 500 mM urea, folding buffer alone, and finally against PBS. Any precipitate was removed by centrifugation and the purified rIL25-His quantified by SDS-PAGE.