

CD4-mediated regulatory T-cell activation inhibits the development of disease in a humanized mouse model of allergic airway disease

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Background: Based on their potency to control allergic diseases, regulatory T (Treg) cells represent a promising target for novel strategies to interfere with allergic airway inflammation. We have previously demonstrated that stimulation of the CD4 molecule on human Treg cells activates their suppressive activity *in vitro* and *in vivo*.

Objective: We sought to determine the effect of CD4-mediated Treg-cell activation on pulmonary inflammation in a humanized mouse model of allergic airway inflammation.

Methods: PBMCs obtained from donors allergic to birch pollen or from healthy donors were injected into NOD-severe combined immunodeficiency $\gamma c^{-/-}$ mice, followed by allergen airway challenges and analysis of airway responsiveness and inflammation. For Treg-cell activation, mice were treated with the CD4-binding, lck-activating recombinant HIV-1 surface protein gp120 after sensitization prior to allergen challenge. Control experiments with CD25-depleted PBMCs were performed to evaluate the role of Treg cells.

Results: PBMCs from allergic donors but not from healthy donors induced airway inflammation and airway hyperresponsiveness. Treatment with gp120 prior to allergen challenge abrogated airway hyperresponsiveness and reduced the inflammatory immune response. In contrast, treatment had no effect on inflammation and airway hyperresponsiveness in mice that received CD25-depleted PBMCs, demonstrating Treg-cell dependency of disease prevention.

Conclusion: Allergic airway inflammation can be prevented by stimulation of human Treg cells by CD4. These results suggest a clinical potential of Treg-cell activation by high-affinity CD4

ligands in allergic diseases. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Humanized animal model, AHR, pulmonary inflammation, regulatory T cells

Regulatory T (Treg) cells play a critical role in the development of allergen-induced airway inflammation¹⁻⁴ and have been found to reverse or dampen established disease upon adoptive transfer.^{5,6} However, *in vitro* propagation of Treg cells for adoptive transfer is laborious and time-consuming and isolated Treg cells can lose their suppressive function.⁷ The risk of adverse reactions by contaminating proinflammatory effector cells has prevented cellular therapies based on Treg cells from becoming a therapeutic regimen in allergic diseases. An alternate, but perhaps more feasible, approach to harness the suppressive activity of Treg cells consists in the activation of Treg cells *in vivo*. In this regard, we recently demonstrated that certain anti-CD4 mAbs have the potential to induce the suppressive function of isolated human Treg cells in a T-cell receptor-independent manner.⁸ In addition, we and others observed that the CD4-binding, lck-activating⁹ HIV-1 surface protein gp120 activates the suppressive function of Treg cells *in vitro* and *in vivo*,¹⁰⁻¹³ signifying that stimulation via the CD4 receptor represents an alternative Treg-cell activating pathway with potential to induce immunological tolerance.

To determine the effect of CD4-mediated human Treg-cell activation on pulmonary inflammation *in vivo* we developed a human/mouse chimeric model of allergic airway disease in NOD-severe combined immunodeficiency (SCID) $\gamma c^{-/-}$ mice. Compared with the NOD-SCID strain that has been previously used to study the induction and modulation of human allergen-specific immune responses *in vivo*,¹⁴⁻¹⁹ NOD-SCID mice additionally lacking the common gamma chain offer superior engraftment with human cells^{20,21} and thus facilitate the investigation of human T-cell responses including Treg cells.²²

Transfer of PBMCs from allergic donors to NOD-SCID $\gamma c^{-/-}$ mice in combination with rhIL-4 and allergen induced airway hyperresponsiveness (AHR) and airway inflammation upon subsequent allergen challenge. The administration of recombinant HIV-1 gp120 after sensitization and prior to allergen challenge abrogated AHR and reduced airway inflammation in a Treg-cell-dependent fashion. Taken together, these results demonstrate the clinical potential of CD4-mediated Treg-cell activation in allergic airway inflammation.

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This study was funded by Deutsche Forschungsgemeinschaft (DFG) (grants Ta275/4-1 and Ta275/5-1 to C.T. and BE-3685/1-1 to C.B.) and Forschungszentrum Immunologie (FZI) Mainz to C.T.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication February 21, 2011; revised September 20, 2011; accepted for publication September 30, 2011.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2011.09.038

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
GvHD: Graft-versus-host disease
MCh: Methacholine
SCID: Severe combined immunodeficiency
SIT: Allergen-specific immunotherapy
Treg: Regulatory T

METHODS**Animals**

NOD-SCID $\gamma c^{-/-}$ mice (Jackson Laboratory, Bar Harbor, Me) were kept in the central animal facility of the University Medical Center of Johannes Gutenberg University in Mainz under specific pathogen-free conditions. Eight- to 10-week-old male mice were used. All animal procedures were conducted in accordance with current institutional guidelines and performed according to the Helsinki convention for the use and care of animals. All procedures have been reviewed and approved by the regional government authorities of Rhineland-Palatinate, Germany.

Patients

Patients with stable allergic asthma diagnosed in accordance with the Global Initiative for Asthma guidelines (www.ginaasthma.com) and proven sensitization against birch pollen, documented by high specific IgE (capacity of binding by the Immuno CAP specific IgE blood test [t3, Phadia Immuno-CAP, Uppsala, Sweden]), were included in the study. Patients' characteristics are given in Table I. All patients were treated with inhaled corticosteroids and inhaled β_2 -agonists. None of the patients received systemic corticosteroid, anti-IgE, or leukotriene receptor antagonists. The local ethics committee approved the experimental protocol, and patients gave their written informed consent. As controls, PBMCs were isolated from sex- and age-matched nonatopic healthy volunteers.

Isolation of human cell PBMCs

Blood was collected in heparinized syringes, and PBMCs were obtained by Ficoll density centrifugation. Cells from different donors were not pooled but separately processed. To determine which cell type was responsible for the induction of allergic response, in some experiments individual cell types were also infused by using PBMCs depleted of CD3⁺, CD4⁺, CD8⁺, or CD25⁺ cells by Dynabead technique (Invitrogen, Darmstadt, Germany). Depletion success was typically around 99% for CD3⁺, CD4⁺, and CD8⁺ cells. CD25 depletion removed all CD25^{high}FoxP3⁺ T cells without affecting cells with lower CD25 expression (see Fig E1 in this article's Online Repository at www.jacionline.org).

Experimental protocol

Adult male NOD-SCID $\gamma c^{-/-}$ mice received intraperitoneal injection of 5×10^6 PBMCs from 1 donor with or without 50 μ g of birch allergen (ALK-lyophilisiert[®], ALK Abelló, Hørsholm, Denmark) or 1000 units of recombinant human IL-4 (rhIL-4, Miltenyi Biotec, Bergisch Gladbach, Germany) or both. The administration of allergen and IL-4 was repeated at day 7. In some experiments, mice received 20 μ g of recombinant soluble HIV-1 envelope protein gp120 (strain JR-FL, kindly provided by F.J. Schneider, Boehringer Ingelheim, Biberach, Germany) on day 18 by intraperitoneal injection. On days 20 to 22, mice were anesthetized with isoflurane (Forene, Abbott, Wiesbaden, Germany) and challenged by intranasal application of 20 μ g of birch allergen. Forty-eight hours later, mice were subjected to airway resistance measurement as previously described.²³ Invasive measurement of airway resistance (R_L) was performed on anesthetized, intubated mice by mechanical ventilation in response to increasing doses of methacholine (MCh) (3.125, 6.25, 12.5, 25, 50 mg/mL) with the Flexi Vent System (Scireq, Montreal, Quebec, Canada). R_L

TABLE I. Characteristics of atopic donors

ID	Sex	Age (y)	Total-IgE (kU/l)	Birch-specific IgE (kU/l)
1	Female	30	1175	>100
2	Male	58	719	50.3
3	Male	44	953	89
4	Female	48	2741	84.2
5	Female	43	339	>100
6	Male	43	451	53.8
7	Male	55	153	51
8	Male	28	641	>100
9	Female	54	489	24.8

was measured every 15 seconds following nebulization. The response to each single dose of MCh was analyzed until a plateau phase was reached. Alanine transaminase was quantified in murine sera by using the Reflovet Plus system (Scil Animal Care Company, Viernheim, Germany).

Bronchoalveolar lavage

After measurement of airway reactivity, lungs were washed with 1 mL of cold PBS through an intratracheal tubus. Bronchoalveolar lavage (BAL) fluid was centrifuged, and cells were resuspended in 200 μ L of PBS and counted. Cytospin preparations of BAL cells were stained with the Microscopy Hemacolor Set (Merck, Darmstadt, Germany), and cell types were counted by 2 investigators blinded to experimental conditions.

Flow cytometry

Reconstitution and organ infiltration with human cells were determined by analyzing collagenase IA (Sigma-Aldrich, Steinheim, Germany)-digested lung cells and splenocytes at indicated time points. Erythrocyte-depleted cells were stained with mAbs against human CD45 (CD45-fluorescein isothiocyanate, BD Bioscience, Heidelberg, Germany) and CD3 (CD3-PerCP, BD Bioscience). Acquisition was performed on an FACS calibur, and data were analyzed by using the Flow Jo software (Tree Star Inc, Ashland, Ore). Further antibodies used for proving the depletion success were fluorescein isothiocyanate-conjugated anti-human CD8, CD25-PE-Cy7 (BD Bioscience), and FoxP3-APC (ebioscience, Frankfurt, Germany) and their recommended isotype controls.

Histology

Lungs were inflated and immersed in 4% formalin and subsequently embedded in paraffin. Lung tissue sections were stained with hematoxylin and eosin, periodic acid-Schiff, and antibodies against human CD45 and human CD3 (both DAKO, Hamburg, Germany). The degree of bronchial inflammation was scaled on slides stained by hematoxylin and eosin by using a scoring system on 5 randomly chosen areas on each slide (0 = no inflammation = to 4 = maximal inflammation) as previously described.²⁴ Numbers of mucus-containing cells per millimeter basement membrane were counted in the primary bronchus and medium-sized bronchi by using an imaging software (Soft Imaging Systems, Stuttgart, Germany). Slides were quantified separately from 2 experienced scientists in a blinded fashion.

Statistical analysis

ANOVA was used to determine the levels of difference between experimental groups. Differences in responsiveness to MCh were assessed by using repeated-measures ANOVA. Comparisons for all pairs were performed by using the Tukey's honest significant difference test. Number of eosinophils, histology score, and number of periodic acid-Schiff-positive cells were initially analyzed by using nonparametric ANOVA (Kruskal-Wallis test) for overall differences. The Mann-Whitney U test was used to elucidate the statistical significance. *P* values were set at .05. Values for all measurements are expressed as the mean $\pm$ SEM.

RESULTS

PBMCs from patients with allergic asthma induce airway inflammation and AHR in NOD-SCID $\gamma c^{-/-}$ recipients following allergen exposure

Mice were transplanted with PBMCs from 9 atopic and 4 nonatopic donors. The profiles of serum IgE levels of atopic donors are shown in Table I. Significant levels of birch-specific IgE were detected in sera only from atopic patients (Table I). Non-allergic healthy volunteers with total serum IgE concentrations less than 25 kU/I and no birch-specific IgE were referred to as nonallergic donors.

Transfer of 5×10^6 PBMCs from donors allergic to birch together with birch allergen (50 μ g) and rhIL-4 (1000 U), repeated administration of allergen and IL-4 on day 7 after transfer, and intranasal allergen challenge on days 20 to 22 resulted in no change in airway resistance at baseline (data not shown) but increased airway reactivity to inhaled MCh (Fig 1, A and B). Increased airway reactivity was observed upon administration of birch allergen or IL-4 alone, but AHR was most strongly induced by the administration of both (Fig 1, B), indicating that both contribute to the inflammatory airway response. Increased airway reactivity was accompanied by the infiltration of human T cells into the peribronchial and perivascular lung tissue (Fig 1, D; see Fig E2 in this article's Online Repository at www.jacionline.org), showing that lung inflammation was either induced or accompanied by homing of human T cells into the lung tissue.

By contrast, transfer of PBMCs from healthy donors did not affect airway reactivity regardless of allergen and IL-4 treatment (Fig 1, C) and resulted in only limited infiltration of human T cells into the lung tissue (Fig 1, D, and Fig E2). In addition, following transfer of PBMCs from allergic donors and subsequent saline exposure, no AHR or increased airway inflammation was detectable (see Fig E3 in this article's Online Repository at www.jacionline.org). Importantly, whereas upon transfer of high human cell numbers ($10\text{--}80 \times 10^6$ PBMCs) mice developed graft-versus-host disease (GvHD) with PBMCs from healthy donors (see Figs E4 and E5 in this article's Online Repository at www.jacionline.org), upon transfer of lower cell numbers (5×10^6 PBMCs) from either healthy or atopic donors no symptoms of GvHD were observed within an experimental period of up to 40 days (Figs E4 and E5; see Fig E6 in this article's Online Repository at www.jacionline.org). Together these findings indicate that allergen-reactive human T cells induce increased airway reactivity in this model.

To further assess the role of T-cell subpopulations, we compared airway resistance and lung infiltration following transfer of CD8 $^{-}$, CD4 $^{-}$, or CD3-depleted PBMCs (Fig 2). Whereas depletion of CD8 $^{+}$ T cells showed no effect, AHR and lung infiltration were abrogated in mice that received T cell (CD3)-depleted or CD4-depleted PBMCs (Fig 2, A and B), demonstrating that airway inflammation in this model is exclusively dependent on human CD4 $^{+}$ T cells. Again, no differences in baseline resistance were detectable between the different groups. In sum these results demonstrate that upon transfer of a low number of PBMCs from allergic donors, nasal allergen provocation induces pulmonary inflammation in NOD-SCID $\gamma c^{-/-}$ mice mediated by recruitment of allergen-reactive human T cells into the murine lung tissue. These observations and the inability of PBMCs from healthy donors to affect lung function show that this human/mouse chimeric model is feasible to study allergen-induced human T-cell-dependent pulmonary inflammation.

Treatment with recombinant HIV-1 envelope protein gp120 prevents development of lung inflammation

We previously showed that HIV-1 envelope protein gp120, a high-affinity ligand for human CD4, is a polyclonal activator of human Treg-cell activity *in vitro* and *in vivo*.¹³ To evaluate the Treg-cell regulating potential of gp120 in the allergic inflammation model, mice were additionally treated with gp120 2 days prior to inhaled allergen exposure (Fig 3, A). Treatment after sensitization and prior to challenge suppressed response in R_L to MCh to the background level (Fig 3, B) and reduced the infiltration of human T cells into the lung tissue (Fig 3, C). By contrast, treatment of naive mice with gp120 did not affect airway response to MCh (data not shown) excluding a direct effect of gp120 on airway smooth muscle.

In addition to AHR, accumulation of inflammatory cells in the BAL fluid was evaluated 48 hours after the last airway challenge. The BAL fluid in untreated mice was mainly composed of macrophages and few neutrophils. Transfer of PBMCs from allergic donors and provocation with allergen and IL-4 increased total cell counts in the BAL fluid compared with the counts in untreated mice but did not affect the composition of the infiltrating cells (Fig 3, D). Treatment with gp120 reduced the total number of cells and particularly decreased the number of macrophages in the BAL fluid (Fig 3, D). These results show for the first time that gp120 is capable of down-modulating human allergen-specific T-cell responses *in vivo* in the presence of the allergen and IL-4.

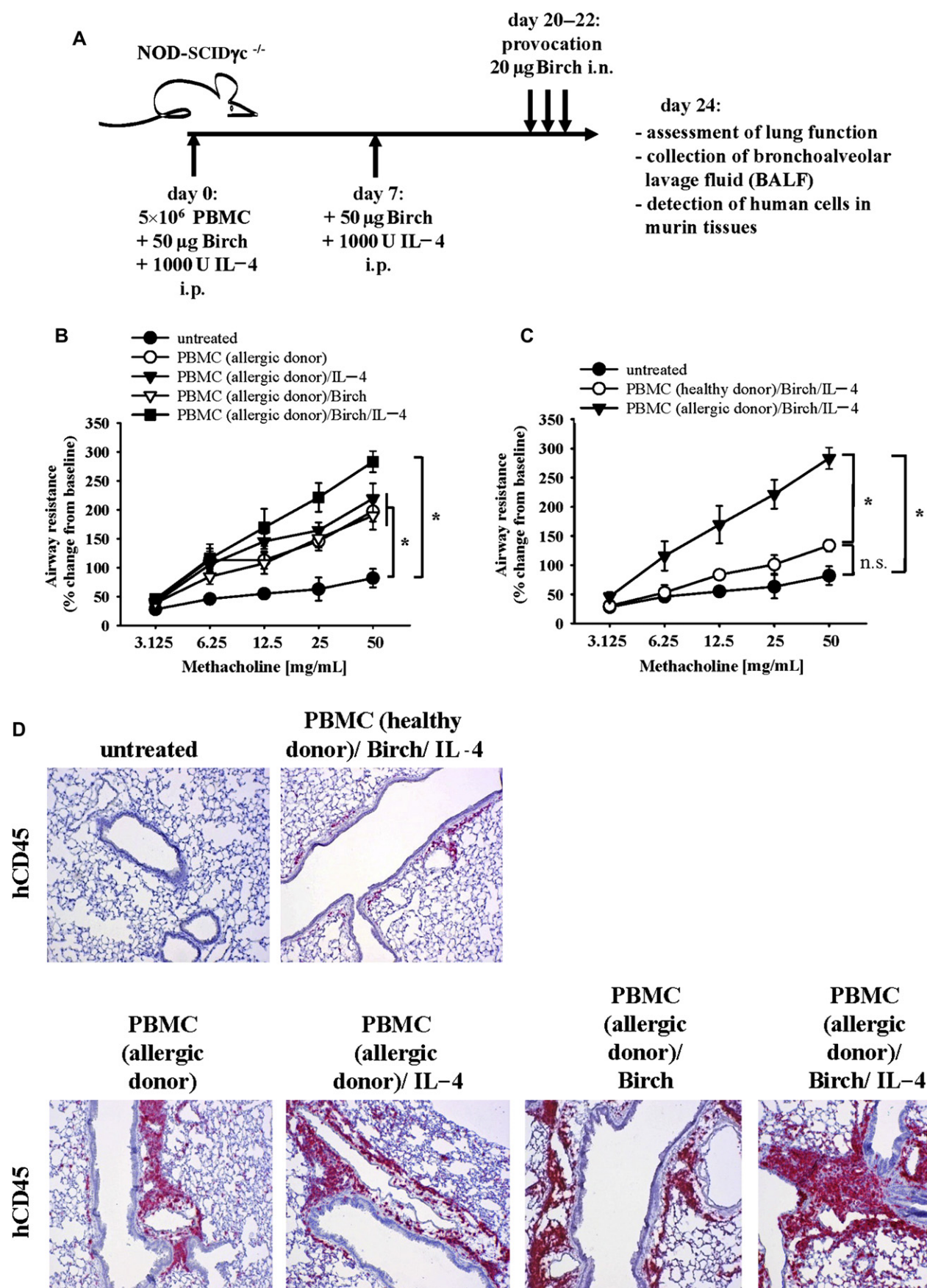
Abrogation of lung inflammation by gp120 depends on Treg cells

To assess the potential Treg-cell dependency of gp120-mediated prevention of pulmonary inflammation, Treg cells were selectively depleted from PBMCs (Fig E1) prior to transfer. Upon transfer of complete or CD25-depleted PBMCs, mice developed comparable AHR and lung inflammation including increased numbers of goblet cells (Fig 4, A-D). Treatment with gp120 abrogated the development of AHR in animals that received complete PBMCs but not in animals that received CD25-depleted PBMCs. Likewise, gp120 repressed other symptoms of lung inflammation in the presence but not in the absence of Treg cells (Fig 4, D; see Fig E7 in this article's Online Repository at www.jacionline.org), proving that suppression of airway hyperreactivity and airway inflammation by gp120 is strictly dependent on Treg cells.

DISCUSSION

In the present study we demonstrate for the first time that allergic airway inflammation can be prevented by CD4-mediated activation of human Treg cells. Our results support the observation that high-affinity CD4 ligands are able to elicit Treg-cell-mediated suppression of effector T cells^{8,10,12,13} and strengthen the concept of non-antigen-specific polyclonal activation of Treg cells to suppress allergic airway inflammation.²⁵

The ultimate goal of any intervention or prevention strategy for allergic diseases is to achieve disease-specific tolerance. So far, patients with allergic asthma are basically treated with anti-inflammatory agents, such as corticosteroids and bronchodilators,



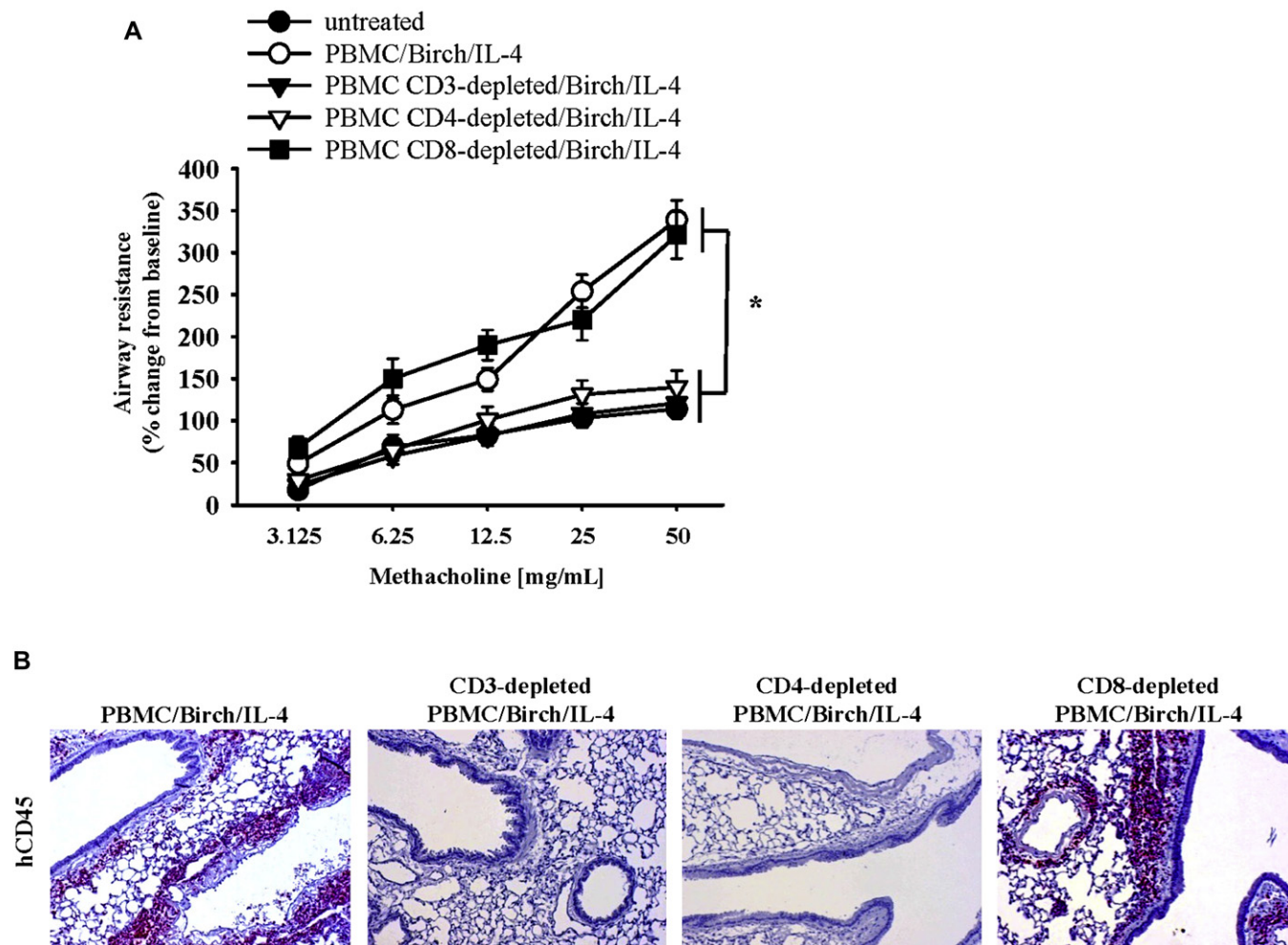


FIG 2. Induction of lung inflammation is CD4⁺ T cell dependent. **A**, Increased airway resistance induced with PBMCs from allergic donors depends on human CD4⁺ T cells. Airway resistance in response to Mch (means $\pm$ SEM) in untreated mice (n = 3) and mice that received complete PBMCs (n = 10), CD3-depleted PBMCs (n = 10), CD4-depleted PBMCs (n = 9), or CD8-depleted PBMCs (n = 9). **P* < .05. **B**, Pulmonary tissue sections stained with an antibody against human CD45 (magnification $\times 100$).

leukotriene receptor antagonists, or mAb against IgE to reduce the inflammation in the airways. The only specific therapy currently available that can ameliorate allergic disease is allergen-specific immunotherapy (SIT). There is considerable evidence that successful SIT is associated with induction of Treg cells, supposedly including thymus-derived Treg cells.²⁶ However, SIT needs to be administered over several years for maximal efficacy, repression of allergic symptoms by SIT is not always complete, and adverse reactions may be induced. Thus, a direct Treg-cell-stimulating approach to establish Treg-cell-mediated dominant tolerance could possibly offer a more efficient solution. To this end, a temporary enhancement of Treg-cell activity in allergic patients by polyclonal CD4-mediated Treg-cell activation

could possibly blunt allergic responses and establish an infectious tolerance toward the allergic trigger.

Earlier reports on anti-CD4-mediated tolerance induction secondary to Treg-cell activation in mice²⁷ and our initial observations demonstrate that human Treg cells can be activated by certain anti-CD4 mAbs.⁸ We recently found that the CD4-binding, lck-activating HIV-1 surface protein gp120 induces the suppressive activity of Treg cells *in vitro* and in a GvHD model *in vivo*.¹³ Since gp120 exclusively binds to human CD4 and its immunoregulatory effect was observed only in the presence of Treg cells, potential additional effects on CD4⁺ T helper cell function clearly did not play a role in the model. Mechanistically, CD4-mediated Treg-cell activation was found to depend on cyclic

FIG 1. Induction of lung inflammation in NOD-SCID $\gamma c^{-/-}$ mice with PBMCs from atopic but not healthy donors. **A**, Experimental setup. **B**, Airway resistance in response to Mch (means $\pm$ SEM) in untreated (n = 5), PBMC + IL-4 (n = 9), PBMC + allergen (n = 10), PBMC + allergen + IL-4 (n = 8) treated mice. **C**, Airway resistance following transfer of PBMCs from atopic donors (n = 8) or healthy donors (n = 5) (means $\pm$ SEM). **P* < .05. **D**, Pulmonary tissue sections from the same mice as under Fig 1 (B and C) stained with an antibody against human CD45 ($\times 100$ magnification). (Fig 1, B-D). Bars represent means. *i.n.*, Intranasal; *i.p.*, intraperitoneal.

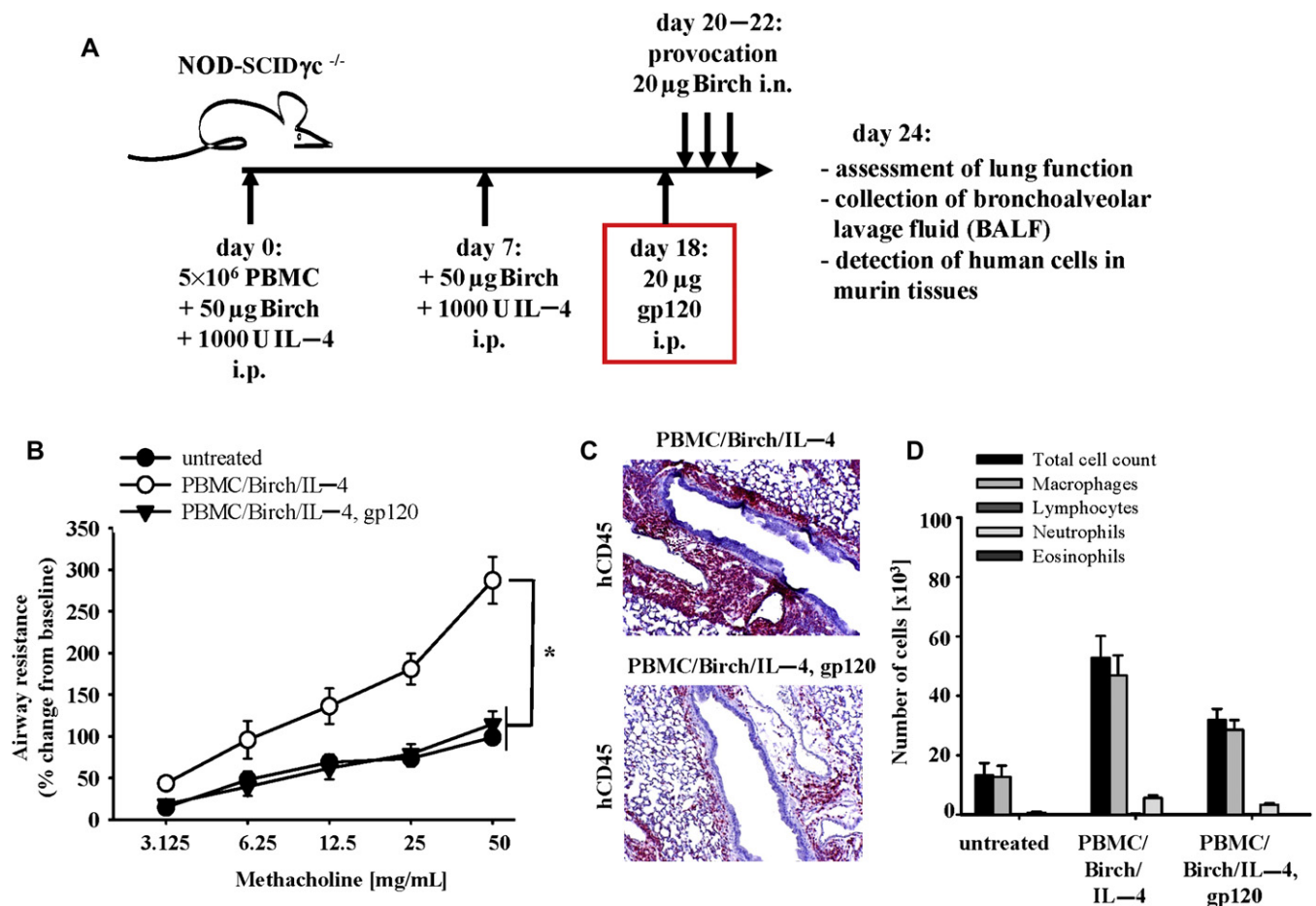


FIG 3. Treatment with recombinant HIV-1 gp120 reduces lung inflammation. **A**, Experimental design. **B**, Injection of recombinant gp120 prior to allergen challenge abrogates AHR. Mch-induced AHR (means $\pm$ SEM) measured in untreated ($n = 5$), PBMC ($n = 15$), and PBMC + gp120 ($n = 12$) treated mice. * $P < .05$. **C**, Pulmonary tissue sections from the same mice as under **B** stained with an antibody against human CD45 (magnification $\times 100$). **D**, Composition and enumeration of cells in the BAL fluid. i.n., Intranasal; i.p., intraperitoneal.

adenosine monophosphate (cAMP) production in Treg cells upon stimulation and could be blocked by an adenylate cyclase inhibitor.²⁸ Furthermore, inhibition of cAMP degradation enhanced the suppressive potential of Treg cells.²⁴ Together these observations indicated that the application of CD4-binding biologicals represents a Treg-cell-stimulating approach that might also be useful to reestablish antigen-specific tolerance in allergic diseases.

Several studies have suggested functional deficiencies in Treg cells in allergic individuals.^{4,25,29,30} However, this hypothesis has been exclusively based on suppression assays performed with isolated Treg-cell populations from atopic patients *in vitro* and has not unequivocally been confirmed.³¹ In lack of an exclusive Treg-cell marker, even highly pure populations of human Treg cells contain a substantial fraction of cytokine-producing proinflammatory effector cells that cannot be distinguished from Treg cells.³² Thus, most likely, functional deficiencies of Treg-cell preparations from allergic individuals result from inaccurate Treg-cell isolation in the presence of phenotypically identical effector T cells.³³

The human/mouse chimeric model described in the present study complements *in vitro* studies on isolated T-cell populations

from atopic donors by enabling to study human allergen-reactive T-cell responses and their regulation *in vivo* without T-cell purification. One concern regarding this kind of model is the potential development of GvHD. However, upon transfer of 5×10^6 PBMCs, no GvHD symptoms could be observed within the experimental period. Thus, although a potential xenogenic response of human immune cells in the lung cannot be formally excluded, airway inflammation in the model was at least clearly allergen induced.

Regarding the proposed functional deficiency of Treg cells in allergic individuals, our findings that polyclonal activation of Treg cells by gp120 suppresses the allergen-reactive T effector cell response in our NOD-SCID γ c $^{-/-}$ model support the notion that Treg cells in atopic donors are not fundamentally functionally altered. However, in contrast to animal models that are thought to mimic the process of human allergic airway disease, increased AHR induced with PBMCs from atopic donors in the NOD-SCID γ c $^{-/-}$ model was not eosinophil dependent, suggesting a different immunopathological pathway. We therefore suggest that the NOD-SCID γ c $^{-/-}$ model particularly emphasizes the role of allergen-reactive T cells and Treg cells.

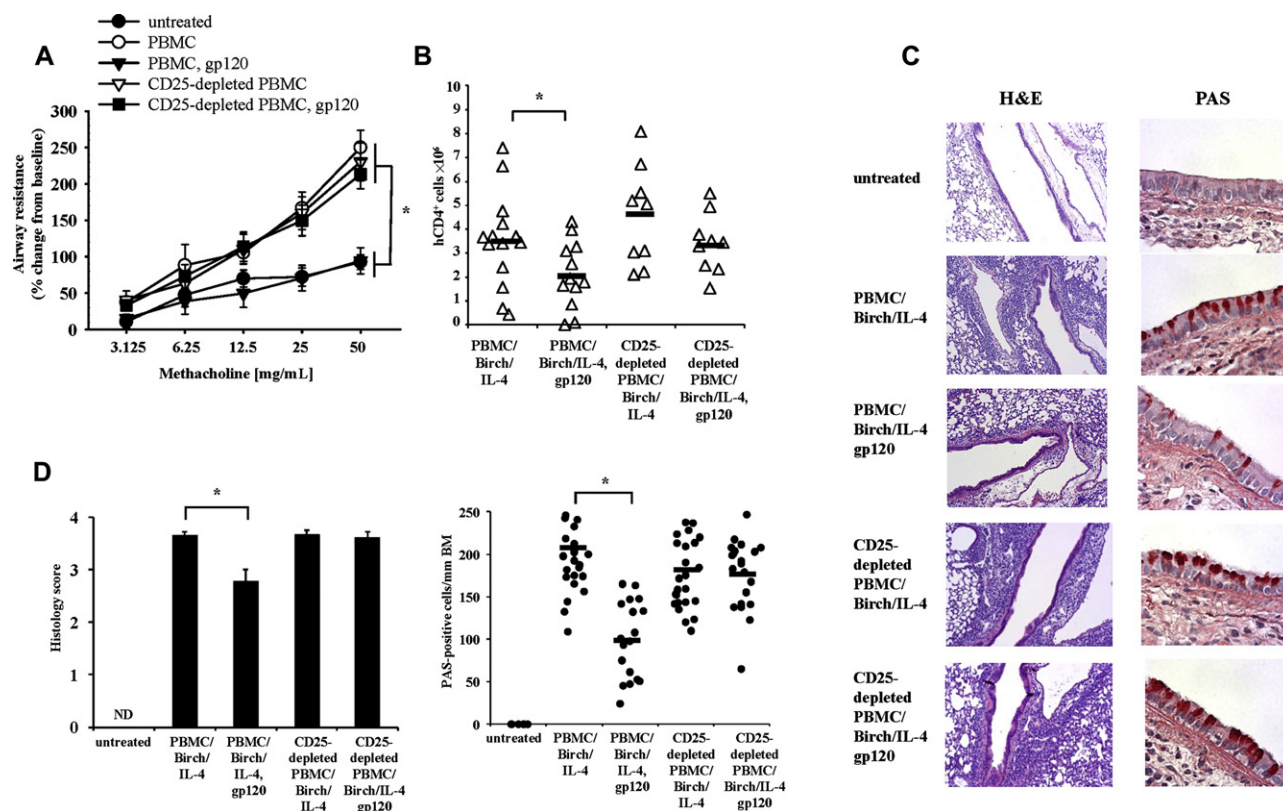


FIG 4. Reduction of lung inflammation by gp120 depends on Treg cells. **A**, Mch-induced changes in airway resistance (means $\pm$ SEM) in untreated ($n = 5$), PBMC ($n = 10$), PBMC + gp120 ($n = 8$), CD25-depleted PBMC ($n = 9$), and CD25-depleted PBMC + gp120 ($n = 9$) treated mice. **B**, Numbers of CD4⁺ cells in whole lung. Symbols represent individual animals; bars represent mean values. * $P < .05$. **C** and **D**, Hematoxylin and eosin- and periodic acid-Schiff-stained lung tissue sections and inflammation scoring in untreated ($n = 3$), PBMC ($n = 10$ -14), PBMC + gp120 ($n = 8$ -12), CD25-depleted PBMC ($n = 9$), CD25-depleted PBMC + gp120 ($n = 9$) treated mice. * $P < .05$. Magnification $\times 100$ and $\times 500$. PAS, Periodic acid-Schiff.

A major consideration in the development of Treg-cell-based therapies is that their effectiveness will critically depend on the stage of the disease. Patients with recent-onset disease and out-of-season patients may represent the clinical settings in which Treg-cell-enhancing therapies may be most advantageously deployed, very similar to specific immunotherapy. Moreover, in addition to the treatment of established allergy, it is important to consider prophylactic approaches before challenge takes place. We demonstrate in the present study that gp120 treatment after sensitization and prior to challenge represents a rational prophylactic approach, ultimately resulting in the prevention of lung inflammation mediated by allergen-reactive T cells.

In summary, our results show that CD4-mediated polyclonal Treg-cell activation blocks the development of allergen-driven lung inflammation and suggest that this tolerizing strategy represents a new approach to prevent allergic airway inflammation.

The authors thank Dr F.J. Schneider, Boehringer Ingelheim, Biberach, Germany, for kindly providing recombinant gp120.

Clinical implications: CD4 stimulation induces immune tolerance through regulatory T-cell activation *in vivo*.

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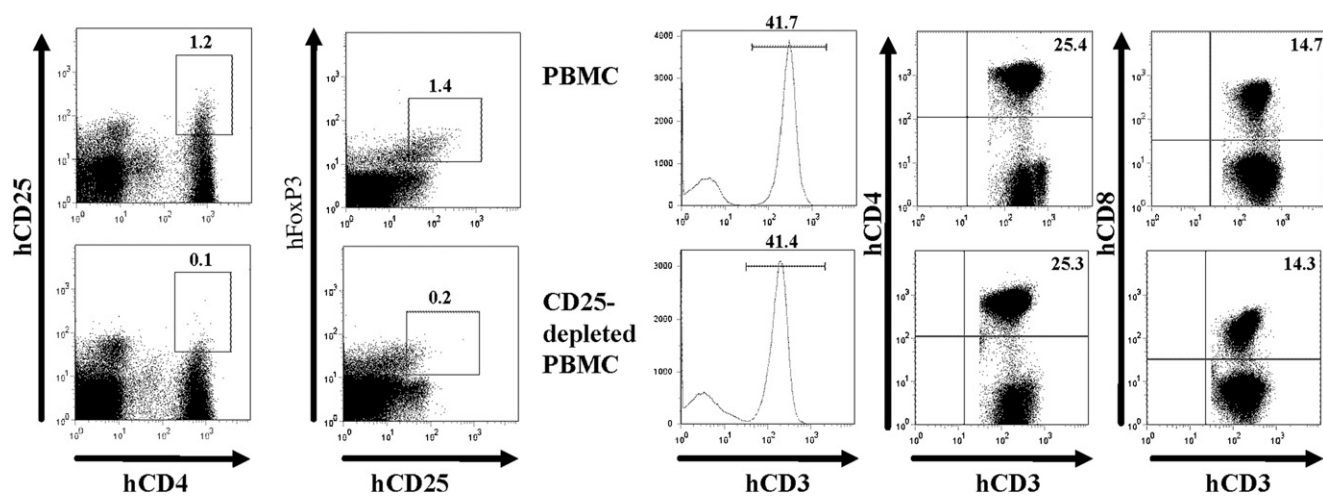


FIG E1. Depletion of CD25⁺ cells from PBMCs. CD25⁺ cells were depleted with Dynabeads. Depletion success is visualized by fluorescence-activated cell sorting staining with antibodies against human CD4, CD25, and FoxP3. Frequencies of gated cells are indicated. Please note that CD25 depletion did not affect the number of CD3⁺CD4⁺ or CD3⁺CD8⁺ T cells and CD25^{intermediate} cells. Depletion success was constant in all experiments.

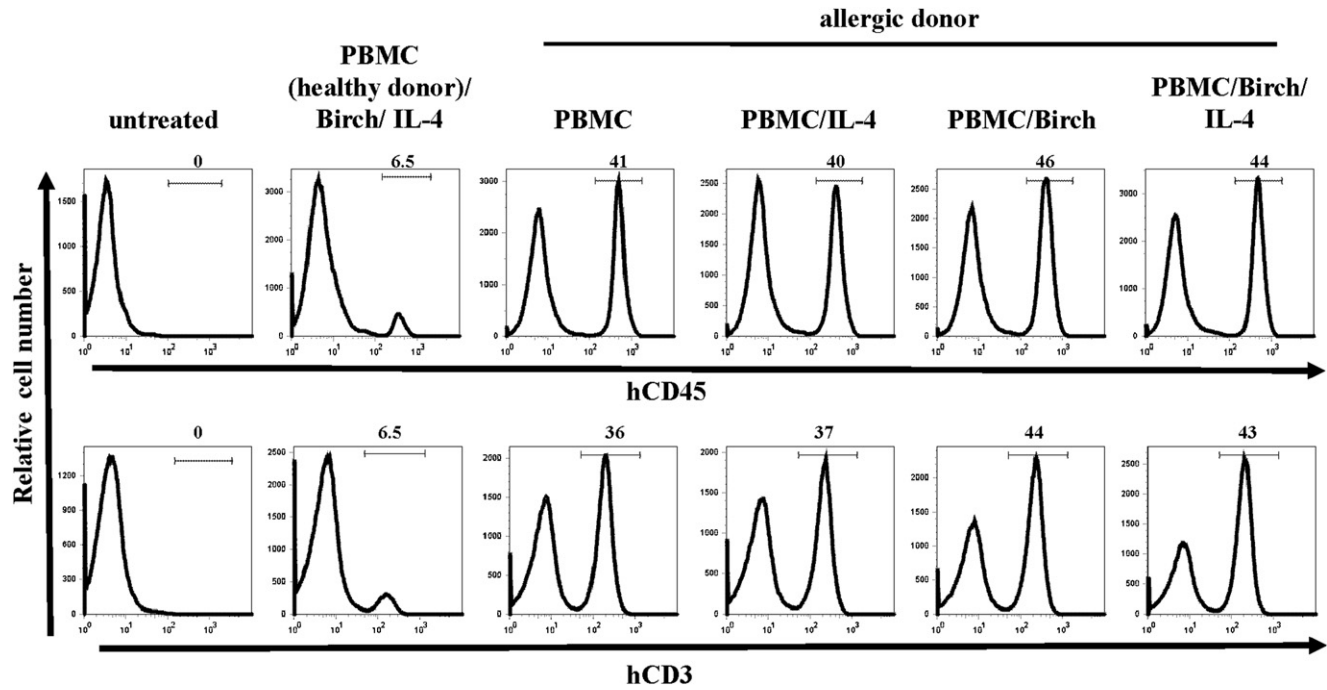


FIG E2. Increased infiltration of T cells from allergic donors into the lungs of NOD-SCID $\gamma_c^{-/-}$ mice. NOD-SCID $\gamma_c^{-/-}$ mice were injected with 5×10^6 PBMCs from allergic or healthy donors together with birch pollen allergen and IL-4 and administration with allergen and IL-4 repeated on day 7. On days 20 to 22, mice were intranasally challenged with allergen. Untreated mice served as controls. Lung cells were prepared on day 24 and stained with antibodies against human CD45 and CD3. Histograms show CD45⁺ and CD3⁺ cells. Dead cells were excluded from analysis. Results representative for 2 experiments with 4 to 5 mice per group.

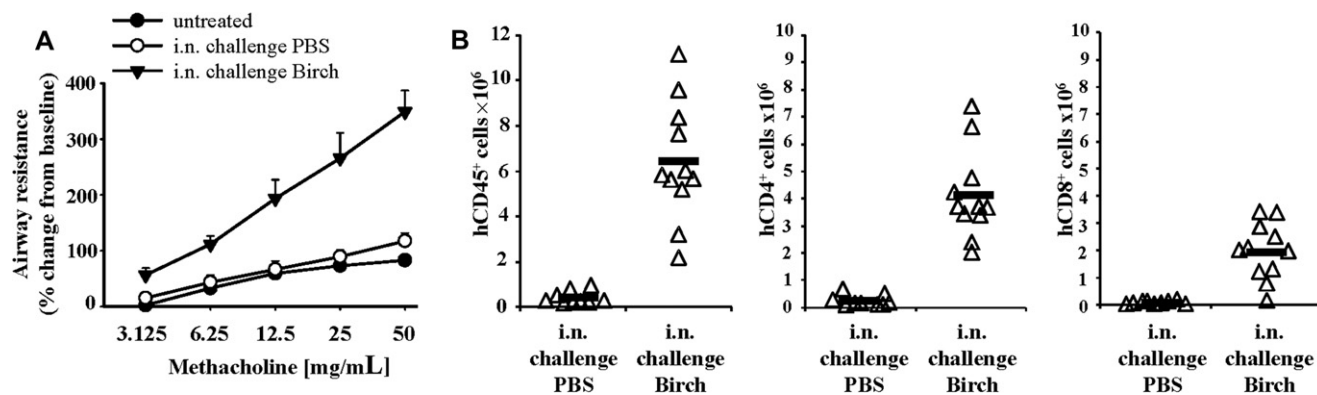


FIG E3. Transfer of human PBMCs and subsequent saline exposure does not increase airway inflammation. NOD-SCID $\gamma c^{-/-}$ mice were injected with 5×10^6 human PBMCs. Following intranasal application of saline on days 20 to 22, no elevated airway resistance (**A**) and airway inflammation (**B**) were detectable. *i.n.*, Intranasal.

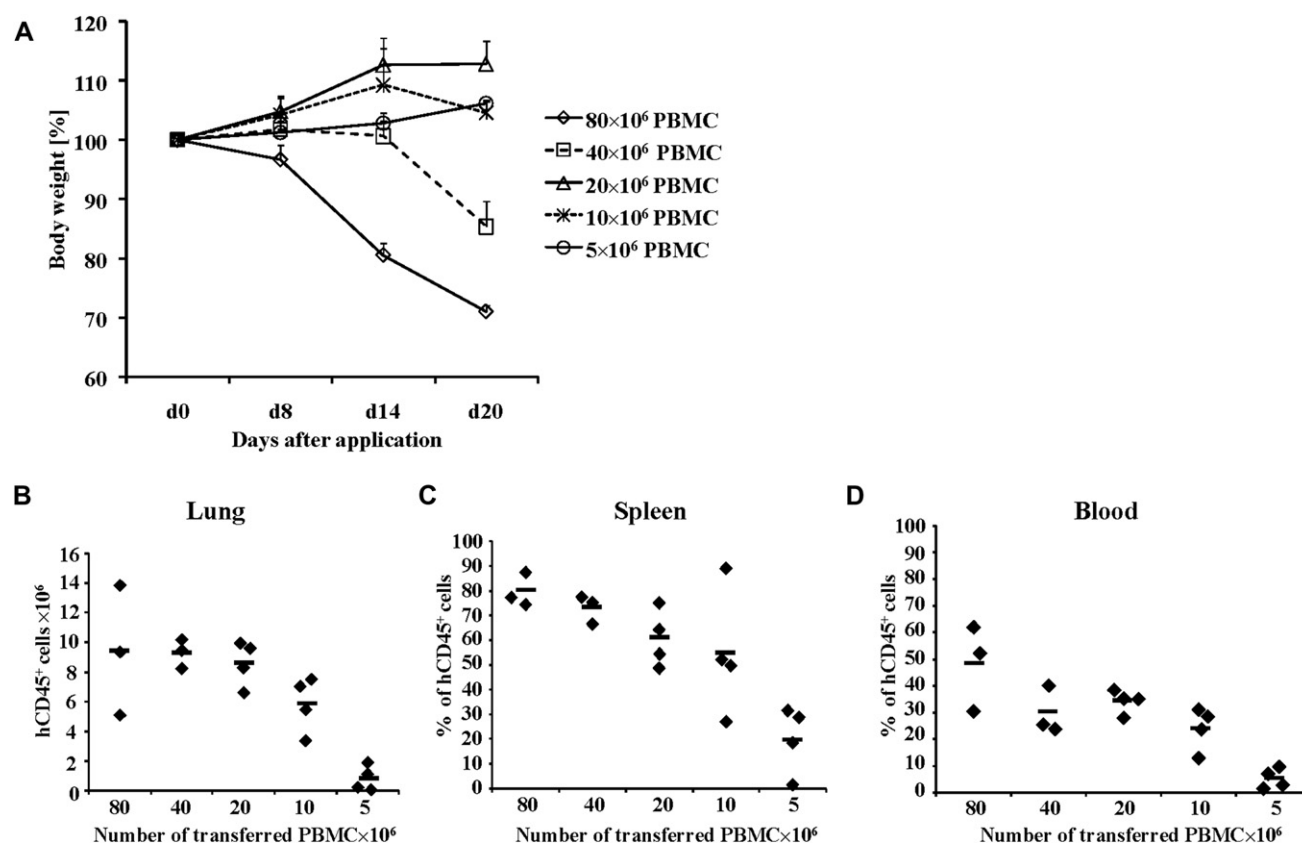


FIG E4. Transfer of different numbers of human PBMCs to NOD-SCID $\gamma_c^{-/-}$ recipients. NOD-SCID $\gamma_c^{-/-}$ mice were injected with different numbers of human PBMCs from healthy nonallergic donors as indicated. **A**, Cumulative mean weight data. Each point represents the cumulative mean weight data of 4 mice per group. Survival of mice: 1 mouse lost in the 80×10^6 and the 40×10^6 group between days 14 and 20. Numbers of human cells isolated on day 24 from lung (**B**), spleen (**C**), and blood (**D**). Each symbol represents 1 individual mouse.

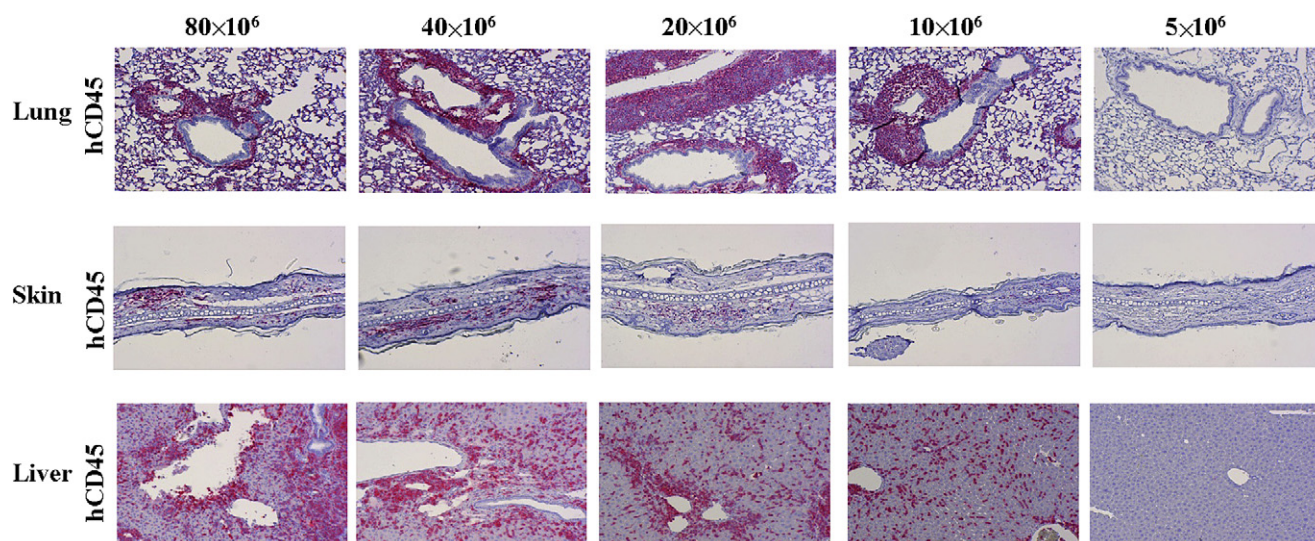


FIG E5. Transfer of 5×10^6 human PBMCs into NOD-SCID $\gamma c^{-/-}$ mice does not result in tissue inflammation. Different numbers of human PBMCs from healthy nonallergic donors as indicated were injected into NOD-SCID $\gamma c^{-/-}$ mice and at day 24 lung, skin, and liver tissue were stained for human CD45 (magnification $\times 100$). Results representative of 2 experiments.

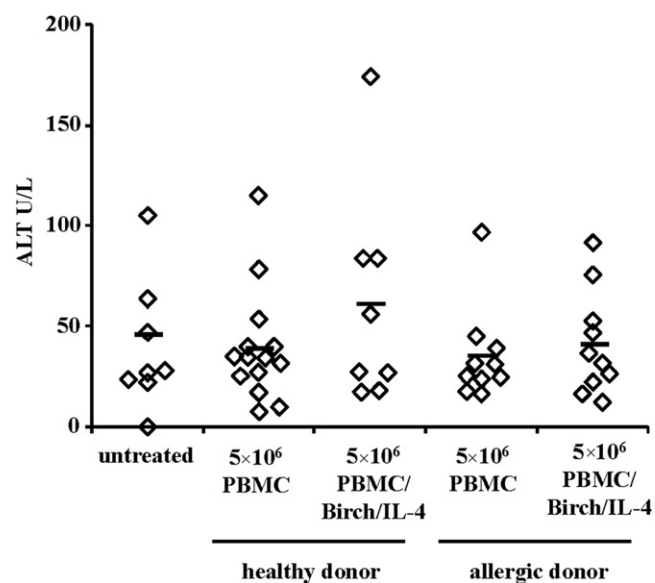


FIG E6. Transfer of 5×10^6 human PBMCs into NOD-SCID $\gamma c^{-/-}$ mice does not result in clinical hepatitis. Alanine transaminase serum levels of mice belonging to different groups at day 24 after PBMC transfer. *Symbols* represent individual animals. One representative result of 3 is shown. The *bars* represent the mean value in the respective groups.

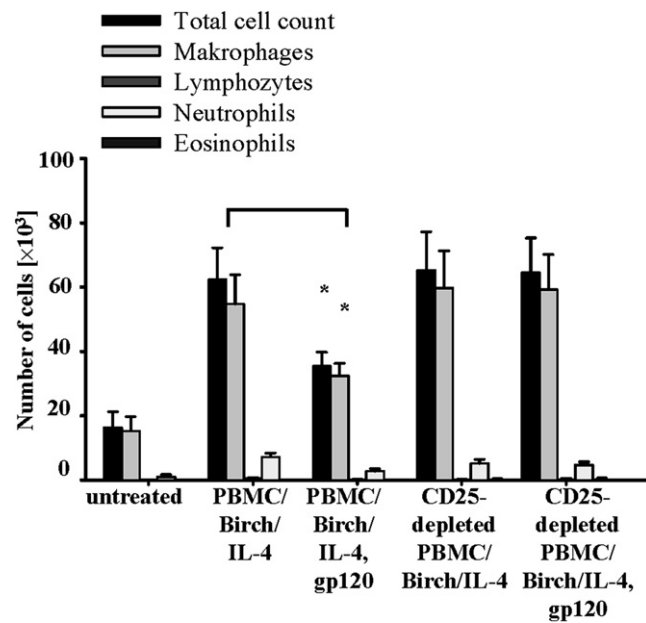


FIG E7. Composition and enumeration of cells in the BAL fluid. NOD-SCID $\gamma_c^{-/-}$ mice were injected with either complete PBMCs or CD25-depleted PBMCs from allergic donors and additionally treated with birch allergen and IL-4. Some animals were additionally treated with gp120. The BAL fluid was isolated on day 24, cytopsin preparations of BAL cells were stained with the Microscopy Hemacolor Set (Merck, Darmstadt, Germany), and cell types were counted by 2 investigators blinded to experimental conditions. * $P < .05$ compared to PBMC/Birch/IL-4.