

Allergen-induced IgE-dependent gut inflammation in a human PBMC-engrafted murine model of allergy

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Background: Humanized murine models comprise a new tool to analyze novel therapeutic strategies for allergic diseases of the intestine.

Objective: In this study we developed a human PBMC-engrafted murine model of allergen-driven gut inflammation and analyzed the underlying immunologic mechanisms.

Methods: Nonobese diabetic (NOD)-*scid-γc*^{-/-} mice were injected intraperitoneally with human PBMCs from allergic donors together with the respective allergen or not. Three weeks later, mice were challenged with the allergen orally or rectally, and gut inflammation was monitored with a high-resolution video miniendoscopic system, as well as histologically.

Results: Using the aeroallergens birch or grass pollen as model allergens and, for some donors, also hazelnut allergen, we show that allergen-specific human IgE in murine sera and allergen-specific proliferation and cytokine production of human CD4⁺ T cells recovered from spleens after 3 weeks could only be measured in mice treated with PBMCs plus allergen.

Importantly, these mice had the highest endoscopic scores evaluating translucent structure, granularity, fibrin, vascularity, and stool after oral or rectal allergen challenge and a strong histologic inflammation of the colon. Analyzing the underlying mechanisms, we demonstrate that allergen-associated colitis was dependent on IgE, human IgE receptor-expressing effector cells, and the mediators histamine and platelet-activating factor. **Conclusion:** These results demonstrate that allergic gut inflammation can be induced in human PBMC-engrafted mice, allowing the investigation of pathophysiologic mechanisms of allergic diseases of the intestine and evaluation of therapeutic interventions. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Humanized mice, IgE, allergen, colon, inflammation, basophils, histamine, platelet-activating factor

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Abbreviations used

APC: Allophycocyanin

DC: Dendritic cell

IMDM: Iscove modified Dulbecco medium

NK: Natural killer

NOD: Nonobese diabetic

PAF: Platelet-activating factor

PE: Phycoerythrin

SCID: Severe combined immunodeficiency

Understanding the immunologic mechanisms of allergic diseases, including intestinal inflammation, is still limited and so are the specific therapeutic approaches. Allergen-specific immunotherapy has been shown to be very efficient for the treatment of venom and respiratory allergy but is not established for IgE-mediated food allergy because of the risk of anaphylactic reactions or because it has only limited effects on concomitant food allergy.¹⁻⁴ Novel therapeutic strategies, such as injection of regulatory T cells, anti-IgE, and cytokines/anticytokines, have been studied in murine models of allergy and asthma.^{5,6} However, mice do not express specific human therapeutic targets, and the investigation of novel drugs in human subjects is limited by ethical and technical constraints. Therefore the development of humanized murine models is very important to study such novel strategies *in vivo* without putting human subjects at risk.⁷

First experiments to analyze allergic immune responses have been performed with severe combined immunodeficiency (SCID) mice, which lack mature T and B cells.⁸ In these mice total and allergen-specific IgE levels, as well as increased airway responsiveness and systemic anaphylaxis induced by the release of mediators from mast cells and basophils, such as histamine, proteases, leukotrienes, prostaglandins, and platelet-activating factor (PAF), could be measured after engraftment of PBMCs from atopic patients but not after engraftment of PBMCs from healthy control subjects.⁹⁻¹⁴ However, no model for the analysis of gut inflammation and anaphylaxis resulting from allergic reactions to food (often associated with pollen allergy⁴) exists thus far. In almost all studies, engraftment of human PBMCs occurred at a low level because of spontaneous generation of murine T and B cells during aging, which is known as leakiness, and the presence of natural killer (NK) cells and other innate immune activity, such as macrophages and neutrophils.

The NK cell activity is lower in nonobese diabetic (NOD)-*scid* mice, which were developed later and allow higher levels of human cell engraftment. Recently, humanization was performed

in NOD-*scid* mice bearing a targeted mutation on the gene encoding IL-2 receptor γ -chain, which leads to severe impairments in T- and B-cell development and function and completely prevents NK cell development, greatly increasing survival of human PBMCs, even at low cell numbers.^{7,15-17} Therefore we used NOD-*scid*- $\gamma c^{-/-}$ mice in this study to develop a human PBMC-engrafted murine model of allergy and demonstrated an allergen-driven inflammation of the intestine, which was dependent on human IgE, human Fc ϵ RI α -expressing effector cells, and the mediators histamine and PAF. Murine basophils/mast cells might also play a role as bystanders. This model provides the possibility to analyze novel therapeutic strategies for intervention in patients with allergic inflammation of the intestine.

METHODS

Blood samples/donors

Heparinized blood was obtained from donors with allergy to grass or birch pollen with allergic rhinoconjunctivitis, asthma, or both or nonallergic healthy control subjects. Some allergic donors also showed cross-reactivity to hazelnut and carrot. Specific sensitization was documented by positive skin prick test responses and detection of allergen-specific IgE in the sera (CAP class ≥ 5 measured with the ImmunoCAP specific IgE blood test; Phadia AB, Uppsala, Sweden). This study was approved by the local ethics committee. Informed consent was obtained from all subjects before the study.

Mice

NOD.CB17-Prkdc^{scid}/J or NOD.CB17-Prkdc^{scid}/J $\gamma c^{-/-}$ mice 4 to 8 weeks of age were obtained from the Central Animal Facility of the Johannes Gutenberg-Universität in Mainz, Germany. Experiments were performed in accordance with current federal, state, and institutional guidelines (authorization 23 177-07/G 08-1-009).

PBMC preparation and purification of dendritic cells and T cells

PBMCs were isolated from heparinized blood by using Ficoll-Paque density centrifugation (1.077 g/mL; PAA Laboratories GmbH, Pasching, Austria). PBMCs (1×10^7 per well) were incubated for 45 minutes in a 6-well plate (Greiner, Frickenhausen, Germany) in Iscove modified Dulbecco medium (IMDM) containing L-glutamine and 25 mmol/L HEPES (PAA Laboratories GmbH) supplemented with an antibiotic-antimycotic solution containing 100 μ g/mL streptomycin, 100 U/mL penicillin, and 250 ng/mL amphotericin B (PAA Laboratories GmbH) and 3% heat-inactivated autologous plasma at 37°C to enrich CD14⁺ monocytes. After washing the nonadherent cells with prewarmed PBS, the remaining monocytes (purity >90%) were incubated in IMDM (3 mL per well) supplemented with 1% heat-inactivated autologous plasma, IL-4 (1000 U/mL; Miltenyi Biotec, Bergisch Gladbach, Germany) and GM-CSF (200 U/mL, Leukine; Immunex Corp, Seattle, Wash). On day 6, the resulting immature dendritic cells (DCs) were pulsed with birch or grass pollen allergen extract (10 μ g/mL; ALK-Abelló, Hamburg, Germany), hazelnut allergen extract (10 μ g/mL; Allergopharma, Reinbek, Germany), or tetanus toxoid (1 μ g/mL; Behringwerke, Marburg, Germany) and further stimulated with TNF- α (1000 U/mL, Miltenyi Biotec), IL-1 β (2000 U/mL, Miltenyi Biotec), and prostaglandin E₂ (1 μ g/mL; Cayman Chemical, Ann Arbor, Mich) to induce their full maturation. Mature DCs were harvested 48 hours after stimulation, washed twice, and used for T-cell stimulation assays. Mature DCs expressed high levels (>90%) of CD80, CD83, CD86, and MHC class II molecules, as controlled by means of flow cytometry.

Autologous CD4⁺ T cells were obtained from PBMCs or PBMC-engrafted murine spleens by using antibody-coated paramagnetic MultiSort MicroBeads (magnetic cell sorting, Miltenyi Biotec), according to the protocol of the manufacturer (purity >98% CD4⁺ T cells).

Reconstitution of mice with PBMCs and rectal or oral allergen challenge

Mice were injected intraperitoneally with 2×10^7 PBMCs or Fc ϵ RI-depleted PBMCs together with 20 μ g of birch pollen, grass pollen, or hazelnut allergen extract in 500 μ L of 0.9% NaCl. Eight days later, mice received an additional intraperitoneal allergen boost of the same amount of allergen in 200 μ L of 0.9% NaCl. Control groups received allergen or NaCl alone. On day 21, sera were collected and stored at -20°C until determination of human allergen-specific and total IgE levels by using the ImmunoCAP specific/total IgE blood test (Phadia AB) or based on β -hexosaminidase release. Then mice were anesthetized and examined with a high-resolution video endoscopic Coloview system (Karl Storz, Tuttlingen, Germany) before and 2 to 6 hours after challenge with 20 μ g of allergen extract in 100 μ L of 0.9% NaCl rectally or with 50 μ g of allergen extract orally, as previously described.¹⁸ Briefly, a mini-endoscope (1.9 mm in outer diameter) was introduced through the anus, and the colon was carefully insufflated with an air pump. Endoscopic pictures obtained are of high quality and allow the monitoring and grading of inflammation of the colon up to 6 cm in depth. Thereafter, endoscopic scoring of 5 parameters from 0 to 3 (translucent structure, granularity, fibrin, vascularity, and stool) was done, resulting in an overall score of 0 (no change) to 15 (severe symptoms).

In some experiments mice were intraperitoneally treated with 250 μ g of human IgG control (Intratec; Biotest Pharma GmbH, Dreieich, Germany) or 250 μ g of the humanized anti-IgE mAb omalizumab (Xolair; Novartis, Hünningue, France) in 200 μ L of 0.9% NaCl 3 days and 1 hour before challenge. Other groups were treated orally with a PAF receptor antagonist (10 mg/kg), ABT491 (Sigma-Aldrich, Taufkirchen, Germany), in 100 μ L of 0.9% NaCl 1 hour before challenge; with intraperitoneal injection of the histamine H₁ receptor antagonist (10 mg/kg) mepyramine (Sigma-Aldrich) and the histamine H₂ receptor antagonist (10 mg/kg) cimetidine (Sigma-Aldrich) in 200 μ L of 0.9% NaCl 30 minutes before challenge; or with all 3 antagonists together.

Depletion of murine or human IgE receptor-expressing effector cells and reconstitution of mice with human pre-enriched basophils

Mice were treated intraperitoneally with 10 μ g of anti-mouse Fc ϵ RI α mAb (MAR-1; BioLegend, San Diego, Calif) or 10 μ g of Armenian hamster IgG as an isotype control (BioLegend) in 200 μ L of 0.9% NaCl on 3 subsequent days beginning 6 days before challenge to deplete murine IgE receptor-expressing effector cells. PBMCs were treated with 10 μ g of mouse anti-human Fc ϵ RI α mAb (CRA-1, BioLegend) and anti-mouse Dynabeads (Dyna, Hamburg, Germany) before injection into mice to deplete human IgE receptor-expressing effector cells. Additionally, mice were treated with 10 μ g of CRA-1 three days and one day before challenge. In some experiments human basophils of the same donor were pre-enriched with the Diamond Basophil Isolation Kit (Miltenyi Biotec), reaching a purity of at least 80% (CD123⁺ and CD203c⁺ cells), and 2×10^5 of these pre-enriched basophils were injected intraperitoneally 4 days before challenge.

β -Hexosaminidase release

Rat basophilic leukemia cells (clone RBL-703/21), which were derived from RBL-2H3 cells by means of transfection with the human high-affinity IgE receptor, were cultivated and analyzed for mediator release (β -hexosaminidase activity) after sensitization with sera from human PBMC-engrafted mice and stimulation with the respective allergen or the unspecific cell degranulator phorbol 12-myristate 13-acetate plus the calcium ionophore A23187 (Sigma-Aldrich), as described earlier.¹⁹

Histologic analysis of colon sections

Colons were removed and put into formalin for paraffin sections. Staining was performed with hematoxylin and eosin (Sigma-Aldrich), Giemsa

TABLE I. Percentage of total human cells in the spleens of NOD-*scid* and NOD-*scid-γc*^{-/-} mice 3 weeks after PBMC engraftment

	NOD- <i>scid</i>	NOD- <i>scid-γc</i> ^{-/-}
CD45	18.1 ± 14.3	51.4 ± 28.8
CD3	17.2 ± 13.6	49.2 ± 28.4
CD4	8.1 ± 6.0	23.5 ± 14.0
CD8	8.7 ± 6.4	29.2 ± 15.5
CD19	0.8 ± 0.7	2.8 ± 2.2

Shown are the means ± SEMs from 24 mice receiving PBMCs from 8 different allergic donors.

(Sigma-Aldrich), or mouse anti-human CD45 (2B11 and PD7/26; DakoCytomation, Glostrup, Denmark), CD3 (LN10; Leica Biosystems Newcastle Ltd, Newcastle, United Kingdom), CD4 (4B12, DakoCytomation), or CD8 (4B11; AbD Serotec, Oxford, United Kingdom) and visualized with the Dako REAL EnVision Detection System (Peroxidase/DAB+, Rabbit/Mouse; DakoCytomation), according to the manufacturer's instructions. Slides were analyzed with an Olympus BX40 microscope (Olympus, Center Valley, Pa) by using the ColorView Soft Imaging system camera and software. Grading of colitis activity was done in a blinded fashion by a pathologist (H. A. Lehr).

T-cell preparation and antigen restimulation *in vitro*

Two hours after rectal challenge and subsequent endoscopy, mice were killed by means of cervical dislocation. Spleens were removed and dispersed through a 70-μm nylon cell strainer (BD Biosciences, San Jose, Calif). After red blood cell lysis, cells were washed twice with PBS and pooled for isolation of human CD4⁺ T cells, as described above. For proliferation assays, 1×10^5 CD4⁺ T cells were cocultured in 96-well plates (Greiner) in triplicates with 1×10^4 autologous unpulsed, allergen-pulsed, or tetanus toxoid-pulsed DCs in 200 μL of IMDM supplemented with 5% heat-inactivated autologous plasma. After 5 days, the cells were pulsed with 37 kBq per well of tritiated thymidine (ICN, Irvine, Calif) for 6 hours, and tritiated thymidine incorporation was evaluated in a beta counter (1205 Betaplate; LKB Wallac, Turku, Finland). For cytokine production assays, 5×10^5 CD4⁺ T cells were cocultured in 48-well plates in the presence of 5×10^4 autologous unpulsed, allergen-pulsed, or tetanus toxoid-pulsed DCs in 1 mL of IMDM supplemented with 5% heat-inactivated autologous plasma. On day 7, T cells were restimulated with 5×10^4 newly generated DCs, and supernatants were collected 24 hours later.

Flow cytometric analysis

Surface phenotyping was performed by staining 5×10^5 CD4⁺ human T cells, 5×10^5 splenocytes, 5×10^4 human DCs, or 5×10^4 pre-enriched basophils with specific mAbs for 20 minutes at 4°C, washing, and analysis in a FACSCalibur (BD Biosciences) equipped with CELLQuest Software. The following mAbs were used: fluorescence isothiocyanate-conjugated anti-human HLA-DR (L243) and anti-human CD3 (UCHT1); phycoerythrin (PE)-conjugated anti-human CD80 (L307.4), anti-human CD83 (HB15e), anti-human CD86 (2331[FUN-1]), and anti-human CD45 (HI30); allophycocyanin (APC)-conjugated anti-mouse CD117 (2B8) and mouse and rat IgG isotype controls (all BD Biosciences); Alexa Fluor 647-conjugated anti-human CD4 (MT310; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif); APC-conjugated anti-human CD8 (MEM-31) and anti-human CD19 (LT19; EuroBioSciences, Friesoythe, Germany); PE-conjugated anti-human CD123 (AC145) and APC-conjugated anti-human CD203c (FR3-16A11, Miltenyi Biotec); and PE-conjugated anti-mouse FcεR1α (MAR-1) and fluorescence isothiocyanate-conjugated anti-mouse CD49b (DX5, BioLegend).

Quantification of cytokine production by means of ELISA

Human IL-4, IL-10, IL-13, and IFN-γ levels were measured by using ELISA, according to the manufacturer's instructions (BD Biosciences).

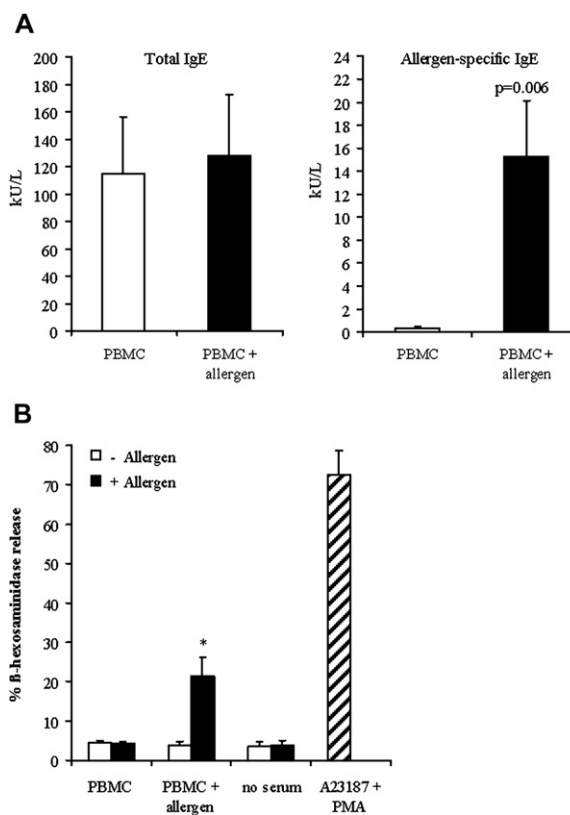


FIG 1. Production of human total and allergen-specific IgE in human PBMC-engrafted NOD-*scid-γc*^{-/-} mice. PBMCs (2×10^7) were injected intraperitoneally with or without the respective allergen, and human IgE levels were measured in murine sera 3 weeks later by using the ImmunoCAP test (A) or β-hexosaminidase activity of FcεR1-transfected RBL cells (B). Shown are means ± SEMs of 9 (8 for Fig 1, B) different experiments. * $P \leq .01$ compared with no allergen stimulation.

Statistics

The Student 2-tailed *t* test was used to test the statistical significance of the results: a *P* value of .05 or less was considered significant.

RESULTS

Detection of allergen-specific human IgE production in sera from allergen-treated NOD-*scid-γc*^{-/-} mice engrafted with PBMCs from allergic donors

In preliminary experiments we had shown that NOD-*scid-γc*^{-/-} mice were superior to normal NOD-*scid* mice concerning the survival of human cells, even at low cell numbers (Table I). Therefore we developed a human PBMC-engrafted model of allergy in this murine strain by injection of PBMCs from donors allergic to grass pollen, birch pollen, or hazelnut and treatment of the mice with the respective allergen or not. To analyze whether and at which time point a humoral allergen-specific immune response was detectable, we measured total and allergen-specific human IgE levels in sera from NOD-*scid-γc*^{-/-} mice weekly after PBMC engraftment. We could demonstrate that total and allergen-specific human IgE was produced from day 14 onward until the end of the experiment in the sera of almost every mouse (data not shown). The presence of the allergen did not change total IgE production in the mice, whereas allergen-specific IgE production was significantly enhanced ($P = .0067$; Fig 1, A).

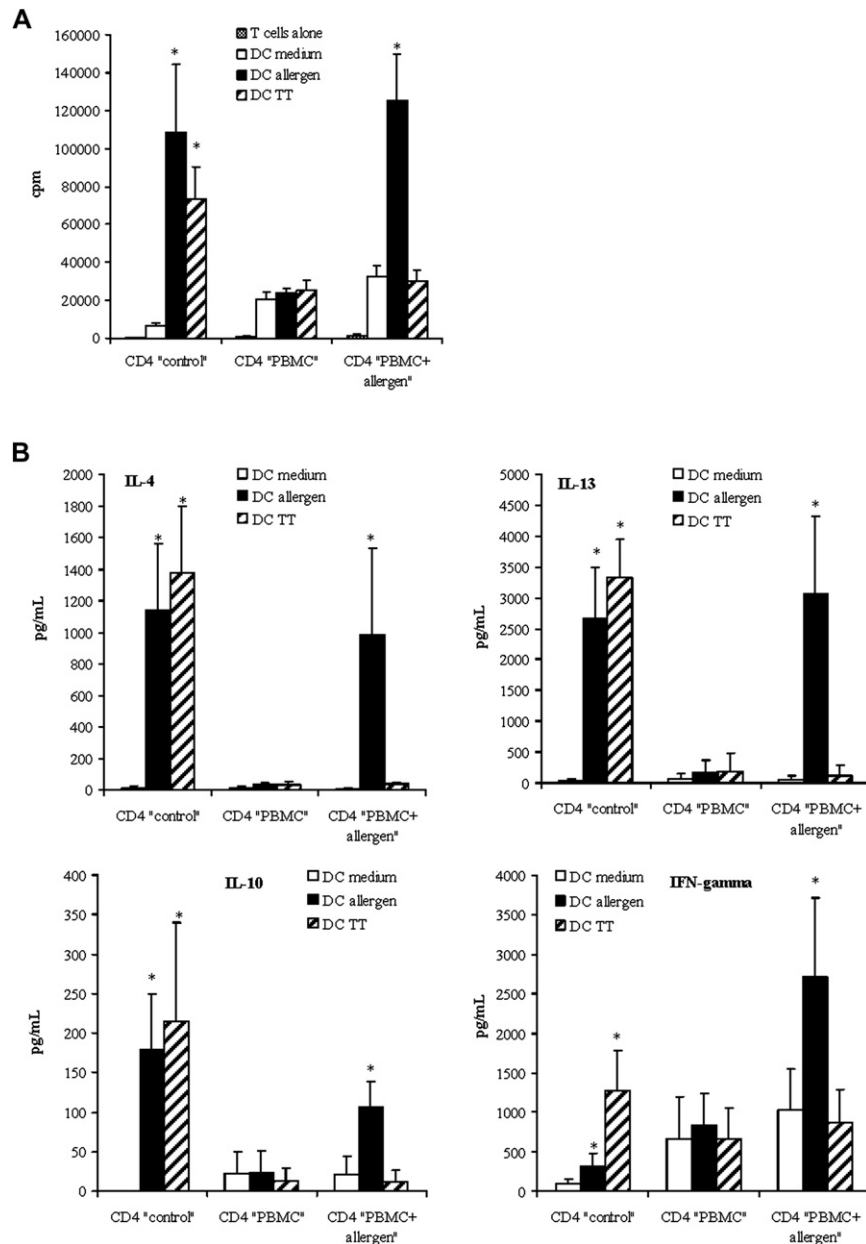


FIG 2. Allergen-specific proliferation and cytokine production in human PBMC-engrafted NOD-*scid-γc*^{-/-} mice. Three weeks after PBMC engraftment, human CD4⁺ T cells were recovered from spleens and stimulated with autologous unpulsed (DC medium), allergen-pulsed (DC allergen), or tetanus toxoid-pulsed DCs (DC TT) to analyze their proliferation (A) and cytokine production (B). Shown are means ± SEMs of 6 different experiments. **P* ≤ .05 compared with DC medium.

Furthermore, β-hexosaminidase release of FcεRI-transfected RBL cells was significantly enhanced only after incubation of the cells with sera from PBMC plus allergen-treated mice and stimulation with the respective allergen (Fig 1, B).

Allergen-specific proliferation and cytokine production by human CD4⁺ T cells isolated from the spleens of PBMC plus allergen-treated NOD-*scid-γc*^{-/-} mice

To analyze proliferation and cytokine production after *in vitro* restimulation of engrafted CD4⁺ T cells, these cells were isolated

from pooled spleens (at least from 2 mice per group) 3 weeks after PBMC engraftment in the presence or absence of allergen and restimulated with unpulsed (medium) or allergen-pulsed autologous DCs. Freshly isolated CD4⁺ T cells from the same donor were used for comparison. Fig 2, A, shows that CD4⁺ T cells were able to proliferate in response to allergen-pulsed DCs *in vitro* only when they were derived from mice that had received PBMCs together with the allergen. Allergen-specific proliferation was comparable with that of freshly isolated CD4⁺ T cells of the same donor.

Similarly, allergen-induced production of IL-4, IL-10, IL-13, and IFN-γ could only be observed in CD4⁺ T cells isolated from

allergen-treated mice and in freshly isolated CD4⁺ T cells (Fig 2, B). Stimulation with tetanus toxoid-pulsed DCs did not lead to proliferation or cytokine production in CD4⁺ T cells recovered from murine spleens, whereas freshly isolated CD4⁺ T cells from all vaccinated human donors responded well to this stimulus (Fig 2), indicating the necessity of antigen-specific stimulation after transfer of human cells into the mice.

Allergen-specific gut inflammation in human PBMC-engrafted mice after rectal or oral allergen challenge

NOD-*scid*- γ c^{-/-} mice were reconstituted with human PBMCs from allergic donors in the presence or absence of the respective allergen and challenged rectally or orally with the allergen or NaCl as a control after 3 weeks to analyze gut inflammation. Before challenge, no inflammation was observed during endoscopic scoring of the colon (the small intestine was not accessible by using our Coloview system) when evaluating translucent structure, granularity, fibrin formation, vascularity, and stool. After rectal allergen challenge, signs of allergen-induced colitis already appeared within 1 to 2 hours, and after oral allergen challenge, these signs appeared within 6 to 24 hours. After rectal but not oral allergen challenge, all mice that had received PBMCs from allergic donors already showed a significantly enhanced clinical score compared with scores seen in control mice that had received allergen alone and no PBMCs. The addition of the allergen at the same time point of PBMC engraftment and 8 days later strongly and significantly increased the endoscopic score in response to both challenge routes (Fig 3, A and B). Importantly, rectal or oral challenge of PBMC- or PBMC plus allergen-engrafted mice with NaCl instead of the allergen did not lead to increased scores compared with those seen in control mice. Mice receiving allergen plus PBMCs from nonallergic healthy donors also did not show enhanced gut inflammation after rectal or oral allergen challenge (Fig 3, A and B).

Histology of the colons showed early inflammatory changes, such as strong mucosal hypertrophy and enhanced wall thickening, together with infiltration of the mucosa with mononuclear cells, especially lymphocytes and neutrophils, in mice that had received human PBMCs from allergic donors but not in mice that had received allergen alone and no PBMCs (Fig 3, C). Further immunohistologic staining revealed a similar infiltration of the colon (Fig 3, D) with human CD45⁺ cells, regardless of whether the allergen was coinjected with the PBMCs during engraftment. Human CD45⁺ cells mainly consisted of CD3⁺CD4⁺ or CD3⁺CD8⁺ T cells (data not shown) and were also present in other organs, such as the spleen (Table I), lung, liver, and skin, indicating some graft-versus-host reactions of human cells against murine MHC molecules in all tissues.

Prevention of allergen-induced gut inflammation by blockade of human IgE or the mediators histamine and PAF

PBMC plus allergen-treated mice were pretreated with the humanized anti-IgE mAb omalizumab, human IgG control, H₁ and H₂ receptor antagonists, a PAF receptor antagonist, or histamine receptor and PAF receptor antagonists together before rectal challenge to assess the role of human IgE and mast cell- or basophil-derived mediator in allergen-specific gut inflammation.

In Fig 4 it is shown that depletion of human IgE by neutralizing antibodies significantly inhibited allergen-induced gut inflammation. The human IgG control had no effect. Blockade of histamine and particularly PAF antagonism also strongly inhibited allergen-induced colitis. Treatment of mice with all 3 pharmacologic compounds resulted in a complete loss of gut inflammation beyond background levels. Of note, the levels of allergen-specific IgE, as well as allergen-specific proliferation and cytokine production, *in vitro* were similar in all groups, indicating equal sensitization (data not shown).

Allergen-driven gut inflammation is primarily mediated by IgE receptor-expressing effector cells among human PBMCs

Because human IgE has been described to not bind to murine FcεRI,^{20,21} we wondered whether IgE receptor-expressing effector cells among human PBMCs are responsible for allergen-driven gut inflammation. To clarify this assumption, we depleted IgE receptor-expressing cells among human PBMCs before injection into mice, by means of intraperitoneal injection of the anti-human FcεRIα mAb CRA-1, or both. Both depletion methods strongly inhibited allergen-driven colitis (Fig 5). To analyze further whether murine basophils, mast cells, or both are involved in the inflammation, we treated the mice with intraperitoneal injection of the anti-mouse FcεRIα mAb MAR-1 on 3 subsequent days, which has been shown to completely deplete basophils and partially deplete peritoneal mast cells already after 4 hours up to 12 days.²² Staining of blood or splenic cells with murine CD117 mAb or CD49b mAb together with MAR-1 also revealed a significant depletion of basophils in the blood and mast cells in the spleen (Fig 6, A and B). However, in Fig 6, C, it is shown that mast cells in the gut were not completely depleted because they were still detectable by staining with Giemsa. Nevertheless, injection of MAR-1 strongly inhibited allergen-driven inflammation of the intestine, whereas hamster IgG isotype control had no effect (Fig 6, D and E). Because MAR-1 also recognizes human FcεRI⁺ cells to some extent, we added pre-enriched human CD123⁺CD203c⁺ basophils (Fig 6, F) together with MAR-1 on the last day of basophil/mast cell depletion. This treatment resulted in a complete restoration of gut inflammation (Fig 6, D and E).

DISCUSSION

In the present study we developed, for the first time, a model of human allergen-specific IgE- and human IgE receptor-expressing cell-dependent inflammation in the guts of human PBMC-engrafted NOD-*scid*- γ c^{-/-} mice using the aeroallergens birch and grass pollen as model allergens and, for some donors, also hazelnut allergen. Enhanced signs of colitis were observed in the colon after rectal or oral allergen challenge only in mice that had been engrafted intraperitoneally with PBMCs from allergic donors but not in mice that had received PBMCs from healthy volunteers or no PBMCs at all. Immunization of mice with the respective allergen during PBMC transfer further increased the endoscopic score. Furthermore, allergen-driven inflammation of the colon correlated with the presence of allergen-specific human IgE in murine sera and allergen-specific T-cell proliferation and cytokine production. Inflammation of the small intestine after oral allergen challenge could not be investigated by means of

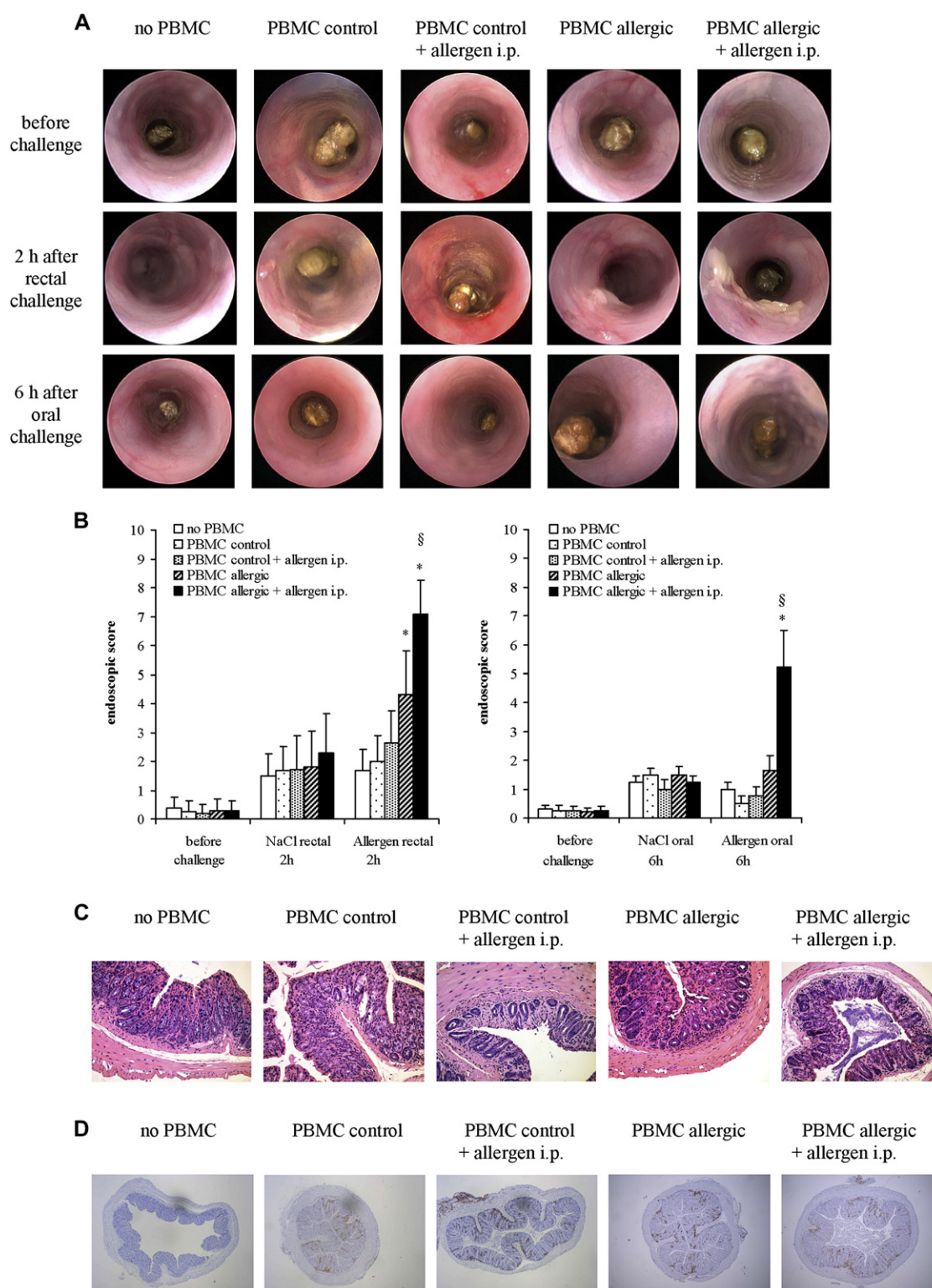


FIG 3. Allergen-specific gut inflammation in human PBMC-engrafted NOD-*scid*- γ ^{-/-} mice. Three weeks after PBMC engraftment, mice were examined with a video miniendoscope before and after rectal (or oral) allergen challenge. **A** and **B**, Shown are the endoscopic pictures (Fig 3, A) of a representative result and the means $\pm$ SEMs of the endoscopic score (Fig 3, B) of 7 (6) different experiments (3 [2] for healthy donors) with at least 2 mice per group. * $P \leq .01$ compared with the "no PBMC" group. § $P \leq .01$ compared with the "PBMC allergic" group. **C** and **D**, Staining of the colon with hematoxylin and eosin ($\times 200$ magnification) or anti-human CD45 ($\times 40$ magnification) after rectal challenge. *i.p.*, Intraperitoneal.

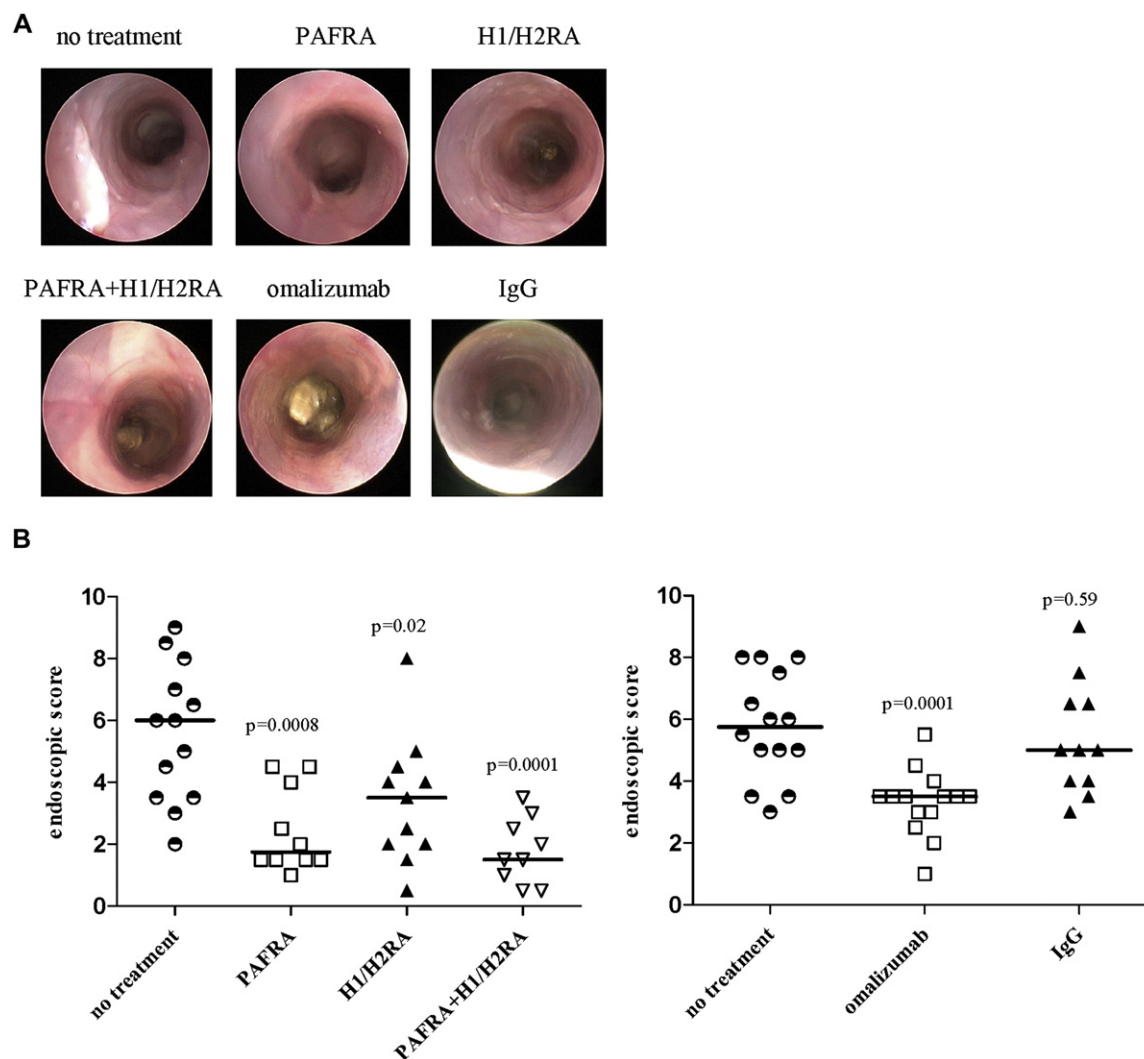


FIG 4. Gut inflammation is mediated by human IgE and the mediators PAF and histamine. NOD-*scid-γc*^{-/-} mice were treated and challenged as described in Fig 3. Human IgG control and the humanized anti-IgE mAb omalizumab were administered 3 days and 1 hour before rectal allergen challenge. PAF receptor antagonist or H₁ and H₂ receptor antagonists were administered 1 hour or 30 minutes before challenge. Shown are the endoscopic pictures (**A**) of 1 representative result and the single values of the endoscopic score (**B**) of 7 different experiments.

colonoscopy, and histologic analysis revealed no signs of inflammation in this organ already in more severe experimental colitis models.²³ In NOD-*scid* mice, which had been used for pilot experiments, similar results could be observed but only in some mice because of limited human CD4⁺ T-cell and B-cell survival and subsequent lack of allergen-specific IgE production in these mice. These findings are consistent with a recent report from King et al¹⁷ also showing a superior human PBMC engraftment in NOD-*scid-γc*^{-/-} mice compared with that seen in NOD-*scid* mice with low intradonor and interdonor variability.

In some experiments, especially after rectal allergen challenge, there was no great difference between the endoscopic scores of NOD-*scid-γc*^{-/-} mice receiving PBMCs alone or those receiving PBMCs plus allergen. This might be due to the fact that too many human cells had migrated into the colons of these mice, probably inducing some unspecific graft-versus-host reactions of human cells against murine tissues.²⁴ Nevertheless, allergen specificity

could clearly be shown by the use of NaCl or another allergen/antigen for challenge or restimulation assays, which was not injected together with PBMCs. The antigen tetanus toxoid, for example, did not increase endoscopic scores, and neither increased proliferation nor cytokine production was observed.

Up to now, allergic immune responses have only been studied in human PBMC-engrafted SCID or NOD-*scid* mice. Because of the high differences in the frequencies and levels of PBMC engraftment depending on donor and recipient variability, it is difficult to use these mice for analysis of novel inhibitory strategies for the treatment of allergic diseases. Here we describe for the first time a reliable and reproducible model of allergic inflammation in NOD-*scid-γc*^{-/-} mice engrafted with PBMCs from allergic donors together with the respective allergen, resulting in proliferation, cytokine production, allergen-specific IgE production, and allergen-induced inflammation in the colon and airways/lung (the latter published separately²⁵). The presented model might be

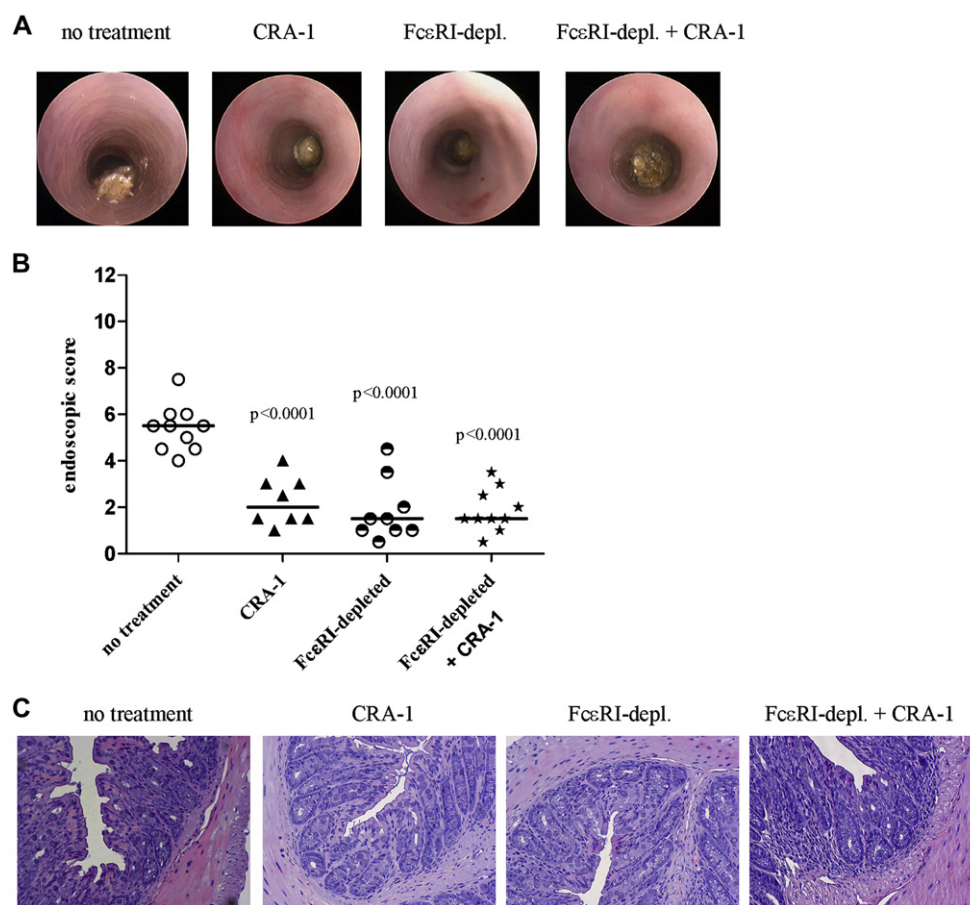


FIG 5. Depletion of human FcεRI-expressing effector cells prevents allergen-specific gut inflammation. NOD-*scid-γc*^{-/-} mice were treated with PBMCs or FcεRIα-depleted PBMCs and challenged as described in Fig 3. Anti-human FcεRIα mAb was additionally applied 3 days and 1 day before challenge. **A** and **B**, Shown are the endoscopic pictures (Fig 5, A) of 1 representative result and the single values of the endoscopic score (Fig 5, B) of 5 different experiments. **C**, Staining of the colon with hematoxylin and eosin (×200 magnification).

useful to study novel immunotherapeutic drugs or strategies for the treatment of allergic diseases, including food allergy. It is now possible to answer the question of whether the allergic immune response can be inhibited by adoptive transfer of regulatory T cells or soluble suppressive mediators, which are involved in the regulation of allergy and induced or expanded during allergen-specific immunotherapy.²⁶⁻²⁸ Studies in murine models of asthma have already demonstrated that IL-10-producing regulatory type 1 cells can prevent allergen-induced airway hyperreactivity and inflammation and that this process is mediated by inducible costimulator-inducible costimulator ligand interaction and by IL-10 derived from pulmonary DCs.²⁹ Alternatively, depletion of the recently defined proinflammatory T_H17 cells or their produced cytokines, which play an important role in chronic tissue inflammation, can be analyzed.³⁰

Analyzing whether the effector mechanisms being activated are human or murine, we could demonstrate that allergen-specific inflammation of the intestine was primarily dependent on human IgE- and human IgE receptor-expressing cells in the PBMCs because depletion of human FcεRIα-expressing cells among human PBMCs before injection into mice decreased the endoscopic score to background levels. Furthermore, the humanized anti-human IgE mAb omalizumab, which reduces the serum IgE

concentration within hours,³¹ significantly inhibited the endoscopic score, whereas the control IgG had no effect. However, depletion or at least inactivation of murine basophils/mast cells by the anti-mouse FcεRIα mAb MAR-1 before rectal allergen challenge also strongly reduced allergen-specific gut inflammation. One explanation of this finding is the fact that MAR-1 also binds to human IgE receptor-expressing cells *in vitro* to some extent (data not shown) and might thus interfere with them *in vivo*. Another explanation could be that activated human effector cells and their mediators initiate additional activation of murine effector cells. However, even if the latter really is the case, murine effector cells can be replaced by pre-enriched human basophils, further “humanizing” the model. Therefore human, but not murine, FcεRIα-expressing effector cells are essential in this model.

In addition to depletion of IgE receptor-expressing cells, decreased inflammation of the colon was further observed through inhibition of the mast cell- and basophil-derived mediators PAF and histamine by pharmacologic antagonists. The endoscopic score was even inhibited to background levels by concomitant injection of PAF and histamine receptor antagonists before rectal allergen challenge. Similar results have recently been reported by Arias et al³² for peanut-induced anaphylaxis in peanut-sensitized mice. Moreover, efficacy of the PAF antagonist ABT-491 has already

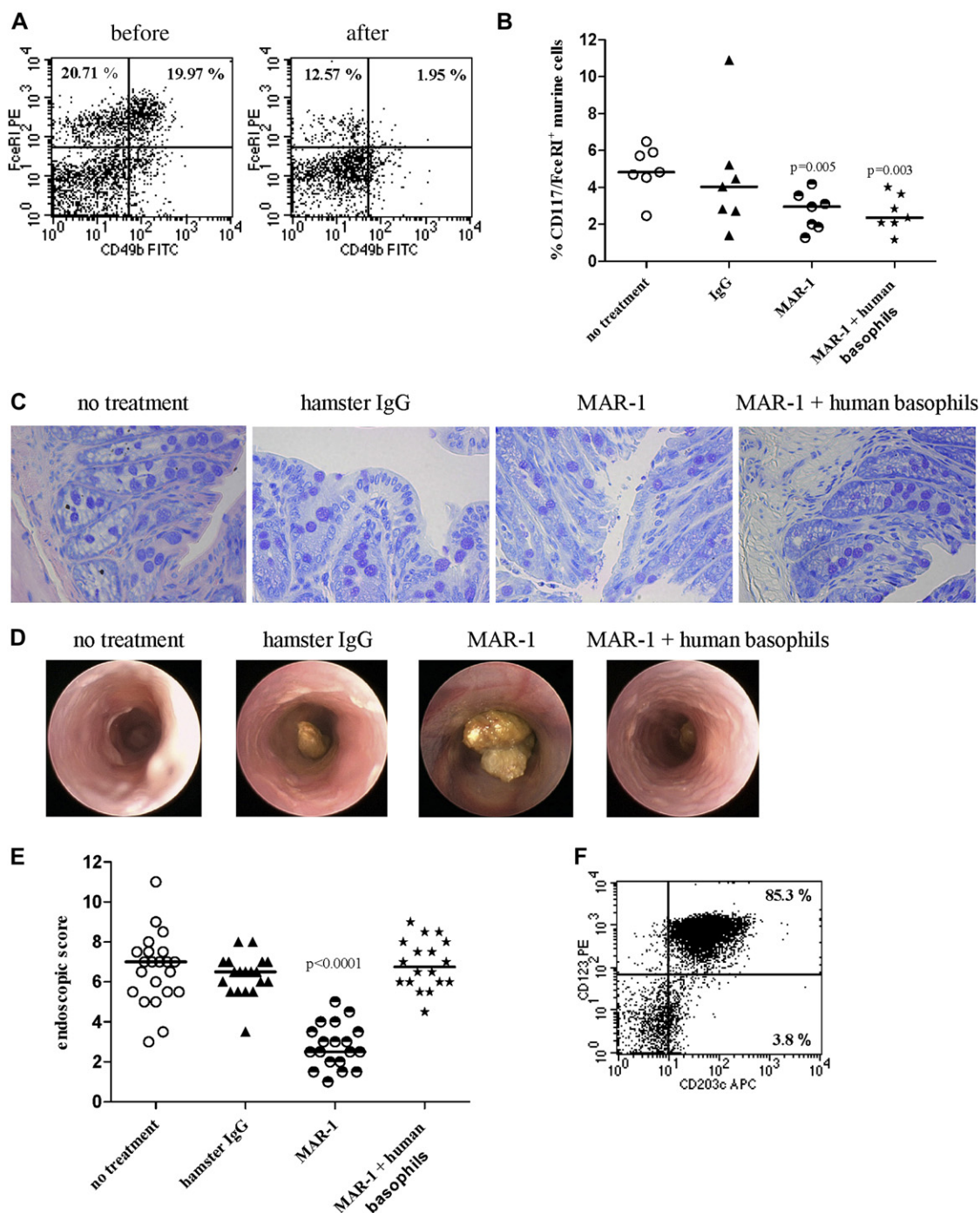


FIG 6. Gut inflammation is prevented by depletion of murine basophils/mast cells but can be restored by addition of human pre-enriched basophils. NOD-*scid*- γ ^{-/-} mice were treated and challenged as described in Fig 3. Anti-mouse FcεRI α mAb or hamster IgG control was administered 6 to 4 days, and pre-enriched human basophils were administered 4 days before challenge. **A**, One representative staining of murine blood before and after depletion. **B**, The single values of the percentage of anti-human CD45⁺, anti-mouse CD117⁺/FcεRI⁺ cells in pooled spleens of 7 different experiments. **C**, Giemsa staining of murine mast cells in the gut ($\times 400$ magnification). **D** and **E**, Endoscopic pictures of 1 representative result and the single values of the endoscopic score of 8 different experiments. **F**, One representative staining of human pre-enriched basophils. FITC, Fluorescein isothiocyanate.

been shown in experimental allergic rhinitis in Brown Norway rats and guinea pigs.³³ In both studies, as well as in our study, coadministration of an antihistamine inhibitor together with the PAF receptor antagonist further enhanced the suppressive properties.

Taken together, the newly developed human PBMC-engrafted murine model of allergy and allergen-driven inflammation of the intestine presented here is a suitable model to study not only existing drugs, such as omalizumab, but also novel

pharmacologic and immunologic intervention for the therapy of allergic diseases, including inflammation of the intestine, also occurring in food allergy, in which therapeutic options are very limited thus far.

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Clinical implications: IgE-mediated allergic colitis can be induced in a human PBMC-engrafted murine model, allowing the analysis of novel therapeutic approaches.

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