

Allergens, IgE, mediators, inflammatory mechanisms

Establishment of human IgE system in severe combined immunodeficient mice with peripheral blood mononuclear cells from asthmatic children

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The frequency of allergic diseases, such as asthma, has increased rapidly during the past decade; however, the exact mechanisms have not been established. In this study we tried to establish an in vivo system to investigate immune regulation of allergic diseases by using severe combined immunodeficient (SCID) mice. Peripheral blood mononuclear cells isolated from asthmatic children or normal adults were injected into peritoneal cavities of SCID mice. Human IgG, IgA, IgM, and IgE could be detected in SCID mice reconstituted with human PBMCs (SCID-PBL-hu mice) 3 weeks later. Moreover, the mice injected with peripheral blood mononuclear cells from asthmatic children had much higher IgE levels than mice reconstituted with cells from normal adults. Phenotypic analysis of spleen cells and peritoneal exudate cells from SCID-PBL-hu mice demonstrated that human lymphocytes could survive in the peritoneal cavity and spleen for several months. After intraperitoneal immunization, mite-specific IgE antibodies could also be detected in SCID-PBL-hu mice. This study indicates that the human IgE system can be established in SCID mice and that this model can be used to study the regulation of IgE production and the immunopathogenesis of human allergic disease. (J ALLERGY CLIN IMMUNOL 1995;95:69-76.)

Key words: Human IgE system, asthma, severe combined immunodeficient mice, *Dermatophagoides pteronyssinus*

Severe combined immunodeficient (SCID) mice derived originally from the IgH^b congenic strain of BALB/c mice are characterized by the absence of functional T and B lymphocytes because of defect in a DNA repair enzyme.^{1,2} The inability to reject xenogeneic tissues makes SCID mice an excellent model with which to study human hematopoietic

Abbreviations used

EBV:	Epstein-Barr virus
LeuLeuOMe:	L-leucyl-L-leucine methyl ester
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
SCID:	Severe combined immunodeficiency
SCID-PBL-hu:	Severe combined immunodeficient mice reconstituted with human peripheral blood mononuclear cells

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and immune systems in vivo.³ SCID mice reconstituted with human fetal thymus or lymph nodes have been successfully used as a model to study human immunodeficiency virus infection and ther-

apy.^{4,5} Mosier et al.⁶ also introduced functional peripheral blood lymphocytes into SCID mice and demonstrated the appearance of tetanus toxoid-specific antibodies. Thereafter, SCID mice have been used as models for studying several human diseases such as autoimmune, tumor, and hematologic disorders.⁷⁻¹⁰

Regulation of IgE production, cytokine secretion, and development of type 2 T helper cells have been the central themes of the pathogenesis of allergic diseases.^{11,12} It has been well documented that allergen-specific T cells derived from atopic patients are type 2 T helper cells. Cytokines such as interleukin-4 and interleukin-5 produced by type 2 T helper cells are important for IgE production and eosinophilia in the inflammatory response. In contrast, allergen-specific T cells derived from nonatopic normal subjects are predominantly type 1 T helper cells.¹³⁻¹⁵ There are several animal models for studying the pathogenesis of atopic diseases¹⁶; however, most of these models still cannot mirror vividly the real human immune system. Therefore establishment of an in vivo human immune system with the use of SCID mice might be of great assistance in investigating the regulation of human IgE production and the immunopathogenesis of atopic diseases.

In this article we reconfirm and extend results of previous studies indicating that the immune response, which is mediated predominantly by type 2 T helper cells, can be reconstituted in SCID mice by injecting intraperitoneally the peripheral blood mononuclear cells (PBMCs) isolated from asthmatic children. This is evidenced by the presence, in the sera of SCID mice, of high levels of human IgE and mite-specific IgE antibody after immunization with mite antigens.

METHODS

Animals

C.B.-17-SCID/SCID mice were purchased from CLEA Japan Corp. (Tokyo, Japan) and maintained in a pathogen-free environment in the animal center of National Taiwan University College of Medicine. The mice were kept in sterilized cages with filter caps under a laminar flow hood and were fed antibiotics (1.5 mg/ml tetracycline) every 2 weeks. All food, water, and instruments were autoclaved or disinfected, and all procedures were performed under a laminar flow hood. Mice aged 5 to 9 weeks old were used in this study.

Subjects

Twenty-two asthmatic children with serum IgE levels greater than 2500 IU/ml were included in this study. All

of them were allergic to mite antigens (*Dermatophagoides pteronyssinus*) as confirmed by skin prick test and RAST (Pharmacia Diagnostic AB, Uppsala, Sweden). Six healthy adults with serum IgE levels less than 20 IU/ml were also included in this study as control subjects. Serum level of IgG antibody against Epstein-Barr virus (EBV) capsid antigens was determined with an indirect immunofluorescence kit (Gull Laboratories Inc., Salt Lake City, Utah). A titer of IgG anti-EBV capsid antigen higher than 1:160 was regarded as positive for EBV infection.

Reagents

Appropriate fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies such as anti-HLe-1 (CD45, clone 2D1), anti-Leu-M3 (CD14, clone MO-P9), anti-Leu 11 (CD16, NKP15), anti-Leu-2a (CD8, clone SK1), anti-Leu-3a (CD4, clone SK3), anti-Leu4 (CD3, clone SK7), anti-Leu-12 (CD19, clone 4G7), anti-Leu 18 (CD 45RA, clone L48), and anti-T-cell receptor 1(α/β , clone WT31) (Becton Dickinson, Mountain View, Calif.) and also anti-H-2K^d (PharMingen, San Diego, Calif.) were used for phenotypic analysis of peritoneal exudate cells and spleen cells isolated from SCID mice. Biotin-conjugated mouse anti-human IgE antibody (clone G7-26, PharMingen) was used in ELISA to detect mite-specific IgE antibody in the sera of SCID mice reconstituted with human PBMCs (SCID-PBL-hu mice).

Cell separation and reconstitution of SCID mice with human cells

Heparinized blood samples were collected from asthmatic children and six normal control subjects. PBMCs were isolated with Ficoll-Hypaque (Pharmacia Diagnostics AB). Briefly, cells at interface were removed carefully and washed 2 times before further experiments were performed. To further define the correlation between cell numbers and the success rate of engraftment, different numbers of PBMCs, ranging from 2 to 8×10^7 cells suspended in 1 ml of Hanks' balanced salt solution were injected into the peritoneal cavities of the SCID mice.

Determination of human immunoglobulin levels in SCID mice

Three weeks after reconstitution, SCID mice were bled retroorbitally to obtain blood samples, and serum levels of human immunoglobulin were determined. Human IgE levels were determined with an IgE radioimmunoassay (Pharmacia Diagnostics AB), and IgG, IgA, and IgM levels were measured with Immunochemistry Systems (Beckman Instruments, Inc., Galway, Ireland). Sera from control mice without any PBMC injection were used as negative controls.

Flow microfluorometric analysis

Some of the mice were killed by cervical dislocation. Peritoneal exudate cells were isolated by peritoneal

lavage, and phenotypic analysis was done by flow microfluorometric analysis. Aliquots of cells (2.5 to 5×10^5 cells) were suspended in 0.1 ml of phosphate-buffered saline (PBS) with 0.1% sodium azide, then incubated at 4°C for 30 minutes with a predetermined optimal concentration of appropriate fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies of CD45, CD14, CD4, CD8, CD3, CD19, CD16, CD45RA, or H-2K^d. The cells were washed and resuspended in 0.5 ml of PBS with 0.1% sodium azide and subjected to flow microfluorometric analysis. A total of $10,000$ cells were counted, and the frequency and mean density of each cell surface marker were determined with appropriate software (FACScan, Becton Dickinson). Controls were cells suspended in medium.

Preparation of mite antigen

The allergen was prepared as described by Lin et al.¹⁸ Briefly, 1 gm of lyophilized whole mite body (Pharmacia Diagnostics AB) was defatted with 100 ml of ether, then homogenized and stirred continuously in 100 ml of PBS for 36 hours. After centrifugation ($10,000$ g), the crude extract was filtered through a 0.22 μm membrane, lyophilized, and stored at -70°C until use. Before use, the lyophilized extract was reconstituted with distilled water, and the protein concentration of mite antigen was determined with a Bio-Rad kit (Bio-Rad Laboratories, Hercules, Calif.).

In vivo immunization

To further enhance the mite-specific antibody production in reconstituted SCID mice, mice were immunized with 100 μg of mite antigen or 100 μg of mite antigen-aluminum hydroxide mixture on the second day after injection of human PBMCs. Three weeks later, the mice were immunized again and were bled 1 to 2 weeks later for determination of mite-specific IgE antibody. Control mice were injected with PBS only.

Determination of mite-specific IgE antibody

ELISA was used to determine mite-specific IgE antibodies in the sera of SCID mice. Briefly, 100 μl of 50 $\mu\text{g}/\text{ml}$ mite antigen in carbonate buffer (pH 9.6) was coated on the ELISA plates (Nunc-Immuno plates; Nunc, Inc., Roskilde, Denmark) at 4°C overnight. After washing 2 times with PBS containing 0.05% Tween 20, the plates were blocked with 1% bovine serum albumin-PBS at room temperature for 2 hours. Then, 100 μl of serial diluted sera from SCID-PBL-hu mice and human donors was added to the plates and incubated at room temperature for 2 hours. After washing 3 times with PBS-Tween, optimally diluted biotin-conjugated mouse anti-human IgE antibody (PharMingen) was added to the plates and incubated at room temperature for 2 hours. Avidin conjugated with horseradish peroxidase (Pierce Chemical Co., Rockford, Ill.) in 1% bovine serum albumin-PBS was added to the plates and incubated at room temperature for 2 hours. After washing 3

TABLE I. Summary of results of reconstituting SCID mice with human PBMCs

Donor	No. of SCID mice	IgE (>2 IU/ml)	IgG (>10 $\mu\text{g}/\text{ml}$)
Asthmatic children*			
$\leq 4 \times 10^7$ cells	9	7/9	7/9
$> 4 \times 10^7$ cells	12	10/12	10/12
Normal control†			
$> 4 \times 10^7$ cells	6	1/6	6/6
Asthmatic children‡			
EBV-negative	9	8/9	8/9
EBV-positive	12	9/12	9/12

*At least 2×10^7 PBMCs were injected into the SCID mice intraperitoneally; serum IgE and IgG levels were not different between mice receiving 4×10^7 PBMCs or less and those receiving more than 4×10^7 PBMCs.

†All were EBV-positive.

‡Both serum IgE and IgG levels were not different between mice receiving EBV-positive PBMCs and those receiving EBV-negative PBMCs.

times with PBS-Tween, o-phenylenediamine (Sigma Chemical Co., St. Louis, Mo.) in adequate buffer was used for substrate development and stopped with 1% H_2SO_4 . Absorbance was determined at 492 nm on a Microplate Reader (Metertech Inc., Taipei, Taiwan). The results of mite-specific IgE antibody analysis were presented as ELISA units (EU/ml) compared with human control serum (with a concentration of 1 U/ml *D. pteronyssinus*-specific IgE antibody, as determined by Pharmacia CAP system).

Statistical analysis

Chi square test and Student's *t* test were used to analyze the data throughout this study.

RESULTS

Appearance of human immunoglobulins in SCID mice

Three weeks after reconstitution, human immunoglobulins could be detected in the sera of SCID mice reconstituted with PBMCs from either asthmatic children or normal adults (Table I). As documented, at least 1×10^7 PBMCs are necessary to achieve successful engraftment of the human immune system in SCID mice.¹⁹ At least 2×10^7 PBMCs from asthmatic children were injected into the peritoneal cavities of the mice in our study. Our results showed that there was no significant correlation between increased cell numbers greater than 4×10^7 and success rate of engraftment. Human immunoglobulin could be detected in 17 of 21 (80.9%) SCID-PBL-hu mice sera 3 weeks after reconstitution (Table I). Of nine EBV-

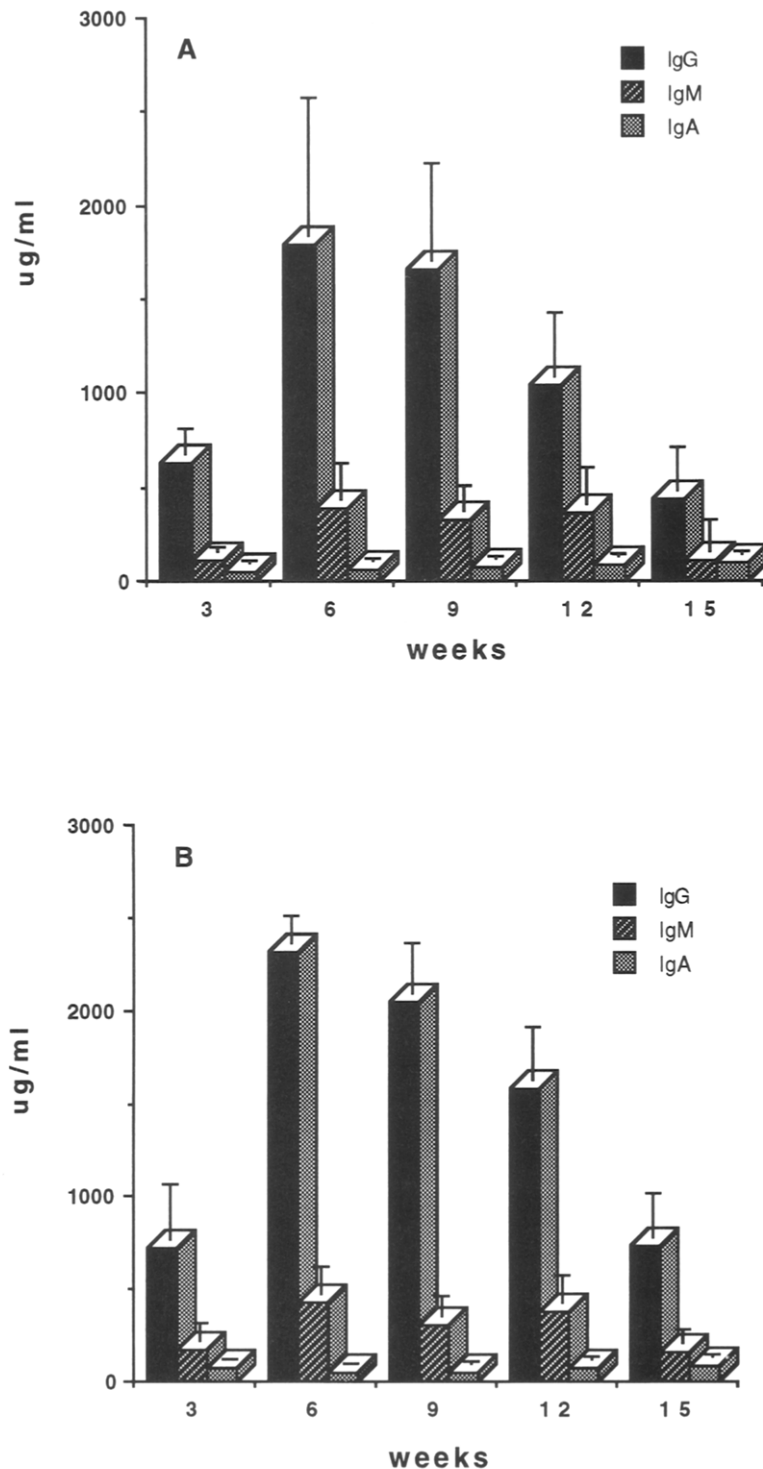


FIG. 1. IgG, IgA, and IgM levels in SCID mice reconstituted with PBMCs from asthmatic children (A) and normal adults (B). The patterns were similar between the two groups. Human immunoglobulins peaked at 6 weeks after reconstitution and declined thereafter.

negative PBMC-reconstituted SCID mice, eight showed the presence of human immunoglobulins. There was no significant difference in the success rate of engraftment between EBV-negative and

EBV-positive PBMCs ($p > 0.05$). Human immunoglobulin could be detected in all six SCID mice injected with PBMCs from normal adults, and the levels of IgG, IgA, and IgM were similar between

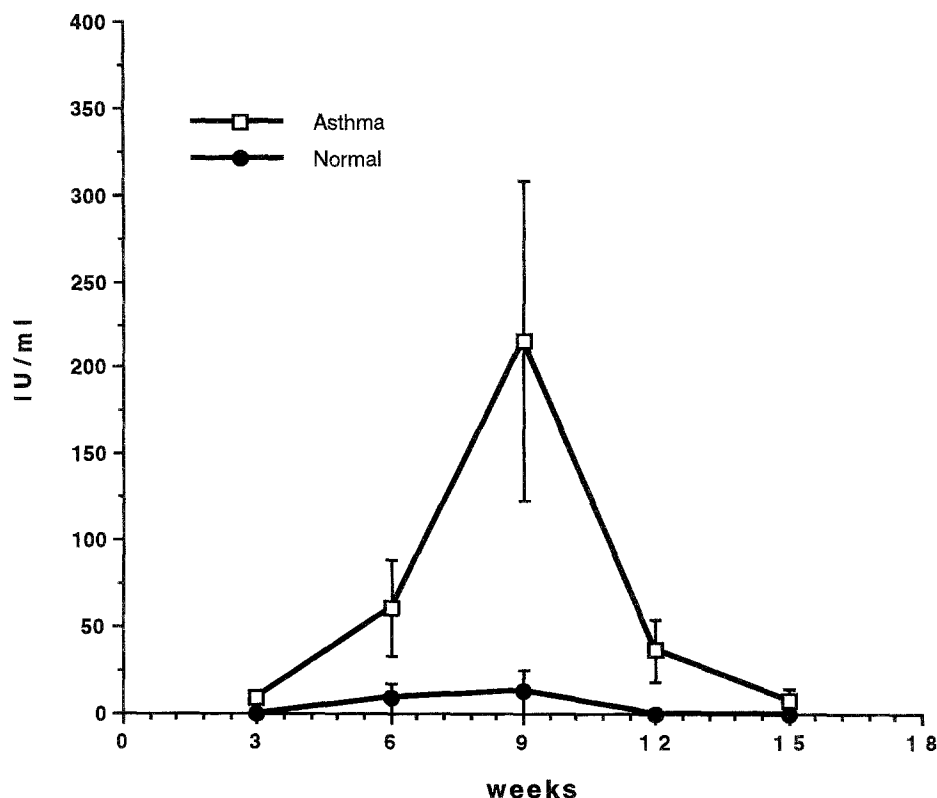


FIG. 2. Human IgE could be detected in mice sera 3 weeks after reconstitution with PBMCs from asthmatic children, and the level peaked at 9 weeks ($n = 21$). In contrast, only a trace of IgE could be detected in sera of mice reconstituted with PBMCs from normal adults ($n = 6$).

TABLE II. Phenotypic analysis of peritoneal exudate cells and spleen cells from SCID-PBL-hu mice

	CD45	CD3	CD19	CD4	CD8	H-2K ^d
Peritoneal exudate cells*						
1 mo	59.7 ± 30.4	56.4 ± 20.3	5.8 ± 7.9	34.6 ± 23.3	23.6 ± 13.9	22.4 ± 11.6
4 mo	26.6 ± 30.4	21.5 ± 21.5	0	12.0 ± 16.0	22.3 ± 20.6	75.9 ± 18.3
8 mo	1.5 ± 1.8	2.5 ± 3.2	0	1.2 ± 1.6	1.2 ± 0.6	93.8 ± 8.3
Spleen cells*						
1 mo	2.2 ± 1.2	1.8 ± 0.4	0.8 ± 0.5	1.7 ± 2.3	1.6 ± 0.4	94.7 ± 6.1
4 mo	1.0 ± 0.2	0.3 ± 0.3	0.8 ± 0.3	0.4 ± 0.1	1.2 ± 1.0	93.7 ± 0.2
8 mo	ND	ND	ND	ND	ND	ND

ND, Not done.

*Three to four mice per group of SCID-PBL-hu mice were killed, and cells were pooled for phenotypic analysis.

atopic and nonatopic groups (Fig. 1, *A* and *B*). The human immunoglobulin started to appear 3 weeks after reconstitution, increased gradually, and peaked at 6 to 9 weeks after injection. The IgG level averaged between 1 and 3 mg/ml (1.8 ± 0.7 mg/ml for SCID-PBL-hu mice reconstituted with PBMCs from asthmatic patients; 2.3 ± 0.2 mg/ml for mice reconstituted with PBMCs from normal adults), and IgA and IgM levels ranged from about 50 to 400 μ g/ml (6 weeks after reconstitution: IgA, 66.4 ± 8.5 μ g/ml; IgM, 392 ± 193.4 μ g/ml). These

data are in agreement with results of previous studies by other groups. It is important to note that only one of six mice reconstituted with PBMCs from normal adults had human IgE in its serum. In contrast, human IgE could be detected in all the mice (17 of 17) that had been successfully reconstituted with PBMCs from atopic patients. In the latter cases IgE levels reached the peak about 9 weeks after reconstitution (215.1 ± 91.3 IU/ml) and declined thereafter (Fig. 2). In addition, there was no significant difference in IgE levels between

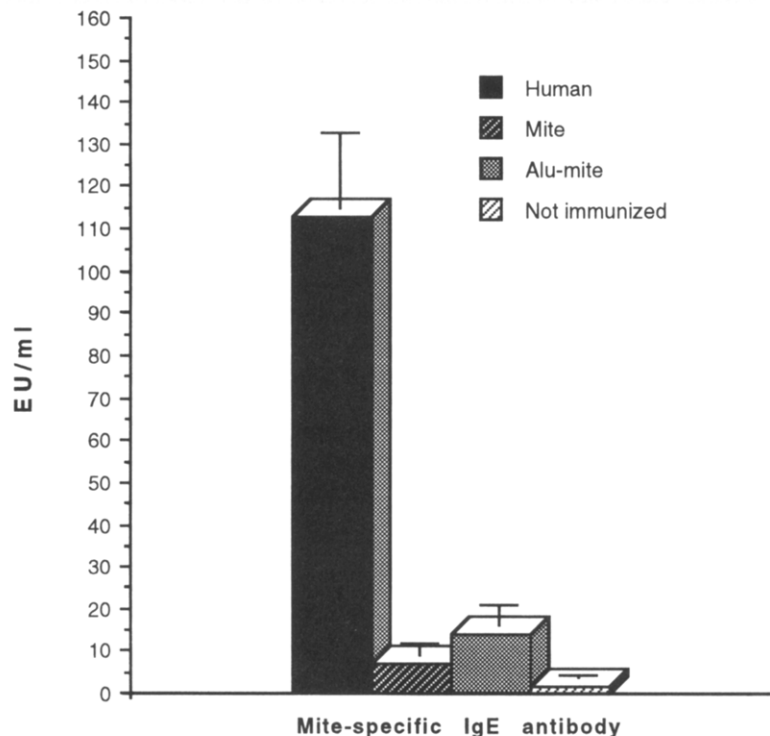


FIG. 3. Serum levels (diluted 1:2) of mite-specific IgE antibodies in human sera of PBMC donors (112.9 ± 17.8 EU/ml, $n = 6$) in SCID-PBL-hu mice immunized with mite-aluminum hydroxide (14.1 ± 4.5 EU/ml, $n = 6$), with mite antigens in PBS (6.9 ± 2.5 , $n = 7$), and with PBS only (1.5 ± 0.4 EU/ml, $n = 5$).

the EBV-negative group (229.77 ± 123.4 IU/ml) and the EBV-positive group (203.3 ± 126.6 IU/ml) at 9 weeks after reconstitution.

Phenotypic analysis of peritoneal exudate cells and spleen cells

Three to four SCID mice were killed separately at 1, 4, and 8 months after reconstitution to obtain peritoneal exudate cells and spleen cells for phenotypic analysis. One month after injection, a very high percentage of the lymphocytes in the peritoneal cavity was of human origin and stained predominantly with T-cell markers ($56.4\% \pm 20.3\%$) with a CD4/CD8 ratio of 1.47 (Table II). A few B cells were detected at 1 month and disappeared rapidly afterwards. In contrast, only few human cells could be detected in the spleen. The presence of human immunoglobulins in SCID mice might be closely related to the survival of human lymphocytes in SCID mice.

Mite-specific IgE antibody

To determine whether an allergen-specific human IgE immune response could be established in

SCID mice, we assayed mite-specific IgE in SCID-PBL-hu mice with and without immunization with mite antigen by using ELISA. The mite-specific IgE antibody could not be detected in SCID-PBL-hu mice without allergen immunization. After *in vivo* immunization with mite antigen on days 1 and 22 after reconstitution, mite-specific IgE antibody could be detected in the sera of SCID-PBL-hu mice (Fig. 3). The mice immunized with mite-aluminum hydroxide mixture (14.1 ± 4.5 EU/ml, $n = 6$) and mite antigen in PBS (6.9 ± 2.5 EU/ml, $n = 7$) showed higher serum titers of mite-specific IgE antibody compared with the mice without immunization (1.5 ± 0.4 EU/ml, $n = 5$). However, the titers in mice sera were still much lower compared with human sera of PBMC donors (112.9 ± 17.8 EU/ml, $n = 6$).

DISCUSSION

SCID mice, characterized by inability to reject xenogeneic tissues, have been used as models to study several human diseases such as acquired immunodeficiency syndrome, systemic lupus erythematosus, primary biliary cirrhosis, and rheuma-

toid arthritis.⁴⁻⁹ Potential use of SCID mice as an animal model for human diseases such as hematopoiesis and cancer has also been suggested.^{10, 20}

For reconstitution of SCID-PBL-hu mice, most studies suggested that intraperitoneal rather than intravenous injection would achieve a much higher success rate. Low-dose irradiation of SCID mice or treatment of PBMCs with LeuLeuOMe before injection to eliminate murine or human natural killer cells has also been shown to increase the chance of engraftment.^{21, 22} However, further studies demonstrated no difference between irradiated and nonirradiated groups in establishing SCID-PBL-hu mice. Previous studies also suggested that EBV infection was another major factor that might affect the outcome of PBMC engraftment in SCID mice. PBMCs from EBV-negative patients survived longer in SCID mice. Nonetheless, EBV-positive B cells can survive in SCID mice for up to 2 months and produce human immunoglobulins.¹⁹ Our results suggested no significant difference in success rate and IgE level between SCID mice reconstituted with EBV-negative PBMCs and those reconstituted with EBV-positive PBMCs. Moreover, in contrast to other reports,^{23, 24} no lymphoproliferative diseases were noted in SCID mice in this long-term follow-up study for up to 10 months. Different strains of EBV endemic to our area or exposure much earlier in life might explain the different manifestations *in vivo*.

Previous reports suggested that low levels of IgE can be detected in the sera of mice reconstituted with PBMCs from normal adults and that the levels were correlated with human serum levels.^{25, 26} Our study has demonstrated that a greater human IgE immune response can be established in SCID mice with PBMCs from asthmatic children. One short report on IgE production in SCID mice with PBMCs from atopic patients has been published recently.²⁷ In that study human IgE production reached the maximum 4 weeks after reconstitution with PBMCs from two patients with atopic dermatitis and declined thereafter. In contrast, human IgE levels in our study reached the peak after 9 weeks. The difference in sample size and number of PBMCs transfused might account for the discrepancy.

Although mite-specific IgG could be demonstrated, no mite-specific human IgE antibody could be detected in sera of mice without antigen immunization, even though human IgE levels in mice sera were greater than 1000 IU/ml. Several studies have suggested that restricted immunoglobulin genes subfamily utilization developed and skewed

as limited B-cell repertoires after reconstitution of PBMCs in SCID-PBL-hu mice.^{25, 28} It is interesting to note that immunization of mice with antigens was essential to rescue antigen-specific antibody response in SCID-PBL-hu mice. Phenotypic analysis also showed that only a few CD14⁺ monocyte lineage cells existed in the peritoneal cavity, and both monocytes and B cells persisted only for a short period. Early immunization with antigen is therefore necessary to elicit an antigen-specific immune response, and immunization with aluminum hydroxide can evoke even higher antibody titers. Although early immunization with mite allergen could lead to mite-specific IgE antibody production in SCID mice, the titer would still be lower than that in human sera. A previous study suggested that *in vitro* incubation with antigens before injection could even rescue antigen-specific antibody even though the human donor's last contact with antigen was more than 17 years ago.²⁹ Therefore *in vitro* incubation with mite antigen before injection may be worth trying, in order to elicit higher antigen-specific antibody production in the SCID-PBL-hu model.

The availability of SCID mice provided a useful *in vivo* model with which to study the pathogenesis of a variety of human diseases. Herein we confirm and extend results of previous studies indicating that not only total human IgE but also mite-specific IgE antibody production can be established in SCID mice reconstituted with PBMCs from atopic patients. This model will provide an excellent tool with which to study the regulatory mechanisms of IgE production, including cytokines and type of T-cell subsets that lead to the development of atopic diathesis.

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