

House Dust Mite–induced Airway Changes in hu-SCID Mice

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SCID (severe combined immunodeficiency) mice reconstituted with peripheral blood mononuclear cells (PBMC) from *Dermatophagoides pteronissynus* (Dpt)-sensitive patients and exposed to Dpt aerosol (allergic hu-SCID mice) develop human IgE and pulmonary inflammation. The present study investigated concomitant changes in airway hyperresponsiveness (AHR). No significant difference in baseline airway responsiveness was seen between nonreconstituted SCID mice exposed or not to Dpt aerosol at Day 35. Allergic hu-SCID mice developed AHR (provocative dose of carbachol causing a 50% increase in lung resistance [$PD_{50} R_L$] = $96.33 \pm 16.88 \mu\text{g/kg}$) compared with nonallergic hu-SCID mice ($PD_{50} R_L$ = $242.03 \pm 37.84 \mu\text{g/kg}$) and nonreconstituted SCID mice ($PD_{50} R_L$ = $297.60 \pm 45.60 \mu\text{g/kg}$) exposed to Dpt aerosol. An inverse correlation was observed between $PD_{50} R_L$ (Day 35) and total human IgE at Day 7 ($r = -0.58$) and Day 15 ($r = -0.64$). However, no correlation existed between $PD_{50} R_L$ and human cell number in the lungs of allergic hu-SCID mice. Moreover, despite the absence of eosinophils, the bronchoalveolar lavage fluid (BALF) of allergic hu-SCID mice had more human interleukin-5 (IL-5) ($3.28 \pm 0.40 \text{ pg/ml}$, $n = 13$) than nonallergic hu-SCID mice ($< 0.5 \text{ pg/ml}$) which inversely correlated with the $PD_{50} R_L$ ($r = -0.61$). No tumor necrosis factor-alpha (TNF- α), IL-6, or IL-4 was detected. These observations indicate that humanized allergic hu-SCID mice may develop AHR after exposure to the relevant allergen, suggesting that this model may improve our understanding of AHR, one characteristic feature of allergic asthma. Duez C, Kips J, Pestel J, Tournoy K, Tonnel A-B, Pauwels R. House dust mite–induced airway changes in hu-SCID mice.

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Allergic asthma is characterized by three main features: specific IgE production, airway inflammation, and airway hyperresponsiveness (AHR) (1).

Several factors including neurogenic abnormalities (2) and airway inflammation characterized by eosinophil infiltration (3, 4), and release of inflammatory mediators and cytokines (5) have been implicated in the development of AHR. By using animal models, our knowledge of mechanisms associated with AHR has been largely improved. Specifically, the use of murine models has extended our understanding of airway inflammation associated with the development of AHR. Indeed, the participation of eosinophils and T lymphocytes was reported as a major feature of AHR. Eosinophils are thought to be major effector cells in the development of AHR because of their ability to release potent mediators, such as major basic

protein, toxic for the bronchial epithelium (6). Epithelial injury may contribute to increases in airway responsiveness because of absence of the epithelial-derived relaxant factor, or activation of denuded intraepithelial nervous terminations (7). $CD4^+$ T lymphocytes are also implicated, probably by inducing an eosinophil infiltrate (8). $CD4^+$ T cells in airway mucosa of asthmatics express increased levels of interleukin-4 (IL-4) and IL-5 messenger RNA (mRNA) (5). These cytokines and particularly IL-5 have been implicated in allergic AHR because IL-5-deficient mice (9) and anti-IL-5 antibody treated mice (10) do not develop AHR. The role of mast cells in AHR is still debated. It seems that this cell may not play a role in the induction of AHR (11) but in its amplification.

However, data derived from animals cannot be directly extrapolated to humans. In an attempt to better approach human allergic asthma, we have developed a model in humanized SCID (severe combined immunodeficiency) mice (12, 13). The SCID mouse is of particular interest because of the absence of mature and functional T and B lymphocytes (14). Because of a defect in the recombinase system, linked to a nonsense mutation of DNA-dependent protein kinase catalytic subunit (15), SCID mice cannot successfully rearrange their immune receptor genes and consequently have no mature T or B cells (16). Thus, SCID mice can tolerate a graft with human cells and particularly those of purified peripheral blood mononuclear cells (PBMC) administered by intraperitoneal injection (17). Consequently, reconstituted SCID mice

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provide a unique model to particularly evaluate the role of human T and B cells in an *in vivo* animal system.

In previous studies (12, 13), we showed that SCID mice reconstituted with 10×10^6 mononuclear cells from asthmatic patients sensitive to *Dermatophagoides pteronyssinus* (Dpt) (allergic SCID mice), and exposed to allergen aerosol developed a specific human IgE response and a pulmonary inflammatory-type infiltrate. The cell infiltrates consisted of human CD45⁺ cells localized in perivascular and peribronchial areas and disseminated in the lung parenchyma, and were mainly of the activated and memory CD4 phenotype. The development of this allergen-dependent infiltrate was associated with the expression of IL-5 mRNA, restricted to a small number of cells, and was correlated with the presence of eosinophils (12, 13).

The purpose of this work was to evaluate for the first time airway reactivity of SCID mice reconstituted with mononuclear cells from Dpt-sensitive patients and exposed to Dpt aerosol.

METHODS

Reconstitution of SCID Mice

Six- to eight-week-old C.B-17 SCID mice were maintained in sterilized isolators (La Calhène, Velizy, France) with sterilized bedding at the Pasteur Institute, Lille. The SCID colony was regularly checked for absence of serum Ig to discard potential leaky animals.

One day prior to reconstitution, SCID mice were treated with 50 μ l anti-asialo GM1 (ASGM1) antibody (Wako Chemicals, Neuss, Germany). Human PBMC were prepared by density centrifugation on Ficoll-Paque (Pharmacia, St. Quentin en Yvelines, France) from blood of donors sensitive to house dust mite (positive skin prick tests toward Dpt allergen, positive RAST and serum IgE concentrations ranging from 400 to 2,173 IU/ml). Human PBMC from nonallergic donors were used as negative controls. Mononuclear cells harvested at the interface were washed three times in sterile RPMI medium before transfer: cells (10×10^6 /mouse) were injected intraperitoneally in 200 to 300 μ l RPMI into SCID mice via a 23-gauge needle. At the same time, 2 Index Reactivity (IR) units of Dpt were injected intraperitoneally.

Inhalation Protocol

Four days after human cell transfer, SCID mice were exposed to daily aerosols of Dpt (D0 to D4). Reconstituted hu-SCID mice were placed in a sterilized air flow-controlled chamber connected to a nebulizer (VarioSonic Peters, Aubervilliers, France) generating 3 to 4 μ m size droplets and were exposed to a solution containing 100 IR units Dpt (100 IR units are equivalent to approximately 200 μ g of protein contained in the Dpt extract) as described (12). One day before airway responsiveness measurement, hu-SCID mice were exposed to another aerosol of 100 IR units of Dpt solution.

Assessment of Airway Responsiveness

Airway responsiveness was measured on Day 35 or Day 60 according to a technique previously described (18, 19). Briefly, animals were anesthetized and a tracheal cannula inserted. They were mechanically

ventilated at a respiratory rate of 145 breaths/min, with a tidal volume of 0.5 ml. Lung resistance (R_L), calculated from measurements of flow, tidal volume, and transpulmonary pressure was continuously recorded. Tidal volume was calculated from the flow at the tracheal cannula, measured using a Fleisch pneumotachograph, Type 00000 (Fleisch, Lausanne, Switzerland). Transpulmonary pressure was measured using a differential pressure transducer, one end of which was connected to the outlet of the tracheal cannula and the other inserted in the pleural space. Blood pressure was recorded with a pressure transducer (Gould Medical BV, Bilthoven, The Netherlands) connected to the femoral artery catheter. The dead space of the equipment was measured to be 0.6 ml. To assess airway responsiveness, carbachol was injected intravenously as a bolus, in increasing doses (40, 120, 400, and 1,200 μ g/kg), until an increase of at least 50% in R_L was observed. The provocative dose of carbachol causing a 50% increase in lung resistance ($PD_{50} R_L$) was then calculated by linear interpolation on a semilogarithmic dose-response curve.

Human IgE Measurements

After reconstitution, mice were periodically bled from the retro-orbital sinus under ether anesthesia. Total human serum IgE was detected by a fluorometric method (CAP system; Pharmacia) using two different mouse monoclonal antibodies specific for the ϵ chain, as previously described (12). The sensitivity of the method was 0.1 IU/ml (0.24 ng/ml). Specific IgE antibodies (Ab) against Dpt allergen were quantified by a modification of the protocol previously described (20). The second antibody used was a peroxidase-labeled monoclonal mouse anti-human IgE Ab (Biomérieux, Lyon, France).

Detection of Human CD45⁺ Cells

Lungs were excised at Day 35, fixed in 4% buffered paraformaldehyde, and processed for paraffin embedding. After deparaffinization and rehydration, paraffin tissue sections (6 μ m thick) were saturated with 20% normal human serum diluted in TRIS-buffered saline (TBS) and incubated at 4°C overnight with monoclonal antibody to human CD45 (Becton Dickinson, Mountain View, CA). Monoclonal

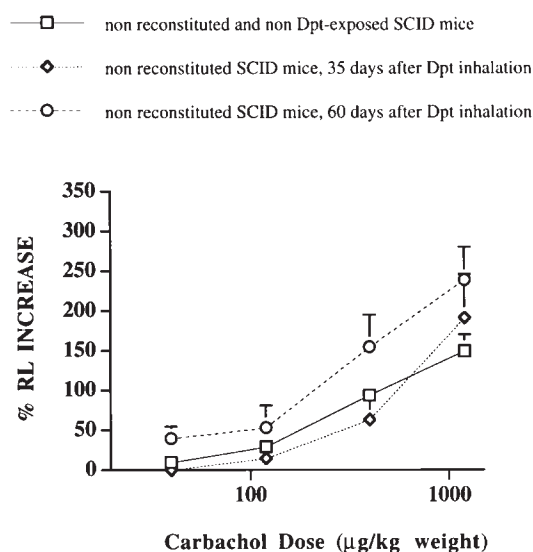


Figure 1. Airway responsiveness of nonreconstituted SCID mice. Airway responsiveness to carbachol was evaluated in nonreconstituted and non-Dpt-exposed SCID mice (square, $n = 13$), or non-reconstituted SCID mice analyzed 35 d after Dpt inhalation (diamond, $n = 4$) or 60 d after inhalation (circle, $n = 8$). Results are expressed in percentage of R_L increase (mean $\pm$ SEM). Differences between the three groups are not significant according to Kruskal-Wallis test.

TABLE 1

HISTOLOGICAL GRADING CRITERIA

Grade 0	No CD45 ⁺ human cells detected in the lungs
Grade 1	1–10 CD45 ⁺ human cells per field (magnification $\times 500$) disseminated in the lung parenchyma
Grade 2	Perivascular CD45 ⁺ human cell infiltrates of less than 40 cells per field (magnification $\times 500$)
Grade 3	Perivascular CD45 ⁺ human cell infiltrates of more than 40 cells per field (magnification $\times 500$)

antibody staining was demonstrated by an alkaline phosphatase–anti-alkaline phosphatase (APAAP) technique (21). Quantification of human cells on murine lung sections by histological score was done as described in Table 1.

Bronchoalveolar Lavage (BAL) and Detection of IL-5, IL-4, Tumor Necrosis Factor-Alpha (TNF- α), and IL-6

Immediately after assessment of AHR at Day 35, the left lung was lavaged via the tracheal tube with Hanks' balanced salt solution (HBSS, 2×0.75 ml, each lavage aspirated immediately and samples pooled), while the right lobes were clamped. A volume of 1 ml of BAL fluid (BALF) from each animal was centrifuged, the pellet resuspended, and cytopspins made. The supernatant was aliquoted in three Rhesus tubes and maintained at -70°C before testing. The undiluted BALF was tested in duplicate according to the manufacturer's indications.

For TNF- α and IL-6, ultrasensitive sandwich type ELISA kits (R&D Systems, Abingdon, Oxon, UK) were used. Briefly, 200 μl of each sample were added per well containing 50 μl of assay diluent and incubated overnight at $+4^{\circ}\text{C}$. After washing, anti-TNF- α or anti-IL-6 conjugate was added. After 3 h incubation, and washing, 50 μl of substrate solution was added and incubated for 1 h. After addition of amplifier solution (50 μl) and a further 30-min incubation at room temperature, the enzyme reaction was stopped by 50 μl of stop solution. The optical density of each well was determined within 30 min using a microplate reader set to 490 nm. TNF- α and IL-6 concentrations were determined from the standard curve.

The presence of human IL-4 and IL-5 was detected in undiluted BALF by using species-specific ELISA (Immunotech, Marseille-Luminy, France). The sensitivity of assay is 0.5 pg/ml. Briefly, 50 μl of undiluted sample or diluted standard was first incubated for 2 h at room temperature. After washing, 50 μl of biotinylated antibody and then 100 μl streptavidin–peroxidase solution were added. After a 30-

min incubation at room temperature, wells were washed. Finally, after addition of the chromogenic substrate of peroxidase (100 μl) and further 30-min incubation at room temperature, the enzymatic reaction was stopped with a double-normal (2N) sulfuric acid solution and absorbance read at 450 nm. Interleukin concentration was determined by interpolation from a standard curve performed in the same assay.

Cells in BAL of Humanized SCID Mice

Cytopspins of cells present in BALF were done (750 rpm, 3 min). Differential cell counts were performed by counting at least 300 cells per slide, after staining by Giemsa. The percentage of cell populations was calculated according to standard hematological procedures.

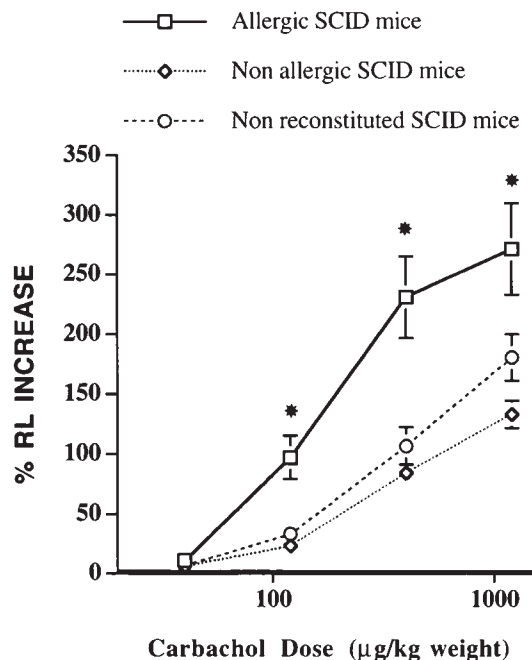


Figure 2. AHR of allergic SCID mice exposed to Dpt aerosol. Airway responsiveness to carbachol was evaluated in allergic SCID mice exposed to Dpt aerosol (square, $n = 12$) in comparison with nonallergic SCID mice exposed to Dpt aerosol (diamond, $n = 10$) and nonreconstituted SCID mice exposed to Dpt aerosol (circle, $n = 12$). Results are expressed in percentage of R_L increase (mean $\pm$ SEM). Airway responsiveness of allergic SCID mice exposed to Dpt aerosol is significantly higher than those of SCID mice in the other three groups, according to Kruskal-Wallis test (* $p < 0.007$).

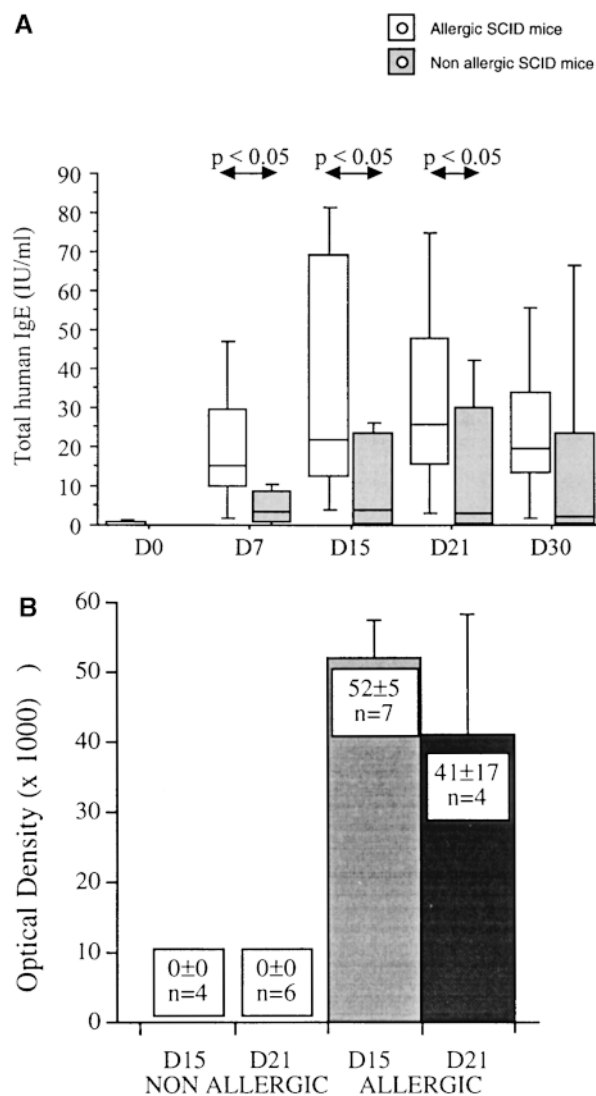


Figure 3. Production of human IgE in allergic or nonallergic SCID mice exposed to Dpt aerosol. (A) Total human IgE were evaluated in two groups of SCID mice reconstituted with PBMC from asthmatic patients sensitive to Dpt (white rectangle, $n = 19$), or from nonallergic donors (gray rectangle, $n = 13$) after Dpt inhalation. Results are expressed in median (IgE concentration for which 50% of values are inferior) and interquartile (median $\pm$ 25%). IgE concentrations are significantly different at Days 7, 15, and 21 according to the Mann-Whitney test ($p < 0.05$). (B) Dpt-specific IgE were evaluated in allergic (gray rectangle) and nonallergic (white rectangle) SCID mice at Days 15 and 21 after Dpt inhalation. Optical density was measured, and results are expressed as means $\pm$ SEM.

Statistical Analysis

The statistical analysis was performed using the Statview F4.11 Software (Apple Company, Cupertino, CA). The Kruskal-Wallis and the Mann-Whitney tests were applied to the quantitative data of the RL increase and total IgE levels, and the Spearman test was applied to evaluate correlations between PD₅₀ RL and IgE or histological score.

RESULTS

Airway Responsiveness in SCID Mice

Baseline airway responsiveness to different doses of carbachol was analyzed in three groups of nonreconstituted SCID mice: SCID mice nonexposed to Dpt aerosols (Group 1, $n = 13$), SCID mice exposed to Dpt aerosols and analyzed on Day 35 (Group 2, $n = 4$) or on Day 60 (Group 3, $n = 8$). No significant difference was observed between the three groups (Figure 1), as indicated by the mean value of PD₅₀ RL: $312.80 \pm 81.80 \mu\text{g/kg}$ (Group 1), $346.53 \pm 112.44 \mu\text{g/kg}$ (Group 2), and $202.85 \pm 25.56 \mu\text{g/kg}$ (Group 3).

As inhalation of Dpt allergen did not seem to significantly modify airway responsiveness of these three groups of SCID mice, they were all considered as nonreconstituted SCID mice.

Evidence of AHR in Allergic hu-SCID Mice

Airway responsiveness of Dpt-exposed SCID mice reconstituted with PBMC from allergic patients ($n = 12$) was analyzed on Day 35. Their airway reactivity to carbachol was significantly increased compared with control animals (Figure 2). Indeed the mean provocative concentration PD₅₀ RL ($96.33 \pm 16.88 \mu\text{g/kg}$) of carbachol was significantly lower than those obtained in both control groups: i.e., nonallergic hu-SCID mice exposed to Dpt aerosol ($n = 10$) $242.0 \pm 36.0 \mu\text{g/kg}$ and nonreconstituted hu-SCID mice exposed to Dpt aerosol ($n = 12$) $297.6 \pm 45.6 \mu\text{g/kg}$ ($p < 0.007$).

IgE Production and Airway Inflammation in Allergic hu-SCID Mice

Measurement of total human IgE levels indicated that allergic hu-SCID mice exposed to Dpt produced higher amounts of IgE than similarly exposed nonallergic hu-SCID mice ($p < 0.05$) (Figure 3A). Moreover, in contrast to nonallergic hu-SCID mice, only allergic hu-SCID mice produced Dpt-specific IgE (Figure 3B). However, no correlation between total human IgE and AHR was evident at Day 35 but total human IgE values detected at Day 7 and Day 15 tended to be inversely related with PD₅₀ RL (respectively, $r = -0.58$, $p = 0.05$ and $r = -0.64$, $p = 0.03$).

Human inflammatory cells were more frequently detected and in a higher number in lungs of hu-SCID mice reconstituted with PBMC from patients allergic to Dpt than in lungs of nonallergic hu-SCID mice (Figure 4). The CD45⁺ human cells were mainly located in perivascular areas and in parenchyma. Only in 1 of 12 animals, were CD45⁺ human cells detected in the peribronchial region (Figure 4B). No eosinophils were detected in the airways. Furthermore although histological mean scores were higher in allergic hu-SCID mice lungs than in nonallergic hu-SCID mice lungs (1.23 ± 0.26 , $n = 11$ and 0.80 ± 0.20 , $n = 8$, respectively, $p = 0.05$), no correlation could be demonstrated between histological grade and PD₅₀ RL.

Presence of Human IL-5 in BALF of Allergic hu-SCID Mice

As T helper cell, type 2 (Th2) cytokines are usually associated with the pulmonary T-cell-dependent inflammation, the presence of human IL-4 and IL-5 was assessed in BALF of hu-SCID mice. The presence of the proinflammatory cytokines TNF- α and IL-6 was also evaluated. Significant concentrations

of human IL-5 were only detected in allergic hu-SCID mice (mean value $3.28 \pm 0.40 \text{ pg/ml}$, $n = 13$) (Figure 5). No significant human IL-5 was evident in nonallergic SCID mice ($< 0.5 \text{ pg/ml}$) ($n = 8$) as well as nonreconstituted SCID mice. The level of human IL-5 was inversely correlated with the PD₅₀ RL of the same mice ($r = -0.61$, $p = 0.006$). In contrast, no significant concentrations of IL-4, TNF- α , and IL-6 could be detected in BALF of allergic hu-SCID mice.

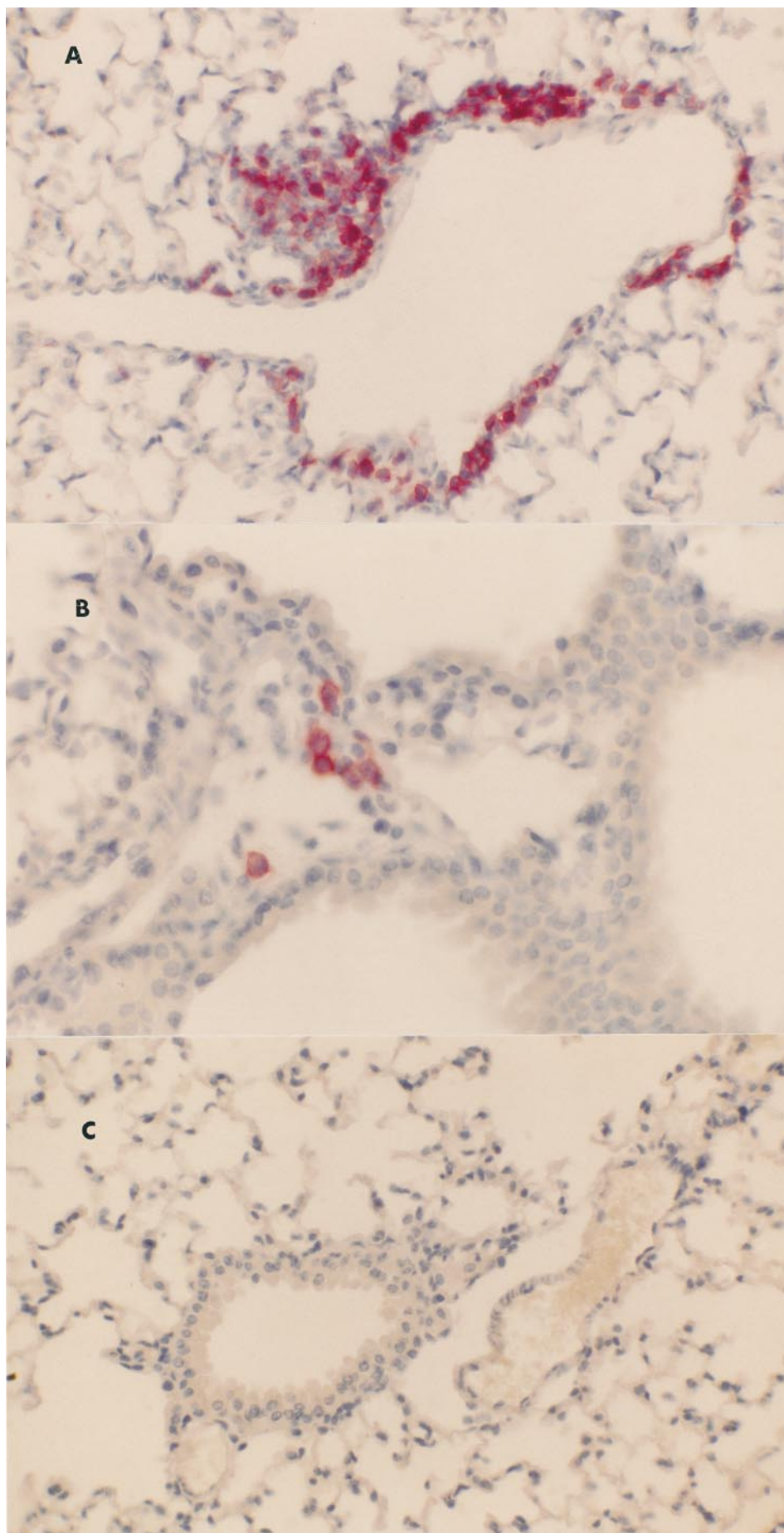
The BALF of these mice was mainly composed of macrophages and no eosinophils were detected (data not shown).

DISCUSSION

In this study, we showed that SCID mice reconstituted with PBMC from patients sensitive to Dpt, and exposed to allergen aerosols, developed AHR in conjunction with production of IgE and airway inflammation when compared with control groups. Several mechanisms could explain the increase in airway responsiveness.

AHR might be due to direct Dpt effect on the bronchial structures. First, the Dpt major allergen, Der p 1, is known to express a thiol-protease enzymatic activity (22), which has the capacity to increase bronchial epithelium permeability *in vitro* (23) and to promote a Th2-type response (24). Second, allergenic extracts used for Dpt inhalation contain variable amount of endotoxin able to nonspecifically stimulate airways. However, AHR was not significantly different between nonreconstituted and nonexposed SCID mice and nonreconstituted SCID mice exposed to Dpt aerosols. Moreover, in the absence of related allergen it has already been shown that both IgE response and human cell infiltrates were lacking (12, 25). Consequently, AHR developed by allergic hu-SCID mice appears not to be induced by the inhalation itself, neither by the direct contact between murine airways and Dpt allergen components.

AHR could be linked to IgE production. Airway responsiveness was not significantly different between nonallergic SCID mice and nonreconstituted SCID mice exposed to Dpt aerosol but was highly different between allergic and nonallergic SCID mice exposed to Dpt aerosol. Nonallergic SCID mice might produce total human IgE after some delay. In previous studies (12, 13), nonallergic SCID mice did not produce any human IgE. In the present work, SCID mice were treated with ASGM1 antibody before reconstitution. ASGM1 treatment increases human cell engraftment, and correlation was demonstrated between human IgE produced in reconstituted SCID mice and total IgE level of the donor, even if the donor level was low (25). However, we were unable to demonstrate Dpt-specific IgE in nonallergic SCID mice contrary to allergic hu-SCID mice. This result is consistent with those we have previously obtained (12). Consequently, human IgE production in nonallergic SCID mice appears to result from a non-specific B-cell activation. As no significant difference was observed in airway responsiveness between nonallergic and nonreconstituted SCID mice, we can hypothesize that the nonspecific activation of human cells in SCID mice alone, or human IgE production alone, was not sufficient to induce AHR as suggested previously (26). However, AHR might be dependent on specific IgE. In a model of ovalbumin (OVA)-sensitized mice, IgE was demonstrated to be necessary to the development of AHR. B-cell-deficient mice sensitized to OVA did not produce specific IgE and did not develop AHR contrary to wild type mice. AHR was restored in B-cell-deficient mice by passive sensitization with anti-OVA-specific IgE during antigenic challenge (27). Moreover, anti-IgE antibody injection to OVA-sensitized mice inhibits eosinophil infiltra-



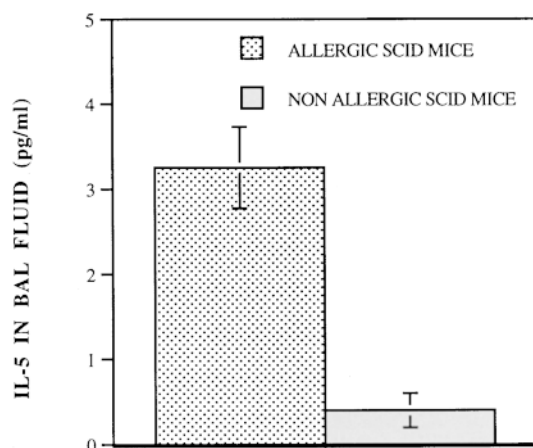


Figure 5. Presence of human IL-5 in BALF of allergic and nonallergic hu-SCID mice. Human IL-5 was evaluated by specific ELISA in BALF at Day 35 in allergic SCID mice ($n = 13$) and in nonallergic SCID mice ($n = 8$). The sensitivity of the ELISA is 0.5 pg/ml. Concentrations in the two groups are significantly different ($p = 0.0117$) according to the Wilcoxon test.

tion and AHR (28). In our work we have demonstrated Dpt-specific human IgE only in allergic hu-SCID mice, and only allergic hu-SCID mice were able to develop AHR. Moreover, PD_{50} RL values at Day 35 tended to be inversely correlated with total human IgE measured at Day 7 and Day 15 in allergic hu-SCID mice. There are at least two explanations. First, IgE may be involved in AHR development, and AHR may thus be a consequence of cellular activation induced by specific IgE. Second, IgE and AHR may only be a reflection of the same cell activation. Passive immunization with human specific IgE or B-cell depletion prior to reconstitution should permit further elucidation of this question.

Because airway responsiveness may be related to airway inflammation, we evaluated the role of human cell infiltration into the lungs of allergic hu-SCID mice in the development of AHR. Differences between the lungs of allergic and nonallergic hu-SCID mice are less evident than in our previous studies (12, 13). However, previous studies were carried out at Day 60 after the Dpt inhalation (75 d after the reconstitution) whereas in this work lung sections were analyzed at Day 35 after the Dpt inhalation (39 d after the reconstitution). Moreover, Dpt was injected intraperitoneally at the time of reconstitution in contrast to previous studies where Dpt was introduced in SCID mice only by aerosol (12, 13). This new protocol may limit the human cell migration from the peritoneum toward lungs but nonetheless the human cell might be more activated. Finally, we did not find any correlation between AHR represented by PD_{50} RL measurement and airway inflammation evaluated by $CD45^+$ cell histological grade criteria. Another explanation for this observation might be that human cell infiltration detected in allergic hu-SCID mice was predominantly perivascular rather than peribronchial. Thus, it appears difficult to correlate a bronchial phenomenon (AHR)

with the preferential migration to the perivascular zone. In addition, the type of mediators released by human cells might be more crucial in the development of AHR than the precise number of human cells infiltrating the lungs of allergic hu-SCID mice.

In order to understand the mechanisms of AHR in allergic hu-SCID mice, we evaluated in BALF the concentrations of different cytokines mainly IL-4 and IL-5 whose involvement in bronchial hyperresponsiveness remains controversial (29–35). Interestingly, whereas IL-4 was undetectable, only low concentrations of human IL-5 were detected in BALF of allergic hu-SCID mice but were correlated with the AHR. In the BALF as well as in the lung tissue of hu-SCID mice no eosinophils could be seen. Moreover, mRNA encoding for IL-5 was always detected in lungs of allergic hu-SCID mice exhibiting AHR in contrast to IL-4 (data not shown). Thus, in this model of hu-SCID mice the participation of eosinophils in the development of AHR seems unlikely, and the true participation of IL-5 requires additional experiments. The effect of antibody directed against IL-5 is under investigation. Nonetheless, though in several models the role of Th2 cytokines in the airways is described as critical to the airway inflammation and AHR, some reports do not support this hypothesis (31–35). Irrespective of the presence of eosinophils, the AHR might be caused by the presence, in and around the airways, of activated T cells whose ability to synthesize a mixture of cytokines depends on the experimental conditions (31). Indeed, by using IL-5-deficient (–/–) or IL-5-sufficient (+/+) mice exposed to OVA it was reported that circulating but not local lung IL-5 is required for the development of antigen-induced eosinophilia (32). In humans dissociation between airway inflammation and AHR in allergic asthma has also been reported; indeed no significant correlation was found between the degree of airway responsiveness and the number of inflammatory cells in sputum or BAL or bronchial biopsy (33). Recently it was shown that after nematode infection eosinophils are not required to induce AHR (34). All these data could partially explain the development of the AHR in humanized allergic SCID mice in absence of eosinophilic lung inflammation. The participation of others factors can not be excluded because it was reported that AHR could be induced without the involvement of Th2 cytokines (35).

In conclusion, we have demonstrated for the first time that only exposure to Dpt aerosol of SCID mice reconstituted with PBMC from Dpt-sensitive patients induces AHR. Consequently, after allergen inhalation, the allergic hu-SCID mice develop three main features of allergic asthma: IgE production, human cell pulmonary infiltrate, and AHR. Such a model could be considered as a potential tool for studying mechanisms involved in the development of allergic asthma.

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Figure 4. Detection of $CD45^+$ human cells in allergic or nonallergic SCID mice exposed to Dpt aerosol. Immunohistochemistry with anti- $CD45$ monoclonal antibody on lung sections counterstained with hematoxylin evidenced human cell infiltrate in allergic hu-SCID mice in perivascular areas (A). In 1 of 12 animals, a few human cells were observed in peribronchial areas (B). On the contrary, very few or no human cells were detected in nonallergic hu-SCID (C).

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