

Increased airway responsiveness, allergy-type-I skin responses and systemic anaphylaxis in a humanized-severe combined immuno-deficiency mouse model

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Summary

Background In patients with allergic bronchial asthma, a strong relationship between elevated serum IgE antibody titres and the development of increased airway responsiveness (AR) has been demonstrated. To further elucidate the relationship between human (hu) IgE and development of increased AR, we developed an *in vivo* model utilizing immuno-compromised severe combined immuno-deficiency (SCID) mice.

Methods SCID mice were either reconstituted with peripheral blood mononuclear cells (PBMC) from non-atopic, healthy or atopic individuals sensitized against house dust mite allergen (Der p), or passively sensitized with plasma from non-atopic, healthy or atopic individuals.

Results In both systems, atopic hu-SCID mice developed increased AR. The following results suggest that these responses were mediated via IgE antibodies: increased AR did not occur after transfer of either PBMC or IgE-negative plasma from non-atopic individuals; increased AR occurred simultaneous with increased serotonin release detected 15 min after allergen-aerosol challenge in bronchoalveolar lavage fluid; and increased AR required at least two allergen-aerosol challenges. SCID mice reconstituted with serum containing anti-Der p IgE antibodies developed positive immediate-type skin test responses to intradermal injection of Der p as well as anti-hu-IgE antibody. In addition, IgE binding to skin mast cells was demonstrated by immunohistochemistry. Furthermore, intravenous challenge of hu anti-Der p positive SCID mice with Der p resulted in systemic anaphylaxis.

Conclusion These data provide evidence that passive immunization of SCID mice with hu IgE alters AR and that T cells and eosinophils were not a requirement for the development of increased AR in this model.

Keywords hu-SCID, IgE, immediate-type hypersensitivity

Submitted 9 January 2003; revised 10 October 2003; accepted 3 November 2003

Introduction

Allergic bronchial asthma is characterized by altered airway responsiveness (AR) influx of eosinophils and T cells bearing the T helper type 2 (Th2) phenotype into the airways, as well as elevated levels of IgE antibody titres directed against environmental allergens [1–3]. One important question in the pathogenesis of allergic bronchial asthma is the relationship between IgE, airway inflammation and development of increased AR. Airway inflammation, especially of the Th2 phenotype and eosinophilia are thought to be critical for the development of increased AR. This hypothesis is based on the significant association of airway inflammation and increased AR in numerous animal models of asthma using conventional and genetically modified mice. Altered AR has been observed

in the presence as well as in the absence of IgE [4–6] eosinophilia, [7] airway inflammation [8], T cell inflammation [9], IL-4 [10–12] IL-5 [6, 13] and IL-13 [14]. Treatment of asthmatic patients with monoclonal anti-IL-5 antibodies resulted in markedly diminished nasal and peripheral blood eosinophilia. However, increased AR and asthma score were not altered by the treatment. This important clinical observation again raised the question about the relationship of eosinophilia and asthma symptoms. The purpose of this study was to assess the role of human (hu)-derived components in the development of increased AR under *in vivo* conditions using a hu-mouse chimera system.

Several experimental strategies have been employed to assess the contribution of hu IgE and airway inflammation to the development of altered AR. One approach represents passive sensitization of isolated hu or rabbit airway smooth muscle via incubation of the tissue with serum from allergic patients. This sensitization procedure alters the mechanical responses of hu isolated airways to allergen and anti-IgE, and also to non-specific broncho-constrictor agents such as

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histamine and neuropeptides [15–19]. In these studies, serum from allergic patients was selected based on elevation of total and/or allergen-specific IgE. Thus, the IgE molecule has been considered to be the serum factor responsible for the altered AR. However, recent experimental data from Watson et al. [20] and the group of Rabe et al. [21] suggest that passive sensitization-induced hyper-responsiveness is not always directly related to IgE.

We and others developed an alternative strategy in which the reconstitution of severe combined immuno-deficiency (SCID) mice with hu peripheral blood mononuclear cells (PBMC) results in hu-mouse chimera with a functional hu antigen-reactive immune system [22,23]. SCID mice engrafted with hu peripheral blood lymphocytes derived from atopic patients, and exposed to allergen aerosol develop a specific hu IgE response. In this model, the production of hu total and allergen-specific IgE depended on: (i) transfer of atopic PBMC (ii) allergen boost, (iii) presence of CD14⁺ antigen-presenting cells and (iv) IL-4 [22]. Hu-PBMC-SCID mice challenged with allergen-aerosol repeatedly develop airway inflammation [24]. This model was employed to further study the relationship between IgE and AR.

Materials and methods

Animals

SCID mice (C.B-17 *scid/scid*) were obtained from Bomholtgard (Ry, Denmark) and maintained under pathogen-free conditions. Animals between 8 and 12 weeks of age were screened for leakage by measurement of mouse IgG antibodies in the serum before the onset of experiments.

Preparation of PBMC

Atopic patients sensitized to house dust mite allergen (Der p) were identified by positive skin prick test (SPT) reactions and elevated serum IgE antibody titres. SPTs were performed with commercial test solutions obtained from ALK-Scherax (Hamburg, Germany). Hu serum IgE antibody titres were measured by fluorescence enzyme immuno-assay (FEIA) (Pharmacia CAP System, Pharmacia, Uppsala, Sweden). Atopic patients responded to Der p with a weal ≥ 10 mm in diameter and antibody titres of at least 410 ng/mL for total IgE and at least 20 ng/mL for anti-Der p IgE. Non-atopic volunteers showed total serum IgE concentrations of ≤ 17 ng/mL, anti-Der p IgE of ≤ 0.8 ng/mL and they had negative skin test reactions to Der p. Heparinized blood (200–250 mL) was collected from atopic and non-atopic donors and mononuclear cells were purified from heparinized blood by LymphoprepTM (Nycomed, Oslo, Norway) density gradient centrifugation as previously described [25].

Reconstitution of SCID mice with hu PBMC

SCID mice were reconstituted as previously described [22]. Briefly, SCID mice were injected intraperitoneally (i.p.) with 9×10^7 PBMC, together with 100 μ g purified house dust mite (HDM) extract referred to as Der p (ALK-Scherax). The extract was calibrated based on the amount of Der p 1 and

diluted in phosphate-buffered saline (PBS). One hundred microgram of the extract contained 5 μ g Der p 1. Eight days later, SCID mice received an additional i.p. allergen boost (100 μ g Der p per injection). For the aerosol-allergen challenge, 100 μ g Der p was diluted in 7 mL PBS. Aerosolization was carried out for 20 min on days 26 and 27 after cell transfer as previously described [26]. AR was assessed on day 28.

Reconstitution of SCID mice with plasma and allergen challenge

SCID mice received three i.p. injections of 200 μ L hu plasma on days 1, 4 and 8, derived from donors sensitized or non-sensitized to Der p. On days 9 and 10, all SCID mice were challenged via the airways by aerosolization of HDM extract referred to as Der p (ALK-Scherax) or with PBS alone.

Measurement of hu IgE and IgG antibodies

The amount of total and allergen-specific hu-IgE antibody titres was determined by FEIA (Pharmacia CAP System). Hu-IgG antibody titres were determined by ELISA. Ninety-six-well microtitre plates (Greiner, Nürtingen, Germany) were coated with anti-hu IgG (Binding Site, Heidelberg, Germany) or anti-hu IgG1 (Dianova, Hamburg, Germany) diluted 1:100 in PBS and incubated overnight at 4 °C. After incubation, plates were washed (0.05% Tween 20/PBS) and blocked with 2% BSA/PBS (w/v) for 4 h at 37 °C. Diluted serum samples and hu Ig standard were added in duplicate and the plates were incubated overnight at 4 °C. After the plates were washed, biotin-conjugated goat-anti-hu IgG or IgG1 was added and incubated for 4 h at 37 °C. After incubation with Streptavidin solution (1:500), phosphatase substrate (Sigma, Deisenhofen, Germany) was added. Microtitre plates were read in an ELISA reader (Dynatech Laboratories, Denkendorf, Germany) at 405 nm. The amounts of Ig were calculated according to standard curves using an RMS computer program (Dynatech Laboratories).

Assessment of immediate-type cutaneous hypersensitivity (ICHS) responses and systemic anaphylaxis

Intracutaneous testing for ICHS responses was performed as previously described [26]. Briefly, before skin testing, 150 μ L of 1% Evans blue dye solution was injected intravenously (i.v.). After 15 min, the abdominal skin was shaved and 50 μ L of test solution was injected intradermally (i.d.). PBS was used as a negative and Compound 48/80 (5 μ g/mL) (Sigma) as a positive control. Skin tests were performed with 15 μ g of Der p extract (ALK-Scherax), anti-hu IgE (Vector Laboratories, Burlingame, CA, USA) or anti-hu IgG4 (Binding Site). After 15 min, vascular leakage was assessed on the inverted skin and scored as positive if the flare reaction was >0.3 cm.

To assess systemic anaphylaxis 100 μ g Der p extract (ALK-Scherax) and Evans blue dye (1%) were injected i.v. in a volume of 150 μ L. Fifteen minutes after allergen/dye injection, mice were killed by cervical dislocation and foot pads were examined for blue colour.

Immunohistological analysis

Skin from SCID mice was covered with an embedding medium, snap-frozen in liquid nitrogen and stored at -80°C . For double visualization of hu IgE and mast cells, cryostat sections ($15\mu\text{m}$) were fixed in acetone and then incubated with rat-anti-hu IgE (BioSource, Camarillo, CA, USA) for 2 h at room temperature in a humid chamber, followed by incubation for 30 min at 37°C with tetra methyl rhodamine isothiocyanate (TRIC)-conjugated F(ab)_2 fragments of goat-anti-rat IgG (Jackson ImmunoResearch, West Grove, PA, USA) at a dilution of 1:200. For the identification of skin mast cells, sections were incubated with the fluorescein isothiocyanate (FITC)-labelled avidin (Vector) diluted 1:1000 in 0.1 M Tris-buffer (TBS), pH 7.4, for 1 h at room temperature [27]. After every incubation, the sections were rinsed in TBS three times, 5 min each. Sections were examined at $\times 400$ magnification under a Zeiss fluorescence microscope. The spleen and lung were prepared and fixed in 4% formaldehyde. Three micrometre paraffin-embedded sections were stained with haematoxylin and eosin (HE) or incubated with normal mouse serum followed by incubation with monoclonal antibodies directed either against hu T lymphocytes (M-742, 1:100, Dako, Hamburg, Germany) or against hu B lymphocytes (M-754, 1:20, Dako) for 30 min at 37°C . Immunostaining was performed by the APAAP technique. Negative controls using Tris-buffer instead of antibodies were free from immunolabelling.

Assessment of AR

Airway smooth muscle responsiveness was assessed by electrical field stimulation (EFS) as previously described [26]. The frequency causing 50% of the maximum contraction was calculated from logarithmic plots of the contractile response vs. the frequency of EFS, and was expressed as ES_{50} .

Bronchoalveolar lavage (BAL) and cell differentiation

Bronchoalveolar lavage (BAL) and cell differentiation were performed as previously described [26]. Briefly, animals were killed immediately after the last airway allergen challenge, the trachea cannulated and BAL performed by two lavages with 0.8 mL ice-cold PBS as described above. A similar volume of BAL fluid ($1.4 \pm 0.2\text{ mL}$) could be recovered from each study group. The BAL fluids were centrifuged and cell-free supernatants were frozen at -20°C .

Determination of mediators in cell free BAL fluid

The serotonin content in BAL fluids was measured 15 min after airway allergen challenge by commercial ELISA according to the manufacturer's recommendation (RD Systems, Wiesbaden, Germany). Murine IL-4, IL-5 and IFN- γ levels were measured by ELISA as previously described [26]. Sensitivities were 40 pg/mL for IL-4, 70 pg/mL for IL-5 and 100 pg/mL for IFN- γ . Hu IL-4, IL-5 and IFN- γ were measured by ELISA as previously described [25]. Sensitivities were 10 pg/mL for IL-4, 30 pg/mL for IL-5 and 12 pg/mL for IFN- γ .

Statistical analysis

The results are presented as mean values $\pm$ SD, unless otherwise stated. The Mann-Whitney U -test was used to determine the level of difference between animal groups. There, appropriate correction for multiple comparison was performed.

Results

The hu-PBMC SCID mouse is a model for increased AR

We have previously demonstrated that SCID mice engrafted with hu PBMC from atopic donors sensitized to Der p produce total and anti-Der p-specific hu-IgE antibodies after boosting [22]. Based on these results, we have now analysed whether hu-PBMC-SCID mice also develop increased AR after airway allergen challenge.

Increased AR was detected in hu-PBMC-SCID mice reconstituted with PBMC from atopic, as indicated by a significant ($P < 0.01$) decrease in ES_{50} values (EFS), causing a half-maximal constriction of tracheal smooth muscle segments (Fig. 1). In contrast, AR in hu-PBMC-SCID mice that received PBMC from healthy donors remained unchanged.

Hu PBMC from atopic patients migrate in secondary lymphoid tissues of SCID mice

To examine the *in vivo* distribution of hu PBMC in SCID mice, sections from secondary lymphoid tissues were

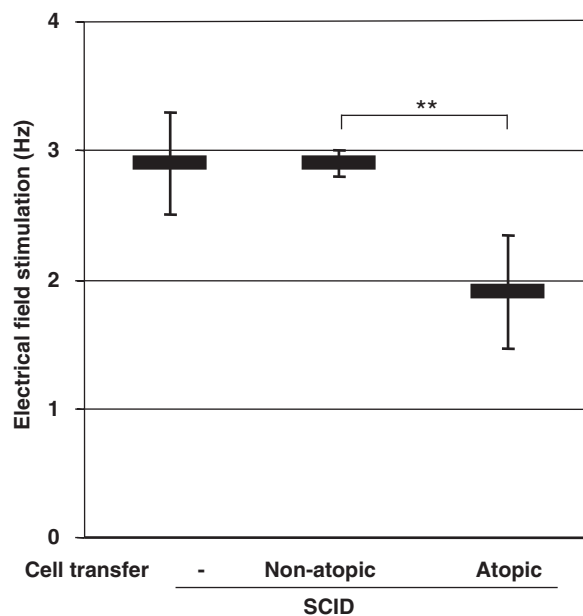


Fig. 1. Assessment of airway reactivity in hu-PBMC-SCID mice. SCID mice were reconstituted with PBMC from healthy, non-atopic ($n = 4$) or atopic ($n = 5$) donors as described in Materials and methods. All animals received two consecutive allergen challenges via the airways at the end of the protocol. Mean $\pm$ SEM stimulation frequencies (Hz) that resulted in 50% of maximal contractility are indicated. Statistical significance ($P \leq 0.01$) is indicated by (**). Selected mice received PBS aerosol. ES_{50} values of the animals were not different from unexposed controls. Hu, human; PBMC, peripheral blood mononuclear cells; SCID, severe combined immunodeficiency; SEM, standard error of the mean; PBS, phosphate-buffered saline.

immunostained with monoclonal antibodies directed against cell surface markers of hu T and B cells. Hu CD3 positive cells were detected in the spleen, hilus lymph node and lung periphery (Fig. 2). Only a few individual cells stained with B cell-specific antibodies (data not shown). Hu T cells appeared to cluster particularly in peripheral lymphoid tissue. Control stainings with irrelevant antibodies and the staining of sections from SCID mice that did not receive any hu lymphocytes indicated the specificity of the staining pattern. Hu CD3 positive T cells were also detected in recipients of hu non-atopic PBMC (data not shown).

Passive sensitized SCID mice develop increased AR after allergen–aerosol challenges

To assess whether allergen-specific IgE is sufficient to trigger the development of AR, SCID mice were reconstituted only with plasma from atopic donors (referred to as 'hu-atopic' SCID mice). Controls were reconstituted with IgE-negative plasma from non-atopics (referred as 'hu-non-atopic' SCID mice) containing no anti-Der p IgE antibodies. After repeated injections, the relative distribution of total and allergen-specific IgE and IgG antibodies mirrored the relative distribution of respective antibodies in the plasma of the donors. Both non-atopic and atopic plasma as well as serum from, respectively, substituted SCID mice always contained anti-Der p IgG and, particularly, anti-Der p IgG4 antibodies (Table 1).

Hu-non-atopic and hu-atopic SCID mice were locally challenged with Der p aerosol, and AR was measured by EFS of the tracheal smooth muscle segments. Non-manipulated SCID mice and airway allergen challenged SCID mice showed a comparable airway response, as reflected by ES_{50} values of 3.0 ± 0.2 and 2.9 ± 0.3 Hz, respectively. In the absence of airway allergen challenges, reconstituted hu-non-atopic and hu-atopic SCID mice responded with the same degree of AR as indicated by ES_{50} values of 2.8 ± 0.4 and 2.9 ± 0.6 Hz, respectively (Fig. 3).

A different pattern was observed following allergen challenge. When hu-atopic SCID mice received two consecutive airway allergen challenges, these animals developed increased AR as indicated by a decrease of the ES_{50} value to 1.7 ± 0.2 Hz ($P < 0.01$). In contrast, hu-non-atopic but airway allergen challenged SCID mice showed normal AR values as indicated by an ES_{50} of 2.6 ± 0.4 Hz (Fig. 3). These data indicate that passively sensitized hu-atopic SCID mice develop increased AR after allergen–aerosol challenges.

Hu-atopic SCID mice develop increased AR in the absence of airway inflammation

To determine whether increased AR in hu-atopic SCID mice is accompanied by lung inflammation, the cellular distribution in BAL fluids was analysed. The total cell number in hu-atopic and airway allergen challenged SCID mice did not

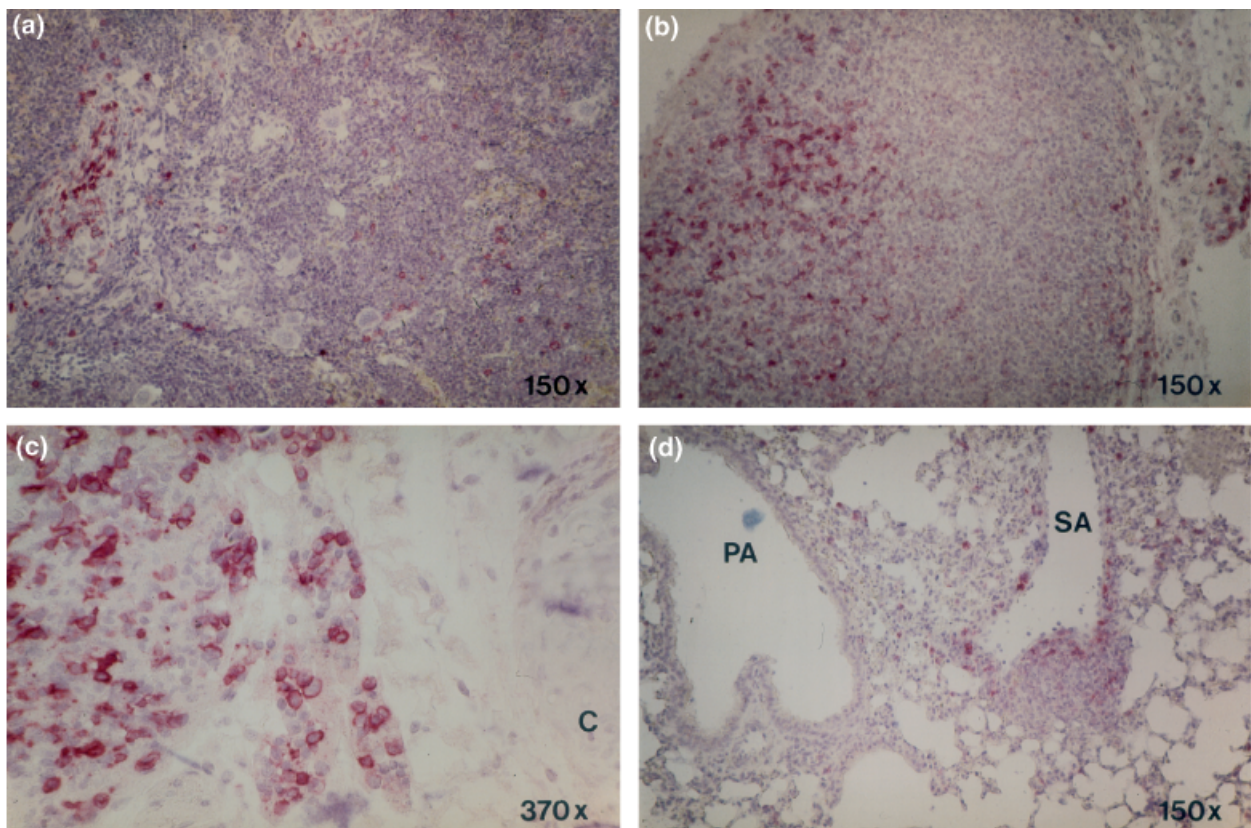


Fig. 2. Detection of human PBMC in lymph node and lung in hu-PBMC-SCID mice. SCID mice were reconstituted with PBMC from an atopic donor as described in Materials and methods. On day 28, animals were killed and 3 μ m sections from secondary lymphoid tissues were immunostained with anti-hu T cell specific antibodies. Hu T cells were detected in the spleen (a), hilus lymph node (b), upper bronchial system (indicated by the presence of cartilage (c)) (c) and in the lung periphery (d) with terminal bronchiolus (PA) and pulmonary artery (SA). The specificity of immunostaining was determined as described in Materials and methods. Hu, human; PBMC, peripheral blood mononuclear cells; SCID, severe combined immuno-deficiency.

Table 1. Hu serum antibody titres in reconstituted SCID mice

	Hu antibody titres in SCID mice			
	anti-Der p			
	Total IgE (ng/mL)	IgE (ng/mL)	IgG (μ g/mL)	IgG4 (%*)
Non-atopic SCID	≤ 0.8	≤ 0.8	5 ± 4	2.0 ± 0.1
Atopic SCID	641 ± 25	75 ± 11	11 ± 1	2.0 ± 0.1

SCID mice were reconstituted with human immunoglobulins by i.p. injection of 200 μ L plasma from one atopic or one non-atopic healthy volunteer as indicated. Hu-IgE antibody titres were examined by FEIA technique as described in Materials and methods. Hu-IgG antibody titres were examined by ELISA technique as described in Materials and methods. Hu, human; SCID, severe combined immuno-deficiency; i.p., intraperitoneal; Der p, house dust mite allergen.

*Anti-Der p IgG4 antibody titre is expressed in percentage compared to an anti-birch pollen IgG4 standard. Mean $\pm$ SD from one out of three experiments are indicated.

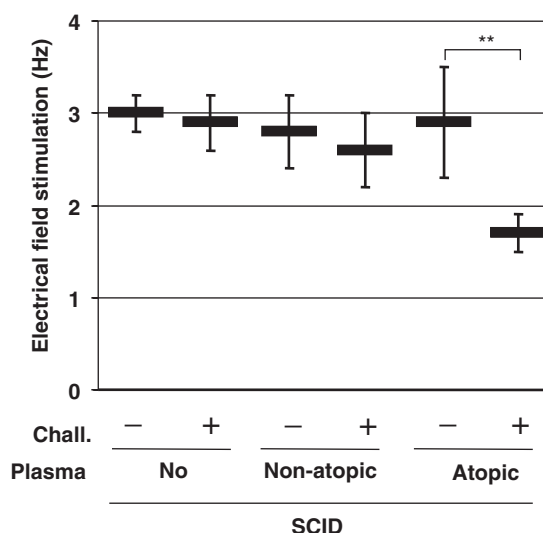


Fig. 3. Assessment of airway reactivity in passively sensitized SCID mice. SCID mice were reconstituted with human immunoglobulins by i.p. injection of plasma derived PBMC from healthy, non-atopic ($n = 8$) or atopic ($n = 14$) donors as described in Materials and methods. As indicated, some mice received two consecutive allergen challenges via the airways. Mean $\pm$ SEM stimulation frequencies (Hz) that resulted in 50% of maximal contractility are indicated. Statistical significance ($P \leq 0.01$) is indicated by (**). ES₅₀ values from PBS exposed mice did not differ from unexposed controls (data not shown). SCID, severe combined immuno-deficiency; i.p., intraperitoneal; PBMC, peripheral blood mononuclear cells; SEM, standard error of the mean; PBS, phosphate-buffered saline.

significantly differ between study groups. Under all conditions, almost all cells were identified as monocytes ($\geq 95\%$) and neither lymphocytes nor eosinophils could be recovered (Table 2). In addition, levels of murine IL-4, IL-5 or IFN- γ production in BAL fluids did not differ significantly ($P > 0.05$) from those of non-challenged SCID mice (data not shown). These data indicate that development of increased AR in hu-atopic SCID mice occurred in the absence of airway inflammation.

Development of increased AR in hu-atopic SCID mice is paralleled by serotonin release in the lung

Serotonin was measured in BAL fluid from airway allergen challenged mice, since it represents an important mediator of

Table 2. Cellular distribution of BAL lavage in reconstituted SCID mice

	Cells/BAL ($\times 10^3$)	
	Control	Atopic
*Cells/BAL ($\times 10^3$)		
Total cells	122 ± 37	215 ± 94
Lymphocytes	3 ± 2	≤ 2
Eosinophils	≤ 1	≤ 1
Neutrophils	≤ 1	≤ 1
Monocytes	119 ± 36	210 ± 94

SCID mice (atopic $n = 6$) were reconstituted with human plasma as described in Materials and methods. *Absolute numbers of leucocytes in BAL fluid/mouse were determined according to standard morphology. BAL, bronchoalveolar lavage; SCID, severe combined immuno-deficiency.

the immediate-type 1 response. Compared with SCID mice that received allergen-aerosol challenges alone and with hu-non-atopic SCID mice, serotonin levels were significantly ($P < 0.05$) elevated in hu-atopic SCID mice 15 min after allergen-aerosol challenges (Table 3). This result indicates that in hu-atopic SCID mice, the development of increased AR was associated with immediate release of serotonin after allergen-aerosol challenges.

Hu-atopic SCID mice develop ICHS reactions and systemic anaphylaxis after allergen challenge

Skin tests for immediate-type hypersensitivity reactions were performed to evaluate the functional relevance of allergen-specific IgE antibodies in SCID mice. Atopic- and non-atopic-SCID mice developed positive skin test reactions to compound 48/80, a non-specific mast cell degranulator, indicating that mast cell reactivity is not altered in SCID mice. After i.d. injection of Der p, 80% of the atopic SCID mice developed positive ICHS reactions (Table 4). After i.d. injection of anti-hu IgE, 36% of the atopic SCID mice developed positive ICHS reactions, whereas no positive response could be detected in non-atopic SCID mice. Anti-hu IgG4 did not elicit positive skin test reactions. Increased vascular permeability, induced by vasoactive mast cell mediators, is a hallmark of systemic anaphylaxis.

Table 3. Determination of serotonin and cytokine release in BAL lavage in reconstituted SCID mice

Plasma	<i>n</i>	Serotonin (ng/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)	IFN- γ (pg/mL)
No	5	1.43 ± 0.57	≤ 40	≤ 70	≤ 100
Non-atopic	6	1.67 ± 1.45	≤ 40	≤ 70	≤ 100
Atopic	6	$2.36 \pm 0.63^*$	≤ 40	≤ 70	≤ 100

SCID were reconstituted with human immunoglobulins by i.p. injection of 200 μ L plasma from atopic or non-atopic healthy volunteers as indicated. Control animals received i.p. 200 μ L PBS. All animals received two airway allergen challenges by aerosolization 48 and 24 h prior to analysis. Fifteen minutes after the last airway allergen challenge, BAL fluid was recovered and serotonin, IL-4, IL-5 and IFN- γ content was measured by ELISA as described in Materials and methods.

*Statistical significance ($P \leq 0.05$) between PBS injected and Der p challenged SCID mice and atopic and Der p challenged SCID mice. BAL, bronchoalveolar lavage; SCID, severe combined immuno-deficiency; hu, human; i.p., intraperitoneally; PBS, phosphate-buffered saline; Der p, house dust mite allergen.

Table 4. ICHS reactions in reconstituted SCID mice

Test solution	Non-atopic		Atopic	
	N	%	n	%
Der p	1/7	14	8/10	80
Anti-hu IgE	0/15	0	5/14	36
Anti-hu IgG4	0/4	0	0/6	0

SCID mice were reconstituted with human serum from atopic or non-atopic healthy volunteers as indicated. Intracutaneous skin testing was performed with Der p, anti-hu IgE and anti-hu IgG4 as described in Materials and methods. All solutions were i.d. injected in a volume of 50 µL. Numbers and percentage of mice that developed weals ≥ 3 mm are indicated. All animals developed positive ICHS reactions after i.d. injection of Compound 48/80 (5 µg/mL), whereas no reaction was observed after i.d. injection of PBS. ICHS, immediate-type cutaneous hypersensitivity; hu, human; SCID, severe combined immuno-deficiency; Der p, house dust mite allergen; i.d., intradermally; PBS, phosphate-buffered saline.

To test for development of systemic anaphylaxis, non-atopic ($n = 3$) and atopic ($n = 3$) SCID mice were i.v. challenged with Der p. To assess vascular leakage, Evans blue dye was co-administered at the same time. Plasma extravasation was assessed by examining the food pads 15 min after allergen exposure. A marked bluing of the feet was observed in all atopic- but not in the non-atopic-SCID mice (Fig. 4).

Hu IgE binds to the surface of murine mast cells

Since IgE-positive but not IgE-negative SCID mice developed positive ICHS reactions to Der p (Table 4), it was examined whether hu IgE could bind to cutaneous mast cells. Skin sections derived from atopic and non-atopic SCID mice were immunostained with an antibody directed against hu IgE, and mast cells were visualized by counterstaining with FITC-avidin. As illustrated in Fig. 5, hu IgE was detected on the surface of mast cells in atopic SCID mice (a, c), whereas immunostaining with anti-hu IgE of mast cells in non-atopic SCID skin remained negative (b, d).

Discussion

Allergic bronchial asthma is defined by altered AR to inhaled allergens, chronic inflammation of the airways with eosinophilia and elevated levels of total and allergen-specific IgE serum antibody titres [28]. In a subset of allergic asthmatic patients, however, altered AR occurs without any signs of lung inflammation, and in cases of intrinsic asthma, increased AR occurs in the absence of IgE. Based on these clinical phenotypes, it is important to assess the pathophysiological relationship between IgE, airway inflammation and the development of increased AR.

The SCID mouse has provided a useful model for analysing hu immune functions *in vivo* [29–34]. We have previously shown that after injection of hu PBMC from atopic patients sensitized to Der p into the peritoneal cavity of SCID mice, there was a marked increase in total IgE and an allergen-specific hu-IgE antibody response was induced [22]. In this study, the immunohistological analysis of major lymphoid tissues revealed that the transferred hu lymphocytes bear the

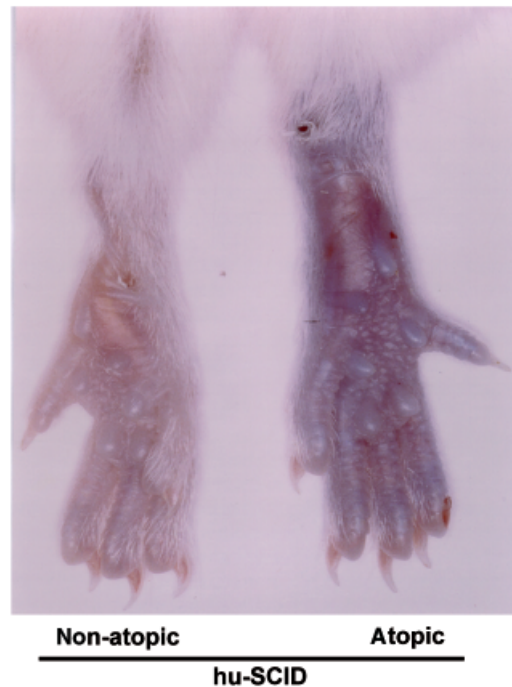


Fig. 4. Development of systemic anaphylaxis in passively sensitized SCID mice. Plasma extravasation during anaphylaxis. The feet of non-atopic and atopic SCID mice 15 min after i.v. injection of Der p in the presence of Evans blue dye. One representative example of non-atopic ($n = 3$) and atopic ($n = 3$) SCID mice is shown. SCID, severe combined immuno-deficiency; i.v., intravenously; Der p, house dust mite allergen.

functional capacity to leave the peritoneal cavity and to harbour actively in various lymphoid organs, including spleen and lymph nodes. Duez et al. [24] have demonstrated that SCID mice engrafted with PBMC from atopics sensitized against Der p develop an intense pulmonary infiltrate of hu cells only after repeated Der p airway allergen challenges.

In our model, we have extended this observation by analysing the development of increased AR. Here, we demonstrate that SCID mice reconstituted either with hu PBMC from allergen-sensitized patients or with serum from atopic patients elicit immediate-type hypersensitivity reactions in the skin and in the lung. This response has the following characteristics: (I) after i.d. injection of the allergen, only atopic SCID mice develop positive ICHS reactions. Even in the presence of allergen-specific hu IgG and anti IgG4 antibodies, no ICHS reactions were observed in non-atopic SCID. (II) Immunohistology revealed that hu IgE has the capacity to bind to skin mast cells. (III) Anti-hu IgE, but not IgG4 antibodies could trigger immediate-type skin test responses in a subset of hu-atopic SCID mice. (IV) Airway allergen challenges resulted in an immediate serotonin release in atopic, but not in non-atopic SCID mice. (V) Two consecutive airway allergen challenges were required to trigger the development of increased AR in these mice, and vice versa, in the absence of airway allergen challenges, AR remained normal in SCID mice, regardless of whether they were reconstituted with atopic PBMC or with atopic plasma. This result confirms the result obtained from the assessment of ICHS reactions. The most likely mechanism underlying these immediate-type hypersensitivity reactions is that, in the presence of allergens, hu IgE is cross-linked on the surface of

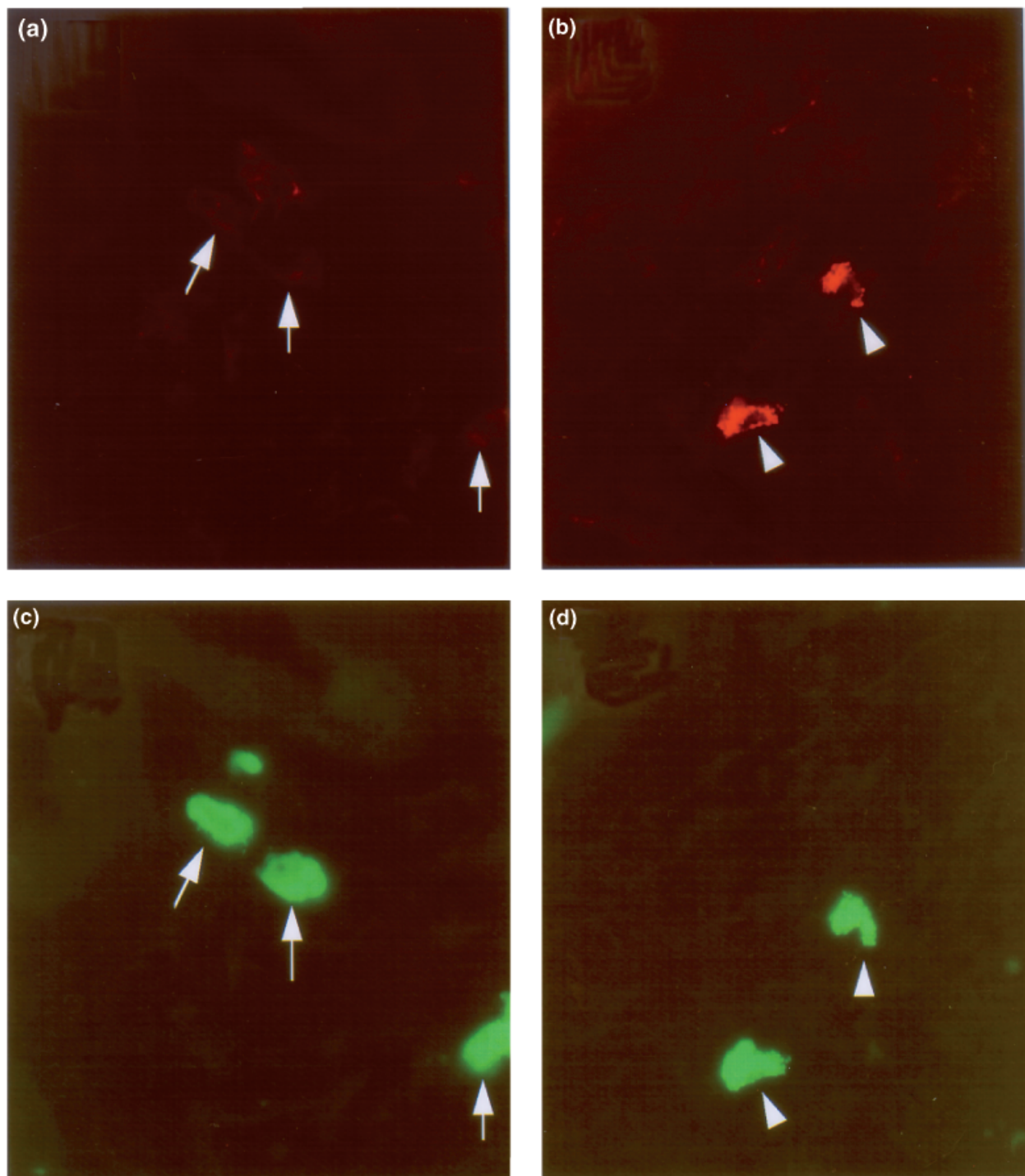


Fig. 5. Detection of human IgE on the surface of murine mast cells. Skin sections from SCID mice reconstituted either with non-atopic (a, b) or atopic (c, d) plasma. Skin sections were incubated with rat anti-hu IgE followed by goat-anti-rat TRITC-conjugated IgG (a, c) and in addition with avidin-FITC interacting with heparin mast cell granules (b, d) as described in Materials and methods. Mast cells, visualized by avidin-FITC staining, did not show anti-hu-IgE immunoreactivity in skin sections derived from non-atopic SCID mice (a, b, arrows), but displayed hu IgE on their surface in skin sections derived from atopic-SCID mice (c, d, arrowheads). The results from one out of three representative experiments. Magnification $\times 400$ are presented. Hu, human; SCID, severe combined immuno-deficiency; FITC, fluorescein isothiocyanate.

mast cells, followed by the release of mediators such as histamine or serotonin. The analysis of serotonin release in BAL lavage indeed indicated an increase of serotonin compared with non-atopic and airway allergen challenged SCID mice.

The presented SCID model has the advantage that functional murine B and T cells are absent in these mice. In contrast, murine mast cells, macrophages, eosinophils and neutrophils are present in normal numbers, and they are functionally active. The finding of elevated serotonin levels in

BAL fluids from atopic and allergen challenged mice 15 min after the allergen challenge most likely reflects degranulation of airway mucosa mast cells followed by mediator release.

Our data indicate that the development of AHR in the hu-SCID-model is associated with increases in serotonin release. Serotonin is one of the major mediators associated with the development of immediate hypersensitivity responses in mice. All species have serotonin in their central nervous system, where it functions as one of the neurotransmitters. In contrast to *hus*, serotonin is present in mast cells in rodents (bound to protein matrix) and also in platelet granules, from which it is released during blood clotting. It still remains unclear whether murine eosinophils or basophils carry serotonin. The data from us and others now lead to the question about the putative role of mast cells in the lung and airways of rodents. Although the identification of mucosal mast cells in rodent airways is difficult and controversially discussed [35–37], it was clearly shown that deletion of mast cell functionality [38,39] abrogates the development of airway hyper-responsiveness in sensitized animals. These data provide further evidence for the concept that mast cells contribute to IgE-associated airway hyper-responsiveness in this model. Together with our own data, serotonin seems to be the most likely mediator of this response.

If allergen challenges were repeated at least twice, such mice developed a state of increased AR. This is in line with the results from studies of *in vitro* passive sensitization of isolated hu airway smooth muscle by incubation with serum from allergic patients containing high concentrations of IgE. A number of studies have demonstrated that passive sensitization alters the mechanical response of hu airway smooth muscle to a variety of non-specific agonists e.g. EFS [15–19]. Since in passive sensitization experiments asthmatic sera are selected on the basis of elevated concentration of total and/or specific IgE, the IgE molecule has been considered to be the serum factor responsible for the alteration of airway smooth muscle mechanical properties. It has been shown that IgE depletion abolished the immediate response to specific antigen [21].

One reasonable hypothesis is that isotypes other than IgE play a role in the development of increased AR [40]. In our model, no immediate-type hypersensitivity reactions were observed in SCID mice that received plasma from non-atopic donors, although allergen-specific hu IgG, and particularly allergen-specific IgG4 antibodies, were present. Several studies have ascribed an important role to pro-inflammatory cytokines such as IL-4, IL-5, IL-13, TNF- α , GM-CSF and others in the pathophysiology of increased AR [12, 14, 41–43]. In our model, the absence of T cells in the lung was expected in SCID mice since they lack mature T cells. If such mediators would have accounted for the effects observed, they would have been present in the serum samples and been of functional activity in the mouse. However, the absence of eosinophils and pro-allergic cytokines such as hu IL-4 and IL-5 is noteworthy. Since in most animal models the development of increased AR generally occurs in the presence of eosinophils, or reportedly is even eosinophil dependent, our model differs from these observations in that here, increased AR occur in the absence of eosinophils. These results suggest that different immuno-pathological pathways regulate increased AR.

Although the functional activity of hu IgE in mice is discussed controversially, our study suggests that functional interactions between hu IgE and murine effector cells, mast cells can indeed occur [44–46]. Both the development of positive skin test responses and the development of increased AR were allergen dependent and specific. The kinetics of skin test responses were similar to the immediate-type skin responses observed in patients following skin prick testing. Furthermore, the experiments with Evans blue indicated that positive skin responses to allergens as well as to compound 48/80 were due to protein extravasation and vascular leakage, likely induced by mediator release from skin mast cells.

The nature of these interactions is one interesting topic for future analysis. For example, hu IgE might interact with various Ig receptors on murine mast cells, including the high-affinity IgE receptor (Fc ϵ RI), the low-affinity IgE receptor (Fc ϵ RII) and the low-affinity receptors for IgG (Fc γ RII/RIII) [47–49]. Alternatively, the development of immediate-type hypersensitivity responses may have been related to binding of hu IgE to hu Fc ϵ R expressed on hu peripheral blood basophils that had migrated into the skin after cell transfer. However, this possibility can be excluded since skin test responses could be induced even in the absence of any hu cells, i.e. by hu atopic plasma alone.

In conclusion, we have demonstrated for the first time that the development of increased AR is a result of immediate-type hypersensitivity responses after IgE cross-linking, in the absence of manifest airway inflammation in a hu–mouse chimera model. This model now enables to assess the contribution of hu IgE for the development of increased AR *in vivo*.

Acknowledgements

We would like to thank C. Seib and R. Strozynski for their excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Grant Re 737/4-4 to HR).

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