METHODS

L major infection

Footpad thickness was measured weekly for up to 8 weeks; parasite burdens and cytokine production from popliteal lymph nodes and spleens were assessed at 3 and 6 weeks after infection. For parasite burdens, spleens and footpads were minced and homogenized in 5 mL of Drosophila medium and serially diluted at 1:2 (spleen) or 1:10 (footpad). Parasite growth was monitored daily by using an inverted light microscope for 2 weeks, and parasite burden is reported as the reciprocal of the final dilution containing viable parasites. Popliteal lymph nodes and spleens were analyzed for IL-4– and IFN-γ–producing cells by using the ELISpot assay, as previously reported.[E1,E2]

Dynamic adhesion assay

Glass slides (35-mm) were coated with 1 μg/mL CD54 overnight at 4°C and blocked with I-block (Applied Biosystems) for 30 minutes. Slides were placed under parallel-plate flow chambers and washed for 5 minutes with PBS (Ca^{++} and Mg^{++} free) to flush the line of particulates. Spleens were harvested from mice, and $CD4^+$ cells were isolated with CD4 (L3T4) microbeads (Miltenyi Biotec, Auburn, Calif). T cells (1×10^6 $CD4^+$ T cells) were injected into the flow line, and steady flow was initiated at a shear force of approximately 1 dyne/cm^2. The flow chamber was contained in a Lucite box–fitted microscope and maintained at 37°C during adhesion experiments. Rolling cells were recorded on high-speed magnetic tapes with phase-contrast video microscopy (Nikon Diaphot-TMD microscope) and a Sony charge-coupled video camera (Sony Corp, New York, NY) and converted to digital video by Image-Pro (Media Cybernetics, Bethesda, Md). Videos were analyzed with ImageJ software (Open source, National Institutes of Health). A rolling/adherent cell was defined as a cell that could be tracked in at least 3 consecutive frames and fit the morphology of a single T cell.

Allergic airway disease

Measurement of changes in respiratory system resistance in response to intravenous acetylcholine challenge, bronchoalveolar lavage fluid differential counts, total bronchoalveolar lavage glycoprotein measurements, and analysis of lung IL-4– and IFN-γ–producing cells were performed, as previously described.[E2,E3] Culture supernatants from lung homogenates incubated overnight at 37°C were analyzed for IL-5, IL-13, eotaxin, thymus and activation-regulated chemokine, and IL-17 by using Luminex (Millipore, Billerica, Mass) with the Bio-Plex System (Bio-Rad Laboratories, Hercules, Calif).

Adoptive transfer of OVA-specific $CD4^+$ T cells

Single-cell suspensions that were prepared from spleens of immunized mice were separated with CD4 (L3T4) microbeads (Miltenyi Biotec).[E1,E4] Selected cells were plated in a 24-well plate (2.5×10^6 $CD4^+$ cells per well) with equal numbers of irradiated $CD4^-$ cells in T_H2-polarizing media (αIL-12 [1 μg/mL, BD Bioscience], αIFN-γ [1 μg/mL, BD Bioscience], rmIL-2 [50 U/mL, BD Bioscience], and rmIL-4 [50 ng/mL, BD Bioscience]) with OVA (1 μg/mL A5503; Sigma-Aldrich, St Louis, Mo) and cultured for 48 hours at 37°C. Cultures were collected, and T cells were separated by using Lympholyte (Cedarlane, Westbury, NY) and counted. Cultured antigen-specific T cells were differentially labeled according to genotype with either PHK-26 or CellVue (5 μmol/L, Sigma-Aldrich) and mixed at a 1:1 ratio. Cells were intraperitoneally injected (2×10^6 cells/mouse) into the strain-specific Rag-deficient mice. Mice were challenged intranasally with 4×10^5 *A niger* and OVA (1 mg/mL) for 3 days and allowed to rest for a day. Lungs were harvested, perfused, and extruded through a mesh, and single-cell suspensions were collected. RBCs were then lysed, and cells were analyzed with an LSR Fortessa (BD, San Jose, Calif) for the presence of fluorescently labeled T cells.

Human SNP analysis and correlation

CAMP was a multicenter clinical trial of the effects of anti-inflammatory medications in children with mild-to-moderate asthma. All participants had asthma defined by symptoms greater than twice per week, use of an inhaled bronchodilator at least twice weekly or use of daily medication for asthma, and increased airway responsiveness.[E5] Of the 1041 children enrolled in the original clinical trial, 968 children and 1518 of their parents contributed DNA samples. Serum IgE levels were measured by using RAST, and the allergy phenotypes were assessed by means of parental self-report of a doctor's diagnosis of these conditions.

Statistical analysis

Mouse data are presented as means ± SEMs. Significant differences are expressed relative to PBS-challenged mice or appropriate control animals (Fig E7) with a power of a *P* value of .05 or less, as measured by using 2-way ANOVA with the Bonferroni multiple comparison.

REFERENCES

E1. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. J Exp Med 1996;183:109-17.

E2. Lee S-H, Prince JE, Rais M, Kheradmand F, Ballantyne CM, Weitz-Schmidt G, et al. Developmental control of integrin expression regulates Th2 effector homing. J Immunol 2008;180:4656-67.

E3. Porter P, Susarla SC, Polikepahad S, Qian Y, Hampton J, Kiss A, et al. Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. Mucosal Immunol 2009;2:504-17.

E4. Lee SH, Prince JE, Rais M, Kheradmand F, Shardonofsky F, Lu H, et al. Differential requirement for CD18 in T-helper effector homing. Nat Med 2003;9:1281-6.

E5. Anonymous. The Childhood Asthma Management Program (CAMP): design, rationale, and methods. Childhood Asthma Management Program Research Group. Control Clin Trials 1999;20:91-120.

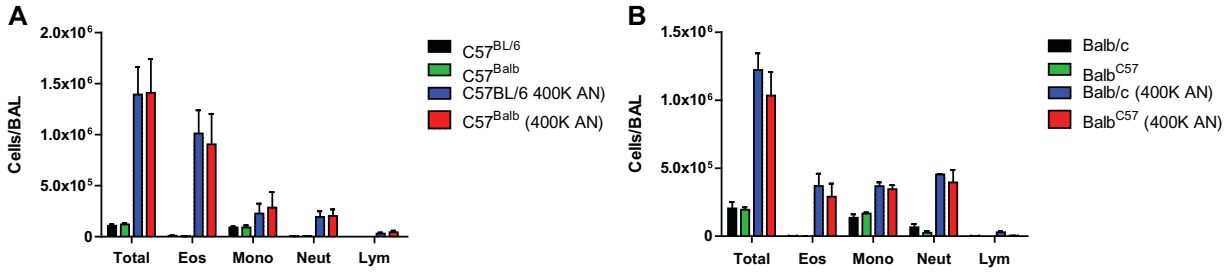

FIG E1. Differential counts of inflammatory cells from bronchoalveolar lavage *(BAL)* fluid of C57BL/6 **(A)** and BALB/c **(B)** background mice challenged with PBS or 4×10^5 *A niger* conidia (*AN*; n = 5 mice per group). *Eos*, Eosinophils; *Mac*, macrophages; *Neut*, neutrophils; *Lym*, lymphocytes.

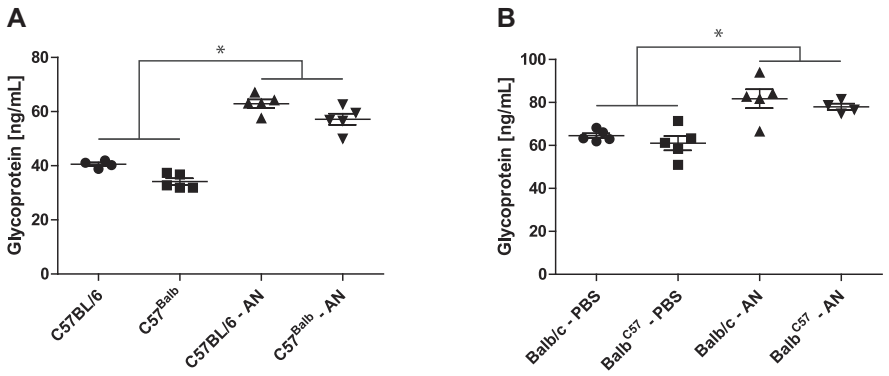

FIG E2. Quantification of total glycoproteins from bronchoalveolar lavage fluid of C57BL/6 **(A)** and BALB/c **(B)** background mice challenged with either PBS or *A niger* conidia *(AN)*. **P* < .05.

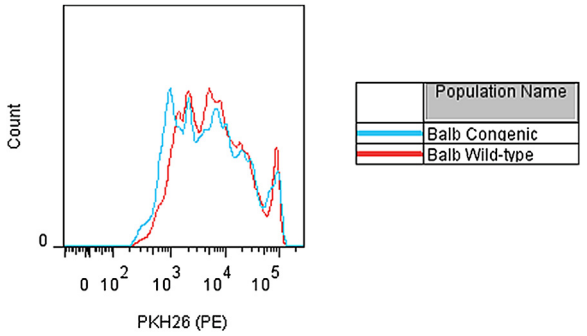

FIG E3. Expression histogram depicting dilution of the fluorescent membrane dye PKH26 in OVA-specific T_H cells obtained from BALB/c *(blue line)* and CD11a congenic $Balb^{C57}$ mice *(red line)* that were cultured for 5 days in T_H2-conditioning medium after labeling.

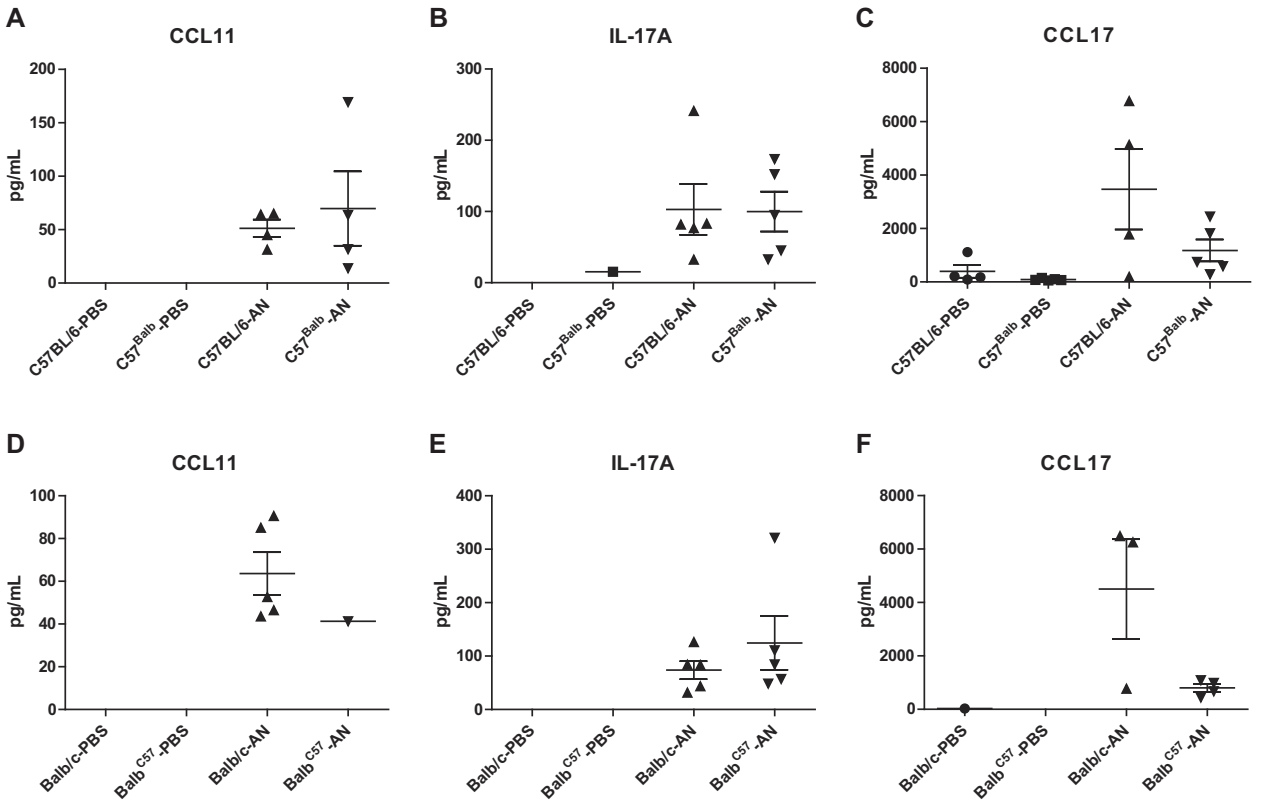

FIG E4. Concentrations of the indicated cytokines and chemokines from lung homogenate supernatants from mice on the C57BL/6 **(A-C)** and BALB/c **(D-F)** genetic backgrounds treated with either PBS or 4.0×10^5 *A niger* conidia *(AN)*.

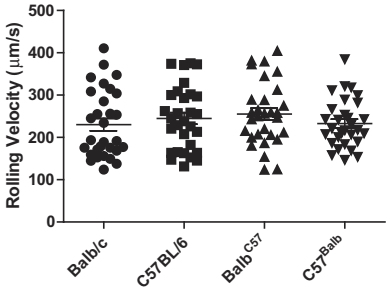

FIG E5. Mean velocity of rolling cells from a dynamic adhesion assay performed with $CD4^+$ T cells from the indicated WT and congenic mice (n = 30 per group).

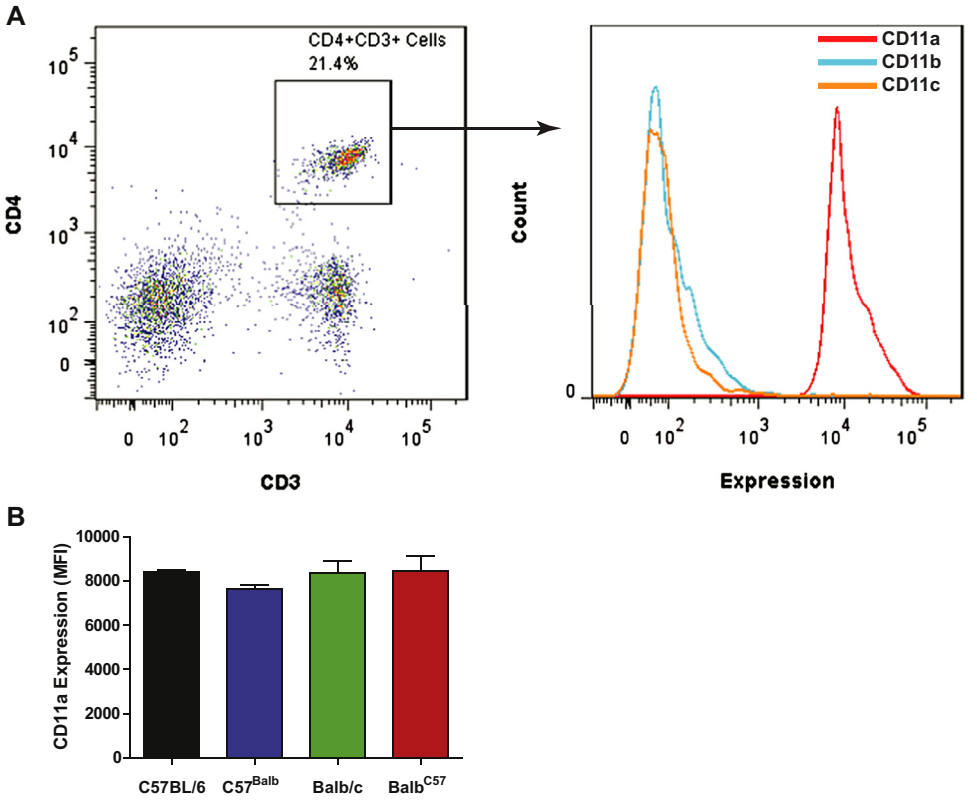

FIG E6. CD11a expression on lymphocytes. **A,** Expression of CD11a, CD11b, and CD11c on $CD4^+CD3^+$ splenocytes from C57BL/6 mice. **B,** CD11 expression on WT and congenic $CD3^+CD4^+$ T cells from the indicated mice. No significant differences were observed (n = 3 per group). *MFI,* Mean fluorescence intensity.

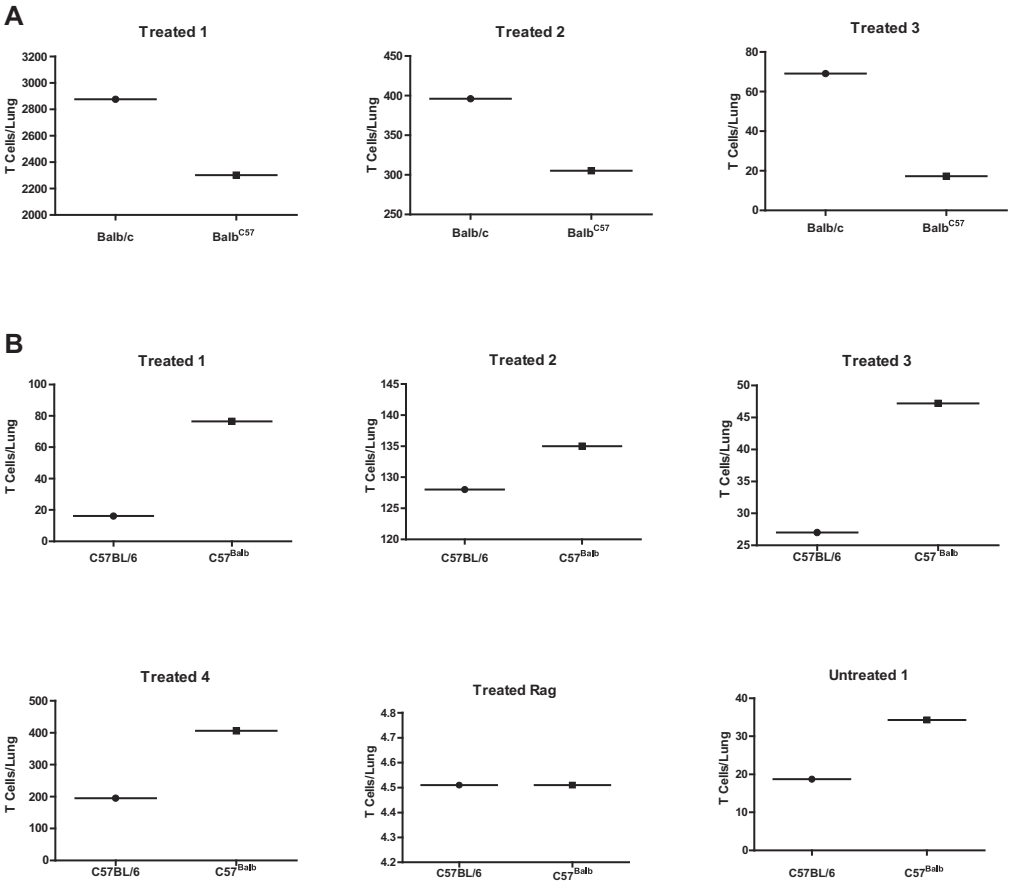

FIG E7. Effect of CD11a polymorphisms on the recruitment of OVA-specific T_H cells to the lung. Rag-deficient mice from the appropriate genetic backgrounds received intraperitoneally a 1:1 mixture of 2 million fluorescently labeled OVA-specific T_H2 cells from WT (PKH26 [PE]) and congenic (CellVue [allophycocyanin]) mice of the indicated backgrounds and intranasally challenged *(Treated)* with *A niger*/OVA to induce T_H2 cell recruitment to lungs. Additional mice received cells but no antigen *(Untreated)* or no T cells *(Treated Rag)*. T cells were enumerated by using an internal fluorescent bead counting standard. Results for individual mice from the BALB/c **(A)** and C57BL/6 **(B)** genetic backgrounds are shown.