

Original article

Functional human IgE specific for *Dermatophagoides farinae* antigen is produced in SCID mice reconstituted with peripheral mononuclear cells derived from healthy persons and patients with asthma

Background: Whether normal peripheral blood mononuclear cells (PBMCs) transferred to severe combined immunodeficient (SCID) mice produce specific IgE remains unclear.

Methods: Mice received injections of *Dermatophagoides farinae* antigen (Df)-stimulated PBMCs from healthy persons (IgE RAST score of 0).

Results: High titers of Df-specific IgE were detected. The Df-specific IgE activity produced was comparable to or higher than that produced by cells from patients with asthma although the time to maximal production was longer. IgE derived from PMBCs of healthy persons or patients with asthma induced histamine release from cultured human basophils that had been stimulated with Df antigen or an anti-IgE antibody. Treatment of Df-stimulated PBMCs with a high dose, but not a low dose, of interleukin-4 stimulated production of Df-specific IgE by PMBCs from healthy persons or patients with asthma. In contrast, intravenous injection of IFN- γ into reconstituted SCID mice decreased Df-specific IgE production by PBMCs from patients with asthma. In PMBCs from healthy persons, IgE class-switching may occur later and block the effects of treatment with IFN- γ .

Conclusions: PBMCs from healthy persons and persons with asthma have clones reactive to allergen and produce functional IgE specific for relevant antigens in mite-sensitive bronchial asthma.

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Severe combined immunodeficient (SCID) mice lack functional T and B cells because of a defect in a DNA repair enzyme (1–3). These mice have been used as a model to study the function of the human immune system. For example, the sera of SCID mice that have received transplants of human peripheral blood mononuclear cells (PBMCs) have been assayed for production of IgG antibodies directed against specific antigens and for total IgE production (1–6). In addition, the regulation of total and specific IgE production in SCID mice has been determined *in vitro* (7, 8). Preincubation of PBMCs with interleukin (IL)-4 before transfer to SCID mice increases IgE production, whereas interferon (IFN)- γ has no effect (6).

Abbreviations. Df: *Dermatophagoides farinae* antigen; IFN- γ : interferon-gamma; IL-4: interleukin-4; OVA: ovalbumin; PBMC: peripheral blood mononuclear cells; PBS: phosphate-buffered saline; RAST: radioallergen sorbent test; SCID: severe combined immunodeficient.

The production of specific IgE antibodies was recently demonstrated in SCID mice repopulated with human PBMCs (9–11). Mice reconstituted with PBMCs from allergic patients sensitive to antigens of *Dermatophagoides pteronyssinus* or *Lolium perenne* and subsequently challenged with *D. pteronyssinus* antigen plus alum or *L. perenne* antigen, respectively, produced antigen-specific IgE antibodies (9–11). These results suggest that reconstituted SCID mice may be a model system for studying antigen-specific IgE production and the regulation of these antibodies during allergic responses.

An interesting question is whether healthy persons without allergies and persons with allergies have clones that are reactive to allergens and produce functional IgE specific for the relevant antigen in allergic diseases, including bronchial asthma. To answer this question, we used SCID mice reconstituted with PBMCs from healthy persons and with PBMCs from mite-sensitive

children with asthma, preincubated with *D. farinae* (Df) antigen, to assay production of Df-specific IgE and to study its modulation by IL-4 and IFN- γ . We also evaluated the histamine-releasing activity of the IgE derived from healthy subjects.

Material and methods

Subjects

PBMCs were isolated from samples of heparinized blood taken from four adult healthy subjects and from six patients with mite antigen-sensitive bronchial asthma, as defined by the diagnostic criteria for bronchial asthma of the American Thoracic Society (Table 1). Patients with active disease had had more than 10 mild or moderate asthma attacks per year. Each patient had a positive skin reaction, defined as an immediate wheal response to a skin prick test with house-dust-mite and Df antigen (Torii & Co., Tokyo, Japan), and had serum IgE scores against mite antigen, as determined with the CAP RAST fluoroenzyme immunoassay system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) (12), of from 3 to 6. None of these patients were having an asthma attack at the time of the examination, and none were receiving oral corticosteroids or antiallergic agents, such as ketotifen (13). Each patient was receiving 15–20 mg/kg per day of theophylline, a β_2 -agonist, or both, either continuously or intermittently. Informed consent was obtained from each patient or from the patient's parents.

In contrast, the four healthy subjects did not have allergic diseases, including eczema, allergic rhinitis, and bronchial asthma, and had no history of urticaria. Each healthy subject had negative skin prick tests with house-dust mite and Df antigen, and had serum IgE scores against mite antigen, as determined with the CAP RAST System (Pharmacia & Upjohn Diagnostics AB) (12), of zero (the value of specific IgE, less than 0.35 Ua/ml; cutoff points for positive RAST, 0.35 Ua/ml; detection limits for specific IgE, 0.15–0.20 Ua/ml) (Table 1).

Antigen stimulation and reconstitution of SCID mice with human PBMCs

PBMCs (1×10^7) were separated from heparinized samples of peripheral blood with Ficoll-Hypaque (Pharmacia) density gradient centrifugation (14) and suspended in 4 ml of serum-free RPMI 1640 medium containing 0, 4, or 40 mg of Df antigen (Torii & Co.) in a culture tube (Falcon Plastic 2054). After incubation for 4 h at 37°C in an atmosphere containing 10% CO₂, the cells were washed four times with phosphate-buffered saline (PBS) and resuspended in 500 ml PBS. Ten million cells were injected intraperitoneally with a

23-gauge needle into 8–12-week-old C.B.-17-SCID mice (CLEA Japan Corp., Tokyo, Japan) maintained under specific pathogen-free conditions without prophylactic antibiotic treatment in the animal facility of Saitama Medical School. Cells incubated with ovalbumin (OVA) (Sigma, St Louis, MO, USA) or Calmette-Guérin bacillus (Japan BCG Laboratory, Tokyo, Japan) at similar concentrations were used as controls. Sera were collected weekly for 6 weeks.

To assay the effects of IL-4 and IFN- γ , Df-stimulated cells were incubated for 4 h in a 10% CO₂ atmosphere with 0.1 or 100 U recombinant IL-4 (Genzyme Corp., Boston, MA, USA) and 100 or 1000 U recombinant IFN- γ (Shionogi, Inc., Tokyo, Japan), and then washed four times with PBS before injection into SCID mice. For further evaluation the effects of IFN- γ , mice received intravenous injections of the same doses of IFN- γ at the same time as they received Df-treated PBMCs and on the following day.

Measurement of specific IgE, IgM, and IgG activity

Levels of Df-specific IgE, IgM, and IgG in sera were measured with a slightly modified solid-phase enzyme-linked immunosorbent assay (ELISA) (15). Briefly, 96-well microtiter plates (Linbro, Flow Laboratories, Inc., MacLean, VA, USA) were incubated with Df (Torii & Co.; 50 μ g/ml in 0.1 M NaHCO₃ buffer, pH 9.6) overnight at 4°C in a humidified box. The wells were washed and incubated with 150 μ l 0.25% gelatin (Wako Pure Chemical Industries, Tokyo, Japan). After 100 μ l of a dilution of SCID mouse serum had been added to each well, the plates were incubated for 2 h at 4°C. The wells were emptied and washed three times with PBS, after which biotinylated mouse monoclonal antibody against human IgE, IgG, or IgM (Pharmingen, San Diego, CA, USA) was added. After incubation for 1 h at room temperature, the wells were washed three times with PBS, and streptavidin-conjugated horseradish peroxidase (Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, MD, USA) was added to each. The plates were incubated at room temperature for 20 min and washed six times with PBS/Tween, and 100 μ l *O*-phenylenediamine (Bethesda Research Laboratories Life Technologies, Inc., Gaithersburg, MD, USA) substrate solution was added to each well. The absorbance at 492 nm was measured with an ELISA reader (Bio-Tek Instruments, Inc., Burlington, VT, USA).

The results are expressed as pg/ml of serum IgE, IgM, and IgG, with human serum with high titers of Df-specific IgE, IgM, and IgG as a standard. The value of Df-specific IgE in standard human serum was evaluated as follows. The absorbance (492 nm) of the standard serum for Df-specific IgE was compared to that of human IgE (purified from myeloma cells) bound to anti-IgE-coated plates in ELISA systems. The value of Df-specific IgM and G was also evaluated in the same fashion. The sensitivity of this assay for specific IgE, IgM, and IgG was approximately 40, 65, and 80 pg/ml, respectively.

The specific activity of anti-Df IgE in human sera was determined with the CAP RAST System (Pharmacia & Upjohn Diagnostics AB) (12).

Evaluation of functional activity of Df-specific IgE

Basophil-enriched umbilical cord blood cells (5×10^5 /200 ml), cultured for 6 weeks in the presence of 1 ng/ml recombinant IL-3 (Kirin Brewery Co., Tokyo, Japan) (16, 17), were incubated for 16 h at 37°C, in a 10% CO₂ atmosphere, with 20 ml sera from PBMC-reconstituted SCID mice. The cells were subsequently incubated with 40 mg/ml Df or anti-IgE antibody for 90 minutes, after which histamine activity in the supernatants was analyzed fluorometrically by a method described elsewhere (18, 19). To verify the specific activity of histamine-release, the sera were incubated in wells coated with Df antigen or anti-IgE monoclonal antibody (Yamasa, Choshi, Japan; different from the antibody used in ELISA) for 3 h at room temperature, and the remaining activity was measured.

Table 1. Characteristics of subjects used in study

	Patients	Controls
Number of patients	6	4
Age (years)		
Median, Ranges	6, 3–10	29, 25–34
Sex (M:F)	3:3	3:1
Disease severity	Mild	(Nonallergy)
Documented attacks* (per year)	More than 10 mild or moderate attacks	
Medication	+ (intermittent or persistent)	—
Serum IgE (U/ml)	1139 $\pm$ 347	112 $\pm$ 51
Df-IgE CAP RAST score	3.7 $\pm$ 0.4	0 $\pm$ 0
Skin prick test (Df)	+	—

* Per year over previous 2 years.

Mean value $\pm$ standard error.

Data analysis

The data were represented as the mean $\pm$ standard error of the experimental value and were analyzed statistically with the two-tailed Student's *t*-test. Differences were considered significant when $P < 0.05$.

Results

Production of Df-specific human IgE in SCID mice reconstituted with patient PBMCs

Although mice reconstituted with both unstimulated and Df-stimulated PBMCs from patients with bronchial asthma produced Df-specific IgE within 1 week of reconstitution, production in mice reconstituted with 10 mg/ml Df-stimulated cells was significantly greater 3 weeks after reconstitution and gradually declined thereafter (Fig. 1). Cells stimulated with 1 mg/ml Df did not augment Df-specific IgE production in this way (data not shown). Because the results were similar in each of six independent experiments with one mouse and a single donor, all the results were pooled (Fig. 1). Although 10^7 cells induced significant Df-specific IgE production, no production was detected when 5×10^6 cells were transferred (data not shown).

To verify the specificity of the IgE produced, the reconstituted mouse sera were incubated for 3 h at room temperature in wells coated with anti-IgE monoclonal antibodies (Yamasa; different from the antibody used in ELISA) or with Df antigen, after which the remaining IgE activity was measured. We found that IgE activity was significantly lower in sera absorbed with Df antigen or anti-IgE antibody than in unabsorbed sera (data not shown). In contrast, we observed no change in activity when these sera were absorbed with anti-IgG polyclonal antibody (data not shown).

Although these data suggest that the sera of SCID mice that have received injections of Df-treated PBMCs are specific for both IgE and the Df antigen, stimulation with any antigen might have induced production of these antibodies. Therefore, to verify that Df-specific IgE is induced by only the Df antigen, SCID mice received injections of PBMCs treated with OVA or Calmette-Guérin bacillus, and the Df-specific IgE activity in sera was measured. We were unable to detect significant Df-specific IgE activity in the sera of these mice (data not shown), a finding that indicates that only Df antigen induces Df-specific IgE.

Induction of Df-specific human IgE in SCID mice reconstituted with PBMCs from healthy subjects

When mice were given injections of unstimulated or Df-stimulated PBMCs from healthy subjects with IgE RAST scores of 0 (Df-specific IgE was not detected or was detected at very low levels), we detected significant production of Df-specific IgE within 1 week of

reconstitution (Fig. 1). In contrast to results with PBMCs from patients with bronchial asthma, Df-specific IgE activity gradually increased from weeks 1 to 5, and decreased slightly thereafter (Fig. 1). Interestingly, the Df-specific IgE activity induced by cells from healthy subjects was comparable to or higher than that induced by cells from patients with bronchial asthma. Because the results were similar in four independent experiments with one mouse and a single donor, all results were pooled (Fig. 1B). In addition, as with mice that received injections of PBMCs from patients, the Df-specific activity was lower in the sera of mice that received injections of Df-treated control PBMCs that had been absorbed with anti-IgE monoclonal antibodies or Df antigen (data not shown).

Induction of Df-specific human IgM and IgG production in SCID mice reconstituted with PBMCs

Assays of isotypes of the Df-specific antibodies induced in SCID mice reconstituted with patients' PBMCs showed significant quantities of Df-specific IgM and IgG as well as IgE (data not shown). Similar results were obtained in mice reconstituted with Df-stimulated PBMCs from healthy subjects (data not shown); the maximal production of Df-specific IgM (day 1) was similar in both groups. Interestingly, however, the levels of Df-specific IgG were higher and were more rapidly induced in mice reconstituted with PBMCs from healthy persons (the maximal production of Df-specific IgG was on day 2 with PBMCs from patients).

Functional analysis of Df-specific IgE

Substantial amounts of histamine were found in the sera of mice that had received injections of PBMCs from patients with asthma or from healthy subjects that had been incubated with basophil-enriched umbilical cord-blood cell populations and treated with Df-antigen or anti-IgE antibody (Fig. 2). Histamine levels in sera collected 3 weeks after SCID mice had been reconstituted with PBMCs from healthy subjects were comparable to those in mice reconstituted with PBMCs from patients with asthma (Fig. 2). In contrast, histamine levels were much higher in week 5 than in week 3 and when mice were reconstituted with patients' PBMCs (Fig. 2). Together, these results indicate that PBMCs from nonatopic subjects induce stronger and more persistent Df-specific IgE responses in SCID mice.

These activities were abolished when the sera were absorbed with anti-IgE antibody or Df antigen; however, treatment with bovine serum albumin did not reduce histamine-releasing activity in the supernatants (data not shown).

Effect of treatment with IL-4 or IFN- γ on Df-specific IgE production

The Df-specific production of IgE was enhanced when

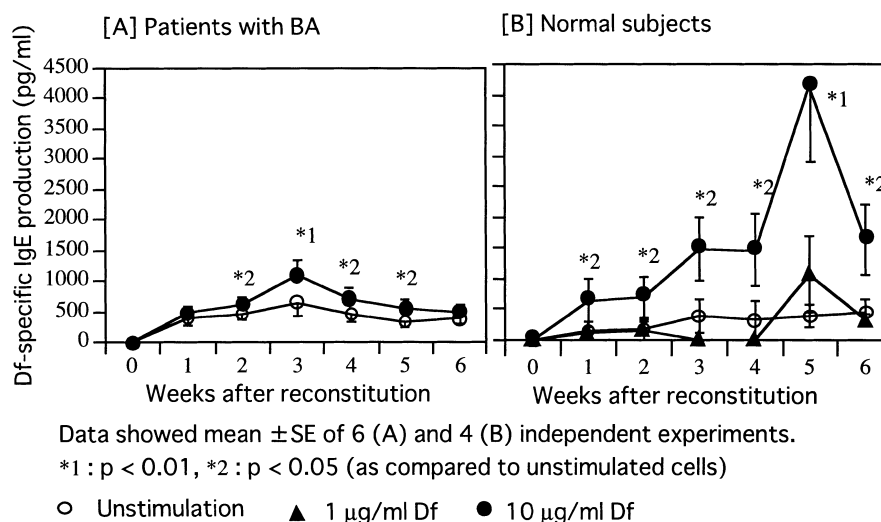


Figure 1. Production of Df-specific human IgE in SCID mice reconstituted with PBMCs. PBMCs (1×10^7) treated with 0, 4, or 40 mg Df antigen in 4 ml serum-free RPMI 1640 medium for 4 h at 37°C were resuspended in 0.5 ml PBS and injected intraperitoneally into SCID mice. Sera were collected on days indicated, and their Df-specific IgE activity was measured with solid-phase ELISA.

Df-stimulated PBMCs from patients or healthy subjects were treated with a high dose (100 U) of IL-4 before injection into SCID mice; enhancement was not observed when PBMCs were treated with a low dose (0.1 U) of IL-4 (data not shown). When PBMCs from patients or control subjects were pretreated with IFN- γ (100 or 1000 units) before injection into SCID mice, IgE production was not affected (data not shown). Therefore, the same doses of IFN- γ , intravenously injected into SCID mice at the same time as they received injections of Df-stimulated, but not unstimulated (data not shown), PBMCs, and on the following day, decreased Df-specific IgE production in mice reconstituted with PBMCs from patients with bronchial asthma (Fig. 3). However, IFN- γ had no effect on Df-specific IgE production by mice receiving injections of PBMCs from healthy subjects (Fig. 3).

Discussion

We have shown that SCID mice that received injections of Df-treated PBMCs from nonallergic healthy persons or patients with bronchial asthma sensitive to mite antigen produced Df-specific human Ig, especially IgE. This finding is somewhat surprising, because the IgE RAST score of each of the healthy persons was zero (Df-specific IgE levels were extremely low or undetectable). Thus, healthy persons appear to have allergen-specific memory lymphocytic populations and can produce Df-specific IgE at levels similar to or greater than those in patients with bronchial asthma.

For ethical reasons, the nonallergic subjects in this study were healthy adults, whereas the atopic subjects were children with asthma. Differences in the magnitude of antibody responses in SCID mice may reflect differences in the strength of immune responses in

adults and children. Similarly, the treatment of patients with theophylline is probably the reason that IgE production with patients' PMBCs was not higher than that with PBMCs from healthy persons. Inherent differences in the extent of human cell engraftment in SCID mice would also affect the magnitude of the antibody response. The data would be more convincing if, as in other studies, SCID mice had been reconstituted with PBMCs from a single donor. However, an interesting question is whether SCID mouse sera derived from PBMCs from healthy persons and containing specific IgE are clinically functional. The Df-specific IgE produced with PBMCs from healthy persons was as functionally active as that produced with patients' PBMC, and both IgEs induced histamine release from basophils.

Injections of Df-pulsed cells from nonallergic healthy persons generated specific IgG more quickly (day 1) than did Df-pulsed cells from patients (day 2). As with Df-specific IgE, the levels of Df-specific IgG were greater in healthy subjects than in allergic subjects; however, the levels of Df-specific IgM were similar in both groups. In contrast, maximal levels of Df-specific IgE were reached more slowly in nonallergic subjects. These data indicate that PBMCs from healthy subjects have clones sensitive to Df and may be primed at a later phase for the class-switching of cells that produce the Df-specific and biologically functional IgE.

Similar studies have been performed (9–11), but not in healthy, nonallergic subjects. Even if investigators examined antigen-specific IgE detected in SCID mice reconstituted with cells from healthy donors, they did not address the maximal production of IgE which occurred 2 weeks later than with cells from atopic donors. Furthermore, their methodology for initiating specific IgE production is different from ours. We found

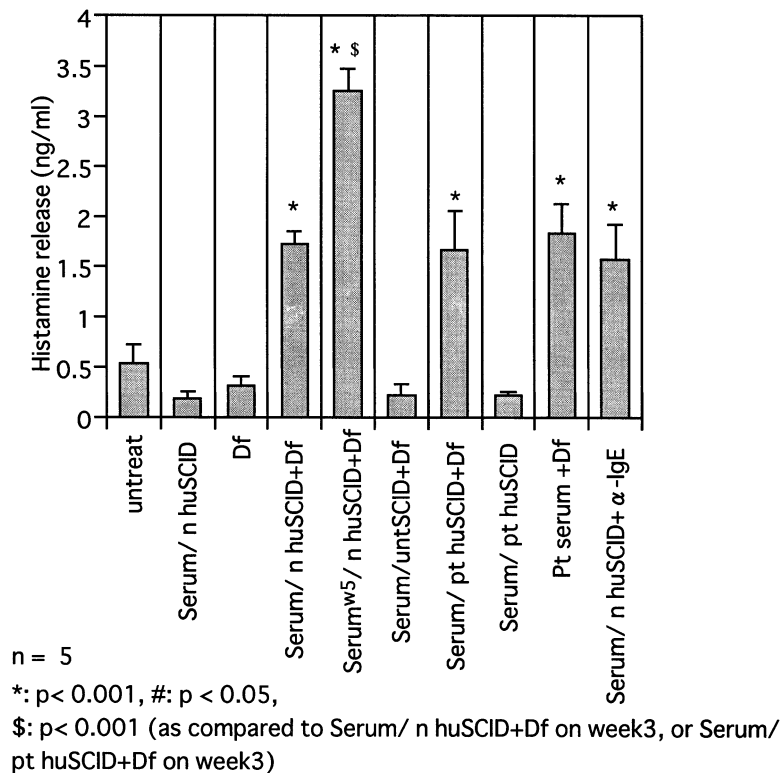


Figure 2. Histamine-releasing activity of sera of PBMC-reconstituted SCID mice. Basophil-enriched umbilical cord blood cells ($5 \times 10^5/200$ ml), cultured for 6 weeks in presence of 1 ng/ml recombinant IL-3, were incubated for 16 h at 37°C in 10% CO_2 atmosphere with 20 ml sera from PBMC-reconstituted SCID mice. Cells were subsequently incubated with 40 mg/ml Df or anti-IgE antibody for 90 min, after which histamine activity in basophil supernatant was measured fluorometrically. pt: patients; n: normal; huSCID: SCID mice receiving injections of PBMC; untSCID: untreated SCID mice; serum: collected on week 3; serum^{w5}, collected on week 5.

that even nonallergic, healthy individuals have clones that react to Df antigen and produce functional IgE specific for mite antigen.

Our finding that IL-4 modulates the production of Df-specific IgE is consistent with earlier studies that showed that incubation of PBMCs obtained from

atopic (6) or healthy (5) donors with IL-4 before reconstitution increases total (but not specific) IgE production. Our results with IFN- γ differed from previous findings, in that IFN- γ administration had no effect on the production of total IgE or IgG by mice reconstituted with PBMCs from atopic donors (6).

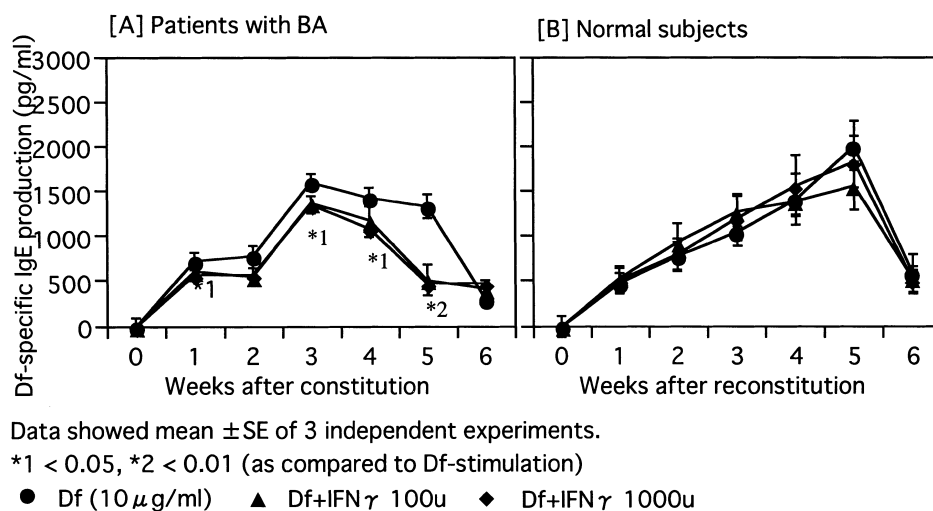


Figure 3. Effect of IFN- γ on Df-specific IgE production. SCID mice were given intravenous injections of 100 or 1000 units of recombinant IFN- γ at same time they were reconstituted with Df-treated PBMC and on following day. Sera were collected on days indicated, and the Df-specific IgE activity was measured as described in legend to Fig. 1.

Unlike our present study, both previous studies found modulation of total, but not specific, IgE production. Exogenous IFN- γ may regulate the allergen-induced class-switching phase of specific IgE synthesis, as suggested by our findings that Df-pulsed PBMCs from patients induced Df-specific IgE and that such induction was suppressed by IFN- γ . With PBMCs from healthy donors, IgE production peaked 2 weeks later than with PMBCs from atopic donors. In healthy donors, the class-switching of IgE probably occurs later, so that treatment with IFN- γ has no effect. When PBMCs were treated with IFN- γ before being injected into the mice, IgE production with either patients' cells or cells from healthy donors was unaffected (data not shown). *In vitro* coculture of PBMCs with IFN- γ probably enhances the ability of antigen-presenting cells to induce specific IgE production and prevents the suppressive effects of IFN- γ . IFN- α is more effective than IFN- γ in suppressing the production of IgE in healthy donors (6). Treatment with recombinant IFN- γ in patients who have atopic dermatitis or allergic rhinitis has varying outcomes (20–23). Our results suggest that the effect of IFN- γ treatment in allergic patients depends on the allergen-induced class-switching phase of the specific IgE production.

Our observations suggest that the reactive T cells from patients with allergy that mediate the IgE response are not fixed and are affected by external factors. In our study, Df-specific IgE was either undetectable or present at very low levels in sera from healthy donors. In contrast, serum levels of Df-specific IgG in healthy donors (22.3 ± 10.6 [SD] ng/ml, $n=4$) were similar to those in patients (46.5 ± 18.9 ng/ml, $n=6$). These results suggest that an IgE suppressor mechanism probably present in healthy donors *in vivo* is eliminated once the cells are injected into SCID mice; for example, IgE precursors might be stimulated in the heterologous environment to proliferate and secrete IgE. Otherwise, a Th2 shift would be expected in the cytokine response of the Df-specific T-cell population of the nonatopic donors isolated from SCID mice, suggesting that the T-cell population is influenced by the cytokine milieu of the donor.

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