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The Allergen-Induced Airway Hyperresponsiveness in a Human-Mouse Chimera Model of Asthma Is T Cell and IL-4 and IL-5 Dependent

Kurt G. Tournoy, Johan C. Kips, and Romain A. Pauwels

Airway hyperresponsiveness (AHR) is a major pathological characteristic of bronchial asthma. It is also a major determinant of asthma symptoms and disease severity. The underlying airway inflammation is thought to be responsible for the AHR. This hypothesis is based on the significant association of airway inflammation and AHR both in human asthma and in animal models of asthma and on repeated observations that treatment with inhaled corticosteroids and allergen avoidance decrease both airway inflammation and AHR. The airway inflammation in asthma is characterized by an increase in activated CD4\(^+\)-Th2 lymphocytes, eosinophils, dendritic cells, mast cells, and monocytes. However, the exact role of the individual cells and their proinflammatory products in the pathogenesis of AHR remains unknown. Based on clinical data and on numerous mouse models of asthma, it is suggested that in allergic asthma, the Th2 lymphocytes induce, via the production of cytokines, an inflammatory cascade comprising eosinophil activation, IgE synthesis, and mast cell activation, which in turn produce the necessary mediators causing the AHR (1, 2). However, more recent animal experiments suggest that neither IgE nor the eosinophil nor the mast cell are necessary for allergen-induced AHR (3–5). The purpose of this study was to identify these components of the human immune system that are responsible for the allergen-induced AHR in vivo using a human-mouse chimera system. The reconstitution of SCID mice with human PBMC (Hu-PBMC) results in a human-mouse chimera with a functional human Ag-reactive immune system enabling the in vivo study of human immune disease (6). We report here that SCID mice, intratracheally (i.t.) reconstituted with Hu-PBMC from nonallergic donors, develop AHR after injection with Dermatophagoides pteronyssinus (Der p) I alum and exposure to house dust mite (HDM) allergen in an IL-4- and IL-5-dependent manner. The AHR in these Hu-PBMC-i.t.-SCID mice was not associated with eosinophilia or increases in allergen-specific IgE. Treatment with human double-mutin-IL-4 (DM-IL-4) or with TRFK-5, molecules inhibiting, respectively, IL-4/IL-13 and IL-5, completely abolished the allergen-induced AHR.

Materials and Methods

Animals

Homozygous male Fox Chase C.B17-SCID mice were obtained from M&B (Møllegaard and Bomholmø Breeding and Research Center, Ry, Denmark). All mice were housed in sterilized cages with filter tops and fed sterilized food and water ad libitum. They were 7 wk old at the beginning of the experiments. The experimental protocols were approved by the local ethical committees of Ghent University (Ethical Committee for Laboratory Animals, project 96/5 and Committee for Medical Ethics, project 96/58).

Reconstitution of SCID mice with Hu-PBMC

Twenty-four hours before reconstitution, the C.B17 SCID mice were injected i.p. with 900 μg TM-β1 (anti-mouse CD122) enabling a long-lasting and recall Ag-reactive graft survival (6). Healthy volunteers (n = 10), without a history of allergic asthma or atopy (Der p I IgE below detection limit) and with a negative skin prick test for HDM allergens, were used as leukocyte donors after informed consent. The Hu-PBMC were isolated from heparinized venous blood (250–350 ml/donor) using Ficoll-Hypaque (density = 1.077 g/ml) (Nycomed Pharma, Oslo, Norway) centrifugation.
The Hu-PBMC were washed and suspended in HBSS so that a concentration of $10^8$ Hu-PBMC/80 μl was obtained. An i.t. instillation technique was applied permitting the administration of a precise graft volume into the mouse lungs (7).

**Allergen challenge protocols**

The lyophilized HDM extract and the major HDM allergen (Der p I) were produced by ALK-Abellô Laboratories (Hørsholm, Denmark). The 25.4-kDa major allergen Der p I was purified from an aqueous extract of mite culture by physicochemical methods as described (5). The measurement of Der p I in the HDM extract was performed with ELISA and indicated a content of 8 ng Der p I per μg HDM. One day after the reconstitution with Hu-PBMC, the SCID mice were either injected i.p. with 10 μg Der p I adsorbed to 1 mg of aluminum hydroxide (Aldrich, Gillingham, Dorset, U.K.) as adjuvant or with adjuvant alone or with Der p I alone. A booster dose was given 10 days after the reconstitution. From day 2 until day 20, the Der p I-injected mice were exposed to a 30-min daily aerosol (Ultra-schallvernebler Sirius Nova; Heyer Medizintechnologie, Bad Ems, Germany) with HDM extract (300 μg/ml), whereas the control mice were exposed to PBS.

**Anti-cytokine therapy of the Hu-PBMC-i.t.-SCID mice**

In some experiments, the Der p I- and HDM-challenged mice were treated with anti-IL-4/IL-13 or anti-IL-5 cytokine therapy. DM-IL-4 (BAY 19-9996; provided by D. Hörlein and J. Peters, Bayer AG, Wuppertal-Elberfeld, Germany) is a mutated human IL-4 protein with amino acid substitutions at positions 121 and 124. As DM-IL-4 binds the IL-4-receptor α-chain without inducing signal transduction, it acts as a competitive antagonist of both IL-4 and IL-13 (8, 9). Blocking the IL-4/IL-13 activity was achieved using Fast Red (Dako) before the sections were counterstained with normal human serum in TBS and incubated with mouse anti-human CD45 (Becton Dickinson Benelux, Belgium). Specificity controls were done for IFN-γ, PE-anti-human-IL-4, and PE-anti-human-IL-5 (Becton Dickinson Benelux). Specificity controls were done for IFN-γ by preblocking with purified unlabeled anti-IFN-γ Ab, for IL-4 by preblocking the PE-anti-human IL-4 Ab with recombinant human IL-4 and for IL-5 by using the corresponding isotype control.

**Measurement of human IgE in Hu-PBMC-i.t.-SCID serum**

After measurement of the airway responsiveness, blood was drawn by cardiac puncture and centrifuged. Human IgE was measured in the serum of the SCID mice using a commercially available ELISA kit (Immunootech, Luminy, France).

**Statistical analysis**

The statistical package SPSS 9.0 (SPSS, Chicago, IL) was used. Results are presented as means ± SEM. Different groups were compared using the Kruskal-Wallis H test. When the Kruskal-Wallis significance level was $p < 0.05$, Mann-Whitney U tests were applied as post hoc analysis (with Bonferroni’s conservative correction). The dose-response curves for the airway responsiveness were compared with the general linear model univariate procedure.

**Results**

**Induction of AHR in Hu-PBMC-i.t.-SCID mice after allergen challenge**

**Graft characteristics.** The Hu-PBMC from eight different nonatopic healthy volunteers were characterized with cytology and intracytoplasmatic cytokine detection. The Hu-PBMC of the different donors had a very constant composition and were exclusively composed of lymphocytes 88 ± 6% and monocytes 12 ± 6%. No eosinophils, neutrophils, or basophils were present. FACS analysis showed that of the CD45+ cells, 63 ± 3% were CD3+ (49 ± 2% CD4+ and 14 ± 1% CD8+), 7 ± 2% were CD19+, 17 ± 2% were CD56+/CD16+, and 11 ± 2% were CD14+. Less than 1% (0.5–0.8%) of the cells were CD1a+/HLA-DR+. Almost no intracytoplasmatic IL-4 was present, but IFN-γ was present in 6 ± 2% of the CD45+ Hu-PBMC before the intrapulmonary grafting (Fig. 1).

**Induction of AHR.** In each of the eight groups of 16–20 SCID mice reconstituted with Hu-PBMC from eight different donors, half of the mice were allergen exposed (two i.p. injections with alum-Der p I and aerosols for 20 days with HDM), whereas the other half were not exposed to allergen (alum alone and challenges with PBS). Irrespective of the donor, the allergen-challenged mice developed a significant AHR to carbachol (Fig. 2). Next, we evaluated the importance of the AHR for induction of the AHR by comparing HDM-challenged mice that were injected with either alum-Der p I or with Der p I alone (Fig. 3A). The mice challenged with Der p I without alum adjuvant failed to develop AHR. The airway responsiveness was also not increased in nonreconstituted
mice that were exposed to allergen adjuvant, excluding that the allergen adjuvant combination on its own could be responsible for the AHR (Fig. 3B). Reconstitution with fewer than $10^6$ Hu-PBMC resulted in a loss of the capacity to induce AHR suggesting that the graft size is also important (Fig. 3C).

**Measurement of human IgE and human peribronchial infiltrates.** Human cells could readily be identified in the mouse lungs 3 wk after reconstitution by means of immunohistochemistry (Fig. 4, anti-human CD45 cell surface marker). Important interdonor variations for peribronchial human cell survival and accumulation appeared. Comparing the HDM- vs PBS-exposed mice, no significant difference in the cell infiltrates could be demonstrated (Fig. 4). Only mononuclear cells with a lymphocyte morphology were observed in the infiltrates. No polymorphonuclear cells such as eosinophils or neutrophils were present. Analyzing the bronchoalveolar lavage fluids, no differences in cell counts between sham and allergen-exposed mice could be identified. Over 99% had a macrophage morphology (data not shown), whereas eosinophils were never identified. Additional stainings with periodic acid-Schiff (PAS) revealed that very few mucus-producing cells were present in the airways and that no differences exist between HDM- and PBS-exposed mice (data not shown). FACS analysis on the lung cells from the Hu-PBMC-it-SCID mice revealed that within the lymphocyte gate, between 0.3 and 1.5% of all the lung cells had the human CD45$^+$, HLA-DR$^+$, CD1a triple-positive cells. These CD45$^+$, HLA-DR$^+$, CD1a$^+$ cells were almost doubled in the allergen-challenged mice vs controls (data not shown). Phenotype analysis of the peribronchial lymph node cells also revealed an infiltration of human cells (Fig. 5A). In contrast with the cells from the lungs, CD45$^+$CD19$^+$ cells were present in the lymph nodes (4–42% of the human cells). Staining the human cells recovered from the SCID mouse lungs for intracellular cytokines revealed a stronger signal for IL-4 and IL-5 in the allergen-challenged mice. In contrast, the human cells in PBS-challenged mice were characterized by a stronger IFN-γ signal (Fig. 5).

**Effect of anti-IL-4/13 and anti-IL-5 in the allergen-challenged Hu-PBMC-it-SCID mice**

**Effect of DM-IL-4 and TRFK-5 on AHR.** Five groups of 8–9 SCID mice were reconstituted with PBMC from the same donor. Treatment of the HDM-challenged mice with DM-IL-4 caused a significant suppression of the AHR. Similarly, antagonizing IL-5 by injecting TRFK-5 but not with the control Ab GL-113 caused a similar down-regulation of the HDM-induced AHR. This is also reflected in an increase of the PD$_{50}$-R L (Fig. 6) and PD$_{100}$-R L (data not shown) in the DM-IL-4- and TRFK-5-treated animals compared with the control-pretreated allergen-exposed animals. The effect of anti-cytokine therapy was similar in different experiments with Hu-PBMC from different donors. The anti-cytokine treatment did not affect the basal airway responsiveness SCID mice (data not shown).

**Effect of DM-IL-4 and TRFK-5 on human peribronchial cell infiltrates, IgE, and weight of the animals.** No significant differences in human cell presence and human peribronchial cell accumulations could be observed between any of the studied groups. The anti-cytokine treatment either with DM-IL-4 or with TRFK-5
FIGURE 2. Airway responsiveness in the Hu-PBMC-it-SCID mice after allergen exposure. Significant increases in airway resistance upon carbachol were noted in the mice exposed to allergen adjuvant. Experiments were performed with eight different donors (16–20 mice per experiment).
did not alter the pulmonary histology. In addition, no influence at all of the anti-cytokine therapy on the human IgE production was observed. No discernible effects of the anti-cytokine treatment on the general condition of the animals were noted (no differences in the weight was observed between any of the groups, data not shown).

**Effect of DM-IL-4 and TRFK-5 on human intracytoplasmatic cytokine profiles.** Treating the mice with DM-IL-4, TRFK-5, or GL-113 did not alter the amount of human cells recovered from the Hu-PBMC-it-SCID mouse lungs. DM-IL-4 treatment did not change the intracellular presence of IL-4 (Fig. 7A) or IL-5 in the human cells when compared with the untreated HDM-challenged mice. Similarly, anti-IL-5 treatment with TRFK-5 did not modify the expression of IL-4 (Fig. 7B) or IL-5. The effect of anti-cytokine therapy on the intracellular cytokine expression was similar in two different experiments with Hu-PBMC from different donors.

**Discussion**

In this study, we show that the i.t. reconstitution of SCID mice with Hu-PBMC from healthy persons with no allergic symptoms, a negative skin prick test, no measurable Der p I-specific serum IgE, and a Th1 cytokine pattern resulted in long-term survival of the engrafted human cells in an Ag-reactive state. The graft of human allergen-reactive cells, the i.p. injection of allergen adjuvant, and the exposure to aerosolized allergen resulted in a significant and consistent increase in airway responsiveness in the mice. The increase in airway responsiveness was critically mediated by the human immune cells because the same procedure of allergen and adjuvant administration in naive SCID mice did not result in changes of the airway responsiveness. Marked differences in the absolute responses as well as in human cell presence and human Ig production were observed between groups reconstituted with cells from different donors, implicating that important donor-dependent factors are involved.

The peribronchial human cell presence was similar in sham and allergen-challenged mice. The finding that the magnitude of the human cell infiltrates is not related to the extent of the AHR is in accordance with findings in asthma patients (11). Moreover, eosinophils (either human or mouse) were never observed in the mouse lungs, whereas the hu-IgE production was comparable in sham and allergen-challenged mice. This indicates that neither IgE nor eosinophils are necessary for allergen-induced AHR. Moreover, we could not show a role for the mucus-producing cells in this model. Although this is the first report based on the use of human cells and clinically relevant allergens in vivo, it confirms the suggestions of some recent murine asthma models (3–5).

Moreover, our findings are consistent with the observations showing the presence of allergen-reactive T cells in the blood of nonallergic persons (12–15) and demonstrate in vivo that T lymphocytes with a Th1 cytokine pattern can be driven to a Th2 pattern after exposure to the combination of allergen and adjuvant. Although IFN-γ production became suppressed in the intrapulmonary human lymphocytes, the Th2 cytokines IL-4 and IL-5 were markedly up-regulated. This allergen adjuvant-driven in vivo change in Th1/Th2 balance of human Th1 cells also confirms the in vitro observation about the reversibility of the Th1/Th2 balance with human cells (16). An important observation was the necessity of the adjuvant-adhered allergen for inducing AHR. Without adjuvant, no AHR could be induced. It is well known that aluminum compounds as vaccine adjuvant are associated with the induction of Th2 responses (17) and with the skewing of an existing Th1 toward a Th2 response (18). Although the underlying mechanisms are not well understood, the adjuvant functions as a long-lasting strong Ag depot and activates local APCs to engage the second (costimulatory) signal (19). Although aluminum without Ag has no major effects on the baseline cytokine production, the adjuvant-Ag combination has proven, also in Hu-PBMC-SCID chimera models, to change the Th1/Th2 balance (18, 20, 21). Another element possibly important for the observed Th2 skewing is the nature of the allergen Der p I from HDM. Der p I has not only the intrinsic capacity to promote Th2 development (22), but facilitates also the trans-epithelial allergen delivery by disruption of the tight junctions (23). How exactly the Ag-adjuvant complex is presented to the T cells in the SCID mouse models remains unknown, but several hypotheses can be formulated. Because human APC can be cotransferred during reconstitution (although scarce in Hu-PBMC), they can constitute an efficient way of local Ag presentation (24). In addition, because 15% of the graft consists of monocytes and because monocytes can mature into functional APC (25), they can represent an additional source of Ag presentation. It has
been shown that a loss of these human APC in the Hu-PBMC-it-SCID models results in a state of T cell unresponsiveness to Ags (26). In our experiments, we were able to demonstrate, within the human cell population in the mouse lung, a fraction of cells that were HLA-DR and CD1a double positive, which is suggestive for such a population. This population was clearly expanded in the allergen adjuvant-exposed mice. In addition, we observed in the peribronchial lymph nodes an important accumulation of human B cells, which also can stimulate the T cells. Another possibility is that the mouse APC took part in the presentation of Ags to the human T cells (27).

This Hu-PBMC-it-SCID model of allergen-induced AHR offers the unique advantage to measure in vivo effects of new therapeutics developed for applications in humans. The relevance of IL-4/IL-13 and IL-5 for AHR in asthma is suggested by their role in several murine models of asthma and by the elevated concentrations in bronchoalveolar lavage fluid and lung tissue of asthma patients (1, 2). An important element about the evaluation of the role of IL-4 is the redundancy of this cytokine with IL-13. This is reflected in the structure of their receptors. IL-4 binds both the IL-4 and IL-13 receptor, whereas IL-13 only binds to its own receptor. Due to this redundancy, it is hard to ascertain the exact role of either cytokine in allergy. The differential effects of anti-IL-4 mAb (28) and of mutant IL-4 (29) or IL-4 receptor antagonists (30) in murine asthma models suggest that IL-4/IL-4 receptor system is of particular importance during the primary sensitization phase, whereas the IL-13 receptor activation (either by IL-4 or by IL-13) plays a more important role during the secondary immune response (31). Based on these observations and on the intention to interfere with a secondary immune response, we opted for DM-IL-4 as a potent antagonist of the IL-4/IL-13 pathway. For blocking the effects of IL-5, a long experience in murine asthma models...
FIGURE 5. Human cell surface makers and cytokines in lymphocyte population from Hu-PBMC-it-SCID mouse peribronchial lymph nodes (A) and lungs (B). When gated on the CD45 pan-leukocyte surface marker in the lymphogate, up to 42% of the human lymphocytes were CD19$^+$ in the lymph nodes, whereas $>99\%$ of the human cells recovered from the pulmonary tissue were CD3$^-$. Figures originate from mice reconstituted with cells from donor 4. Allergen-exposed mice (lower panel) had more IL-4 and IL-5 but less IFN-$\gamma$ when compared with sham-exposed mice (upper panel). Isotype controls are shaded gray. The latter figures originate from SCID mice reconstituted with cells from donor 5.
exists with TRFK-5, which inhibits the binding of mouse and human IL-5 to the IL-5 receptor. Conflicting data from the murine models exist on the effect of TRFK-5 and therefore on the importance of IL-5 in AHR (28, 32–34).

Here we provide evidence that, by blocking the pathways of human IL-4/IL-13 or IL-5, an inhibition of the allergen-induced AHR is achieved. Thus, it appears that human IL-4/IL-13 and IL-5 are both critically involved in the induction of allergen-induced AHR. IL-5 has been shown to increase the responsiveness of airway smooth muscle to contractile agonists in vitro (35), whereas a pretreatment with an IL-5 receptor Ab decreased the altered responsiveness of atopic asthmatic sensitized airway smooth muscle (36). Because the airway smooth muscle cells express receptors for IL-5 (35) and because there is a cross-activity of human IL-5 on...
the murine IL-5 receptor (37), we can explain the allergen-induced AHR in the current Hu-PBMC-it-SCID model by the direct effect of IL-5, secreted by allergen-specific human Th2 lymphocytes, on the murine airway smooth muscle.

The effect of allergen-induced IL-4 production on the airway responsiveness might be more indirect. Although the smooth muscle cells carry the IL-4 receptor, which is even up-regulated in sensitized tissue (35), and although increased smooth muscle shortening velocity has been linked with IL-4 levels (38), other experiments showed that IL-4 neutralizing mAb had no significant effect on the altered responsiveness of atopic airway smooth muscle (36). Therefore, a possible explanation for the remarkable effects of DM-IL-4 in our experiments can be found in the neutralization of the up-regulation of the IL-5 secretion from the human Th2 lymphocytes by IL-4 (39). This fits with the clinical observation of increased airway responsiveness after IL-4 inhalation, which was associated with an IL-5-driven eosinophil influx (40). DM-IL-4 also blocks all activities of IL-13 including its possible direct effects on smooth muscle cells on which the IL-13 receptor was demonstrated (41). Therefore, an important finding of this study is that the Th2 lymphocytes alter the airway responsiveness by the secretion of IL-4 and IL-5 without the involvement of other inflammatory cells such as eosinophils. This finding is in accordance with some recent findings in mouse asthma models (5).

Strikingly, the intracellular human Th2 cytokine production under continuous allergen adjuvant exposure did not change after the anti-cytokine treatment. This can be explained by the findings that allergen adjuvant is capable of inducing Th2 cytokine profiles in an IL-4/IL-13- or IL-5-independent manner (34). The fact that IL-4 production remains unchanged during DM-IL-4 therapy is in agreement with previous reports on the role of IL-4 in the secondary immune response (42) and proves that a recall immune response and not a primary immune response is involved in this model. Earlier reports suggested also that a primary immune response can be achieved in SCID-hu models, but not in Hu-PBMC-SCID models that we used (43).

In summary, these data demonstrate that allergen-reactive cells from nonallergic donors can be triggered in vivo to produce Th2 cytokines after allergen adjuvant exposure. Moreover, the production of Th2 cytokines is critically associated with the induction of AHR because blocking of the Th2 cytokine activity reduced the AHR to baseline. Despite the beneficial effects of DM-IL-4 and TRFK-5 on the allergen-induced AHR, the Th2 cytokine production remained stable, suggesting that these possible therapeutic agents do not influence the ongoing Th2 response.

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References


