A Therapeutic inflammation protocol



FIG E1. Induction of chronic allergen-induced airway inflammation and remodeling. Airway inflammation and remodeling were induced in female BALB/c mice. Therapeutic inflammation protocol (A): Therapeutic transfer of CD4+CD25+ regulatory T cells or PBS as a control took place on day 26 after the acute challenge phase, and mice were killed for analysis on day 33. Airway remodeling protocol (B): Cells were transferred at day 26 and mice were killed for analysis at day 53 to assess the effect of transfer of CD4+CD25+ regulatory T cells or PBS on day 46 and were killed for analysis on day 53 to determine the effect of CD4+CD25+ regulatory T cells on the development of airway remodeling.



FIG E2. Time course of inflammatory and airway remodeling changes during prolonged allergen challenge. Mice were assessed at days 26, 33, 46, and 53 to determine the degree of airway inflammation and remodeling throughout this prolonged allergen challenge model. BAL eosinophil numbers (**A**) were assessed by means of differential counting. BAL fluid T_H2 cytokine levels (**B**) were assessed by means of ELISA. Lung mucus production (**C**) was assessed in periodic acid–Schiff-stained lung sections. Total lung collagen (**D**) was quantified by using a biochemical assay. Data are expressed as means \pm SEMs (n = 6-25 mice per group).



FIG E3. Transfer of CD4⁺CD25⁺ regulatory T cells reduces BAL fluid eosinophilia and T_H2 cytokine expression. Airways underwent lavage, and eosinophil numbers (**A**) were determined by means of differential counting. IL-5 and IL-13 (**B**), IL-10 (**C**), and TGF- β 1 (**D**) levels were measured in BAL supernatant by means of ELISA. End points were measured on day 33 after the therapeutic inflammation protocol. Data are expressed as medians (Fig E3, *A*) or means ± SEMs (Fig E3, *B-D*). Results from 8 to 12 mice per group from 2 separate experiments are shown. **P* < .05 in comparison with OVA-sensitized mice that received PBS instead of CD4⁺CD25⁺ regulatory T cells.



FIG E4. Transfer of CD4⁺CD25⁺ regulatory T cells has no effect on serum IgE levels. Levels of total IgE (**A**) and OVA-specific IgE (**B**) were measured in sera by means of ELISA on day 33 after the therapeutic inflammation protocol. Data are expressed as means \pm SEMs (n = 8-12 mice per group from 2 separate experiments.



FIG E5. Therapeutic transfer of CD4⁺CD25⁺ regulatory T cells has no effect on established AHR. Cells were administered on day 26, and mice were killed on either day 33 (**A**; therapeutic inflammation protocol) or day 53 (**B**; airway remodeling protocol). AHR was measured and expressed as mean lung compliance (*Cdyn*) summarized for the 10 mg/mL methacholine dose. Data are expressed as means \pm SEMs (n = 11-25 mice per group from 2 separate experiments).



FIG E6. Transfer of CD4⁺CD25⁺ regulatory T cells during established remodeling (day 46) has no effect on allergen-induced airway inflammation or remodeling. AHR was measured and expressed as mean lung resistance **(A)** and compliance **(B)** summarized for the 10 mg/mL methacholine dose, and total lung collagen **(C)** values were quantified at day 53 after transfer of regulatory T cells by using the established remodeling protocol. Data are expressed as means \pm SEMs (n = 4-6 mice per group).