Supporting Information

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SI Materials and Methods

Mice. C57BL/6 were purchased from Oriental yeast, *Rag2*$^{-/-}$ and *gc*$^{-/-}$*Rag2*$^{-/-}$ mice were from Taconic. All animal experiments were performed in accordance with guidelines of the Institutional Animal Care Committee of Hyogo College of Medicine (Hyogo, Japan).

Reagents. Chitin was purchased from New England Biolabs. Fluorescent-labeled antibodies for CCR3, and Siglec F were purchased from BD Biosciences; Gr-1, CD45, Sca-1, CD3, CD4, CD8, CD19, NK1.1, c-Kit, and B220 were from BioLegend; T1/ST2 was from MD Biosciences; and IgE (23G3) was from Southern Biotechnology Associates. Recombinant human IL-33 (rhIL-33) was made by Hokudo Co.

Generation of *Il33*$^{-/-}$ Mice. The *Il33* gene was isolated from genomic DNA extracted from ES cells (GSI-I) by PCR. The targeting vector was constructed by replacing a 2.9-kb fragment encoding the *Il33* ORF with a *neomycin-resistance* gene cassette (*neo*), and a herpes simplex virus thymidine kinase (HSV-TK) driven by the PGK promoter had been inserted into the genomic fragment for negative selection. After the targeting vector was transfected into ES cells, G418 and gancyclovir doubly resistant colonies were selected, screened by PCR. and further confirmed by Southern blotting. Homologous recombinants were microinjected into blastocysts from C57BL/6 female mice, and heterozygous F1 progenies were intercrossed to obtain *Il33*$^{-/-}$ mice. *Il33*$^{-/-}$ mice under 129Sv × C57BL/6 background were backcrossed to C57BL/6 for four generations and their littermate controls were used for the experiments.

Helminths Infection. At indicated time points after infection, the lungs were prepared for histology or analysis of protein or RNA after perfusing the mice via the right ventricle with 10 mL PBS under anesthesia. Bronchoalveolar lavage was performed and bronchoalveolar lavage fluid (BALF) cells were analyzed with flow cytometry. Sera were collected at 0, 7, 10, and 14 d after infection and measured the concentrations of IgE and mouse mast cell protease 1 (mMCP-1) by ELISA (1, 2). Seven days after infection, mesenteric lymph nodes (mLNs) were harvested and cell suspensions were enriched for CD4^{+} cells by positive sorting on AutoMACS (Miltenyi Biotec) with magnetic beads conjugated with anti-CD4 mAb. The CD4^{+} cells were stimulated with anti-CD3 and anti-CD28 antibodies. The culture supernatants were harvested 24 h later and measured the concentrations of IFN-γ, IL-4, and IL-5. In IL-33 reconstitution experiment, *Il33*$^{-/-}$ mice received intranasal administration of 4 μg rhIL-33 at 1 d before and 0, 1, 2, and 3 d after infection.

Chitin Administration. Mice were intranasally administered 50 μL of 0.75 mg chitin or 2-mg glass beads under anesthesia, and at indicated time points, histological and BALFs analysis were performed.

Histological Analysis. Lungs were fixed with 4% (wt/vol) paraformaldehyde and embedded with paraffin. Deparaffinized sections were microwave-heated in citrated buffer (pH 6.0) for antigen retrieval, then blocked with 1% BSA, stained with affinity-purified rabbit anti-mouse IL-33 polyclonal antibody, followed by biotinylated goat anti-rabbit antibody (Vector Labs) and Alexa-555–labeled streptavidin (Invitrogen). For the double-staining experiment, IL-33–stained sections were blocked and stained with anti-Pro surfactant Protein C antibody (Millipore), following biotinylated goat anti-rabbit antibody and Alexa-488–labeled streptavidin (Invitrogen), then mounted with Prolong Antifade Gold with DAPI (Invitrogen). For macrophage staining, frozen sections of freshly isolated lung specimens were incubated with F4/80 mAb, biotinylated anti-rat IgG, and then Alexa Fluor 488-conjugated streptavidin (Molecular Probes). Nuclei were stained with DAPI (KPL). The immunostaining of each section was evaluated under microscope Zeiss LSM 510 (Carl Zeiss). Computer software, Zeiss LSM 510 ver. 3.2 (Carl Zeiss), was used for image processing and analysis.

Flow Cytometry. BALF cells were stained with antibodies for CD45, CD3, B220, CCR3, and Gr-1, examined by FACS Calibur (BD Biosciences) and classified as follows. CD45^{+}CD3^{-}B220^{-}CCR3^{+} cells: eosinophils; CD45^{+}CD3^{+}B220^{+}CCR3^{-} cells: lymphocytes; CD45^{+}CD3^{-}B220^{-}CCR3^{-}Gr-1^{high} cells: neutrophils; and CD45^{+}CD3^{-}B220^{-}CCR3^{-}Gr-1^{-} cells with high background intensity: monocytes. Monocytes were further confirmed with CD11a expression. Natural helper (NH) cells in BALF were examined for the expressions of lineage markers (CD3, CD4, CD8, CD19, Gr-1, Siglec F, IgE, NK1.1), Sca-1, c-Kit, and ST2. For intracellular IL-5 staining, BALF cells were incubated in the culture medium [10% (vol/vol) FCS-RPMI containing 2-mercaptoethanol, L-glutamine, penicillin, and streptomycin] for 3 h and stained surface antigens in the presence of monensin. Cells were fixed with 4% PFA, permeabilized with 0.1% saponin buffer (PBS with 0.1% saponin, 1 mM Hepes and 0.1% BSA) and stained with anti–IL-5 or control Ab (rat IgG1; BD Biosciences).

Quantitative RT-PCR. Total RNA was extracted with RNeasy Mini Kit (Qiagen) and the cDNA was synthesized using SuperScript III (Invitrogen). The expressions of genes were quantified with TaqMan Gene Expression Assays (Applied Biosystems). The result were shown as relative expression standardized with the expression of the gene-encoding eukaryotic 18S rRNA or β-actin. Specific primers and probes used for quantitative RT-PCR were *Il33*, *Il5*, *Il13*, *Epx*, *Prg2*, *Ccl11*, *Il18*, *Tslp*, *Il25*, *Actin-b*, and *18S* rRNA (Applied Biosystems).

1. Matsuba-Kitamura S (2010) Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. *Int Immunol* 22:479–489.

2. Sasaki Y (2005) IL-18 with IL-2 protects against *Strongyloides venezuelensis* infection by activating mucosal mast cell-dependent type 2 innate immunity. *J Exp Med* 202: 607–616.

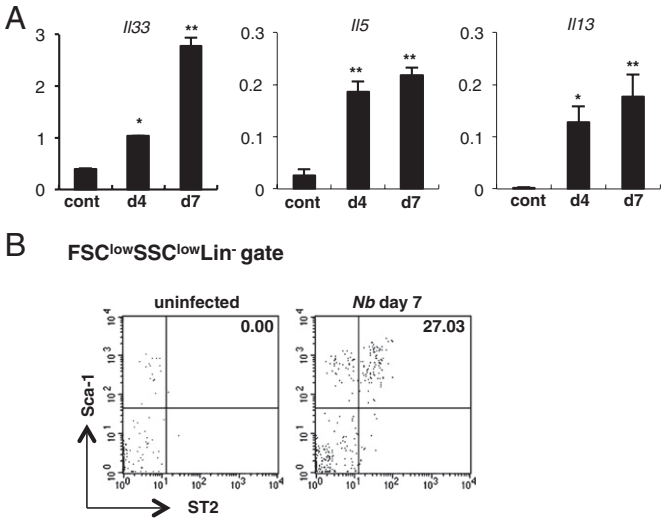

Fig. S1. IL-33 induction in *Nippostrongylus brasiliensis* infected mice. (*A*) Total RNA was prepared from the lungs of normal (cont) or *N. brasiliensis* infected BALB/c mice and the expression level of mRNA for *Il33*, *Il5*, *or Il13* was determined by quantitative RT-PCR. Data are representative of three independent experiments and expressed as the means ± SD. $*P < 0.01$, $**P < 0.001$ (one-way ANOVA with Dunnett's posttest). (*B*) Flow cytometric analysis of the expression of Sca-1 and ST2 by BALF cells from *N. brasiliensis* infected B6 mice at 7 d postinfection. Cells were gated on the $FSC^{low}SSC^{low}Lin^{-}$ subset.

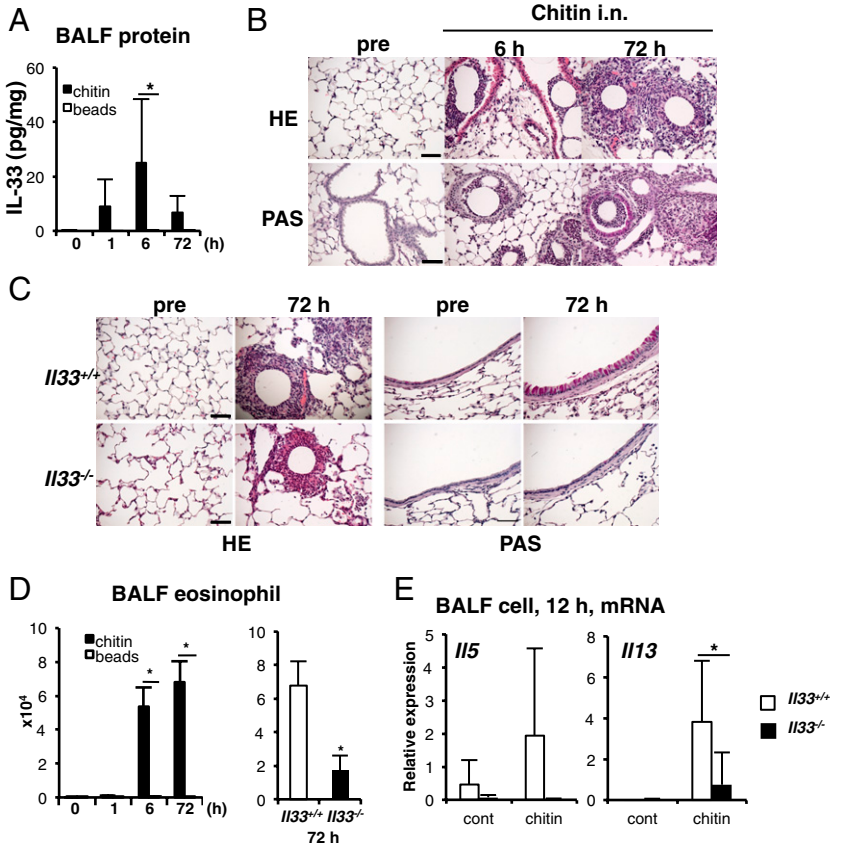

Fig. S2. Intranasal administration of chitin induces IL-33 production in the lungs. (*A*) Chitin was intranasally administered into WT mice. IL-33 concentrations in the BALFs at indicated time points. The concentrations of IL-33 in the BALFs were normalized by the total protein concentration ($n = 5$). Data are representative of two independent experiments. (*B* and *C*) Chitin was intranasally administered into WT (*B*, $n = 4$), $Il33^{+/+}$ (*C*, $n = 4$), or $Il33^{-/-}$ (*C*, $n = 5$) mice. Histological analysis of the lungs was performed at the indicated time points. (Scale bars, 50 μm.) (*D*) Number of eosinophils in BALF cells from chitin- or glass bead- (2 mg) treated mice at indicated time points ($n = 5$). (*E*) The expression levels of *Il5* or *Il13* mRNA in BALF cells from $Il33^{+/+}$ or $Il33^{-/-}$ mice ($n = 3$). Data are expressed as the means ± SD. $*P < 0.05$ (Student's *t* test).

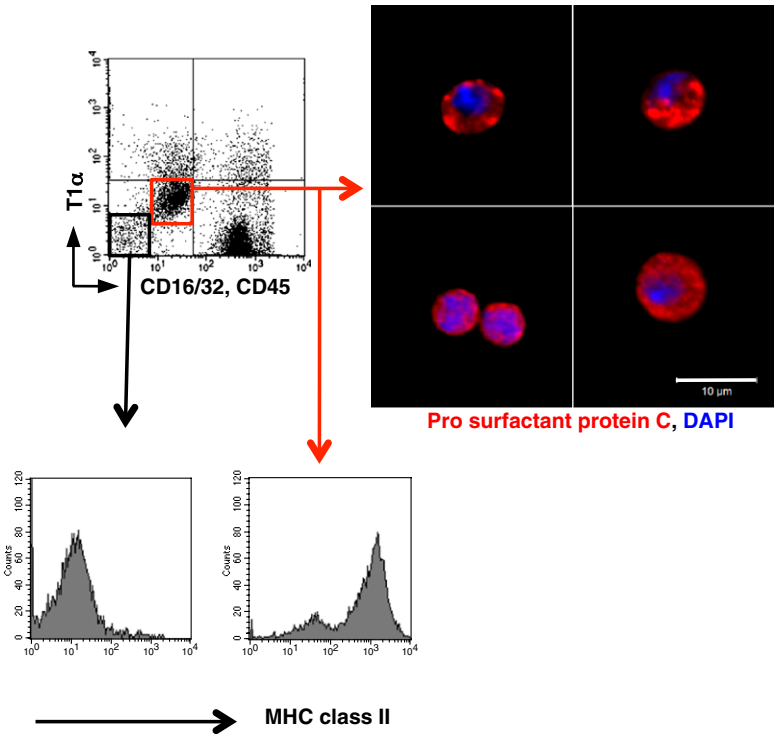

Fig. S3. Characterization of alveolar epithelial type II (ATII) cells. Flow cytometric analysis of the expressions of CD45, C16/32, and T1α by freshly prepared lung cells from naive B6 mice. MHC class II expression by cells in each gate was shown. $CD45^{-}CD16/32^{-}T1\alpha^{-}$ cells in red gate were sorted and stained with anti-SPC antibody and DAPI, and subjected to confocal microscopic examination. (Scale bar, 10 μm.)

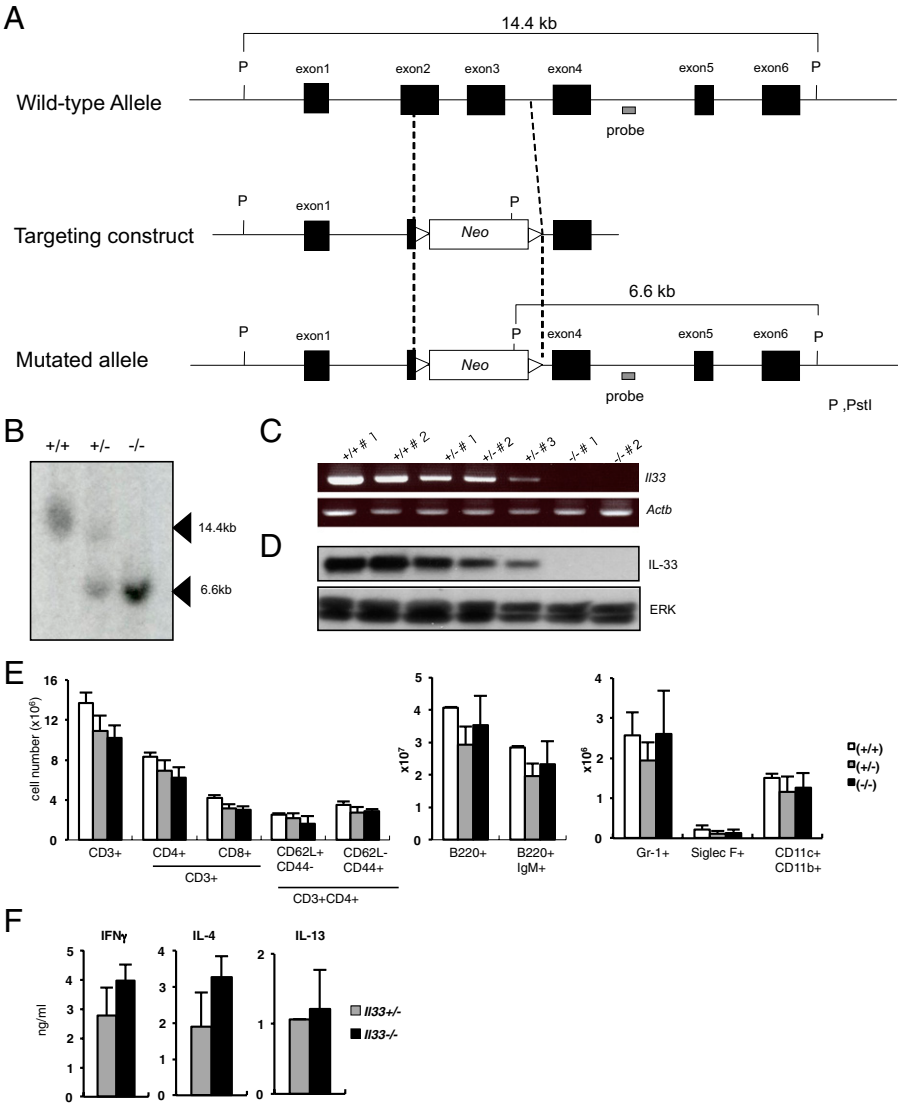

Fig. S4. Generation of *Il33*$^{-/-}$ mice. (*A*) Structure of the mouse *Il33* gene, the targeting construct and the disrupted gene. (*B*) Southern blot analysis of offspring from the heterozygote intercrosses. Genomic DNA was extracted from mouse tails, digested with PstI, separated by electrophoresis, and hybridized with the radiolabeled probe indicated in *A*. (*C*) RT-PCR for analysis of the expression levels of *Il33* mRNA in the lungs from mice of indicated genotypes. Total RNAs isolated from the lungs were subjected to RT-PCR for the expression of *Il33* and *Actin-b*. (*D*) Immunoblotting for analysis of the levels of IL-33 protein in the lungs from mice of indicated genotypes. Lung homogenates were immunoprecipitated with polyclonal anti–IL-33 antibody followed by immunoblotting with anti-IL-33 antibody (Nessy-1). The same homogenates were subjected to immunoblotting with anti-ERK antibody. (*E*) The comparison of cell populations of each subset for T cells, B cells, granulocytes, and dendritic cells in splenocytes of *Il33*$^{+/+}$, *Il33*$^{+/-}$, and *Il33*$^{-/-}$ mice. (*F*) Splenic CD4^{+} T cells from *Il33*$^{+/+}$ and *Il33*$^{-/-}$ mice were stimulated with anti-CD3 and anti-CD28. Twenty-four hours later, the culture supernatants were collected and measured concentration of IFN-γ, IL-4, and IL-13 by ELISA.

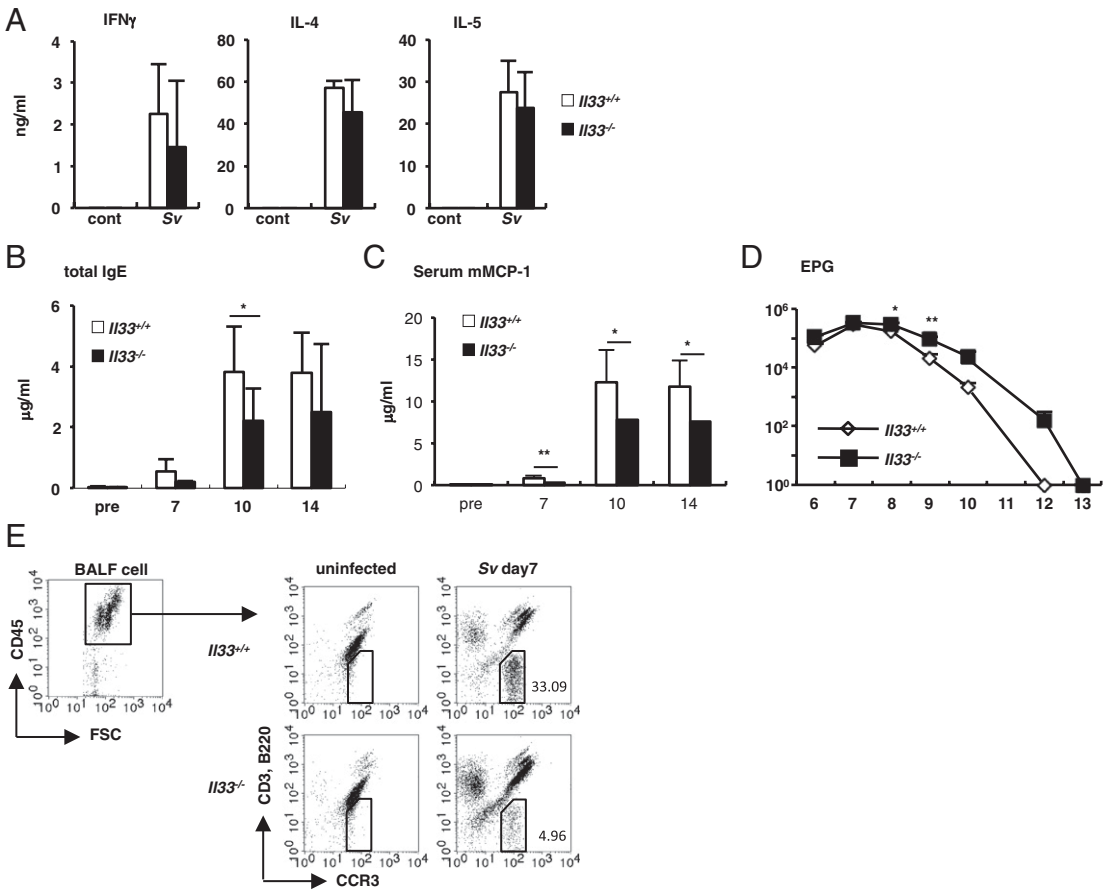

Fig. S5. *Il33*$^{-/-}$ mice show normal Th2 differentiation in the mLNs but reduced systemic Th2 responses to *Strongyloides venezuelensis* infection. (*A*) CD4^{+} T cells in mLNs from *S. venezuelensis* infected mice were labeled with anti-CD4 magnetic beads, purified with AutoMACS, and stimulated with plate bound anti-CD3 and anti-CD28 antibody for 24 h. Supernatants were harvested and tested for IFN-γ, IL-4, and IL-5 production by ELISA. (*B* and *C*) *Il33*$^{+/+}$ ($n = 8$) and *Il33*$^{-/-}$ ($n = 7$) mice were infected with *S. venezuelensis*. IgE (*B*) or mMCP-1 (*C*) concentrations in the sera at indicated time points were measured by ELISA. Data are expressed as the means ± SD. *$P < 0.05$, **$P < 0.01$ (Student's T-test). (*D*) The numbers of eggs per gram feces were counted daily from day 6 after *S. venezuelensis* infection. Data are representative of two independent experiments and expressed as the means ± SD. *$P < 0.05$, **$P < 0.01$ (Student's *t* test). (*E*) *Il33*$^{+/+}$ ($n = 4$) and *Il33*$^{-/-}$ ($n = 5$) mice were infected with *S. venezuelensis*. Flow cytometric analysis of CD45^{+} BALF cells from uninfected or *S. venezuelensis* infected mice (*Sv*). Numbers indicate proportion of eosinophils (box).

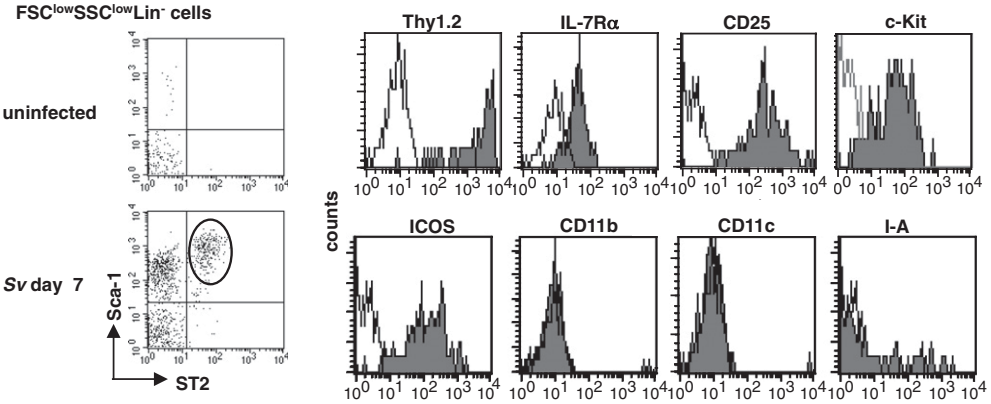

Fig. S6. Flow cytometry of BALF cells from noninfected or *S. venezuelensis* infected WT mice ($n = 5$). Cells were pooled in each group and gated on FSC^{low}SSC^{low}Lin^{-} fraction and were stained for ST2 and Sca-1. Cells in ST2^{+}Sca-1^{+} fraction were examined for their expression of indicated antigens (filled histograms). Empty histograms: control antibody.