Deficient cytokine response of human allergen-specific T lymphocytes from humanized SCID mice and reconstitution by professional antigen-presenting cells

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Background: Hu-PBL-SCID mice generated by the transfer of PBMCs from atopic individuals may provide a physiologic in vivo model for investigating human responses to allergens and potential approaches toward immunotherapy.
Objective: This study was undertaken to investigate the functional activity and cytokine profile of human allergen-reactive T lymphocytes isolated from hu-PBL-SCID mice.
Methods: PBMCs from allergic individuals were coinjected with allergen into SCID mice. Human lymphocyte migration and phenotype were established by reverse transcription–PCR and immunohistochemistry, IgE levels in sera were determined, and the frequency of allergen-reactive cytokine-producing T lymphocytes was established.
Results: After immunization with allergen, specific IgE levels in hu-PBL-SCID sera were comparable with levels in donor sera. Although the majority of lymphocytes remained in the peritoneum, significant numbers of T lymphocytes were located in the spleen, where human IL-4, IL-5, and IFN-γ messenger RNA expression was detected after stimulation with PHA and phorbol myristate acetate. Failure to induce cytokine production by human T lymphocytes isolated from the peritoneum and spleen of hu-PBL-SCID mice by allergen was reversed by stimulating with allergen in the presence of exogenously added IL-2 and antigen-presenting cells (APC), particularly CD14$^+$ monocytes. Under these conditions, allergen-reactive T cells expressed a T_H2-like phenotype.
Conclusions: These data suggest that, after initial activation and induction of antibody production, human T lymphocytes enter a state of unresponsiveness, arising from a loss of human professional APC, in hu-PBL-SCID mice. The use of hu-PBL-SCID mouse models in studies on therapeutic approaches for allergy may benefit from the additional transfer of human professional APC. (J Allergy Clin Immunol 2000;105:967-74.)

Key words: *Allergy, anergy, hu-PBL-SCID, IgE*

Abbreviations used

APC:	Antigen-presenting cells
cDNA:	Complementary DNA
DC:	Dendritic cells
hu-PBL-SCID:	Humanized–peripheral blood leukocyte–severe combined immunodeficient
mRNA:	Messenger RNA
PMA:	Phorbol myristate acetate
RT:	Reverse transcription
TBS:	TRIS-buffered saline solution

Investigations of immune mechanisms underlying pathogenic human allergic responses are limited to the use of in vitro culture systems, which do not reflect complex cellular interactions occurring in vivo. Although they have provided considerable insight into mechanisms underlying allergic disease, animal in vivo models do not accommodate intrinsic differences between species in their physiologic features. This has led to increasing interest in the use of humanized severe combined immunodeficient (SCID) mice as a means of investigating human allergic responses and therapeutic approaches in a more physiologic environment. Because of an autosomal recessive mutation these mice lack T and B lymphocytes[1] and are therefore incapable of rejecting xenogeneic grafts.[2] It is therefore possible to establish human responses in SCID mice after the intraperitoneal transfer of PBMCs.[3] A number of groups have demonstrated that human IgE responses can be established in SCID mice after transfer of PBMCs from allergic individuals.[4-7] However, little is understood about the functional activity of allergen-reactive human T lymphocytes in SCID mice.

Atopic individuals are characterized by production of high levels of the T_H2 cytokines IL-4 and IL-5 and low or negligible levels of IFN-γ by allergen-reactive CD4$^+$ T cells after stimulation. These cytokines are largely responsible for the pathogenesis of allergic disease.[8,9] Because IgE production in humanized–PBL–immunodeficient (hu-PBL-SCID) mice is transient, with levels rapidly falling within 4 weeks after cell transfer, we were interested in investigating the cytokine response of aller-

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gen-reactive $CD4^+$ T lymphocytes, which are central to the induction and regulation of IgE production by B cells. A number of studies have shown that a proportion of human lymphocytes engrafted in hu-PBL-SCID mice is xenoreactive.[10,11] This raises the question as to what extent the microenvironment of the SCID mouse and the presence of human T_H1-mediated graft-versus-host responses, which are associated with high levels of IFN-γ production, influence the cytokine phenotype of human allergen-reactive T cells in hu-PBL-SCID mice.

With use of PBMCs from atopic individuals, we have established the hu-PBL-SCID mouse as an in vivo model for investigating human responses to allergens and potential immunotherapeutic approaches. The aim of our study was to investigate the functional activity and cytokine profile of allergen-reactive T lymphocytes isolated from hu-PBL-SCID mice.

METHODS

Animals

C.B.-17 scid/scid mice, 6 to 8 weeks old, were obtained from M&B A/S (Ry, Denmark) or the Medizinische Hochschule Hannover. All mice were screened for "leakiness" by analysis of serum IgG levels. The mice were kept in microisolator cages and were provided with autoclaved food and water. All experimental procedures were performed in a laminar air-flow hood. Animal experiments were approved by the regional Ethics Commission according to German federal law.

Donors

PBMCs were obtained from atopic donors sensitized to aeroallergens of house dust mite *Dermatophagoides pteronyssinus* or Timothy grass pollen *Phleum pratense.* The donors had a history of atopic dermatitis and/or asthma and rhinitis, exhibited a positive skin-prick test, and radioallergosorbent test scores for specific IgE between 2 and 4. The study was approved by the regional Ethics Commission. Written informed consent was obtained from the blood donors.

PBMC isolation, cell transfer, and immunization

PBMCs isolated from heparinized blood by density centrifugation were transferred intraperitoneally ($5\text{-}10 \times 10^7$) into SCID mice, which were immunized with 20 μg of either *D pteronyssinus* or *P pratense* or grass pollen extract (kind gifts of Dr S. Sparholt, ALK, Horsholm, Denmark), at the time of transfer and 7 days thereafter. At weekly intervals mice were bled from the tail vein, blood samples were centrifuged, and the serum was stored at –20°C.

Determination of total and allergen-specific human IgE levels in sera

Total and allergen-specific IgE levels in sera were determined by the total-IgE JEMA and the AT-PLUS allergy assay EAST, respectively, according to the protocol of the manufacturer (DDV Diagnostika, Marburg).

Immunohistochemical analysis

Organs were removed from hu-PBL-SCID mice and snap-frozen in liquid nitrogen. Cryostat sections (5 μm) were mounted on glass slides, air dried overnight, and fixed in anhydrous acetone for 10 minutes at room temperature. Tissue sections were washed in TRIS-buffered saline solution (TBS, 50 mmol/L TRIS-[hydroxymethyl]aminomethane–hydrochloric acid, 150 mmol/L sodium chloride, pH 7.6). Unless mentioned otherwise, reagents were purchased from DAKO, Hamburg. Antibodies were diluted in TBS/1% BSA (Sigma Chemical, St Louis, Mo). To prevent nonspecific binding of antibodies, sections were blocked for 30 minutes with TBS/1% BSA containing 10% hu-PBL-SCID serum and 20% rabbit serum. The sections were incubated for 1 hour with mouse antibodies specific for human molecules CD45 (HI30), CD3 (UCHT1), CD4 (MT 310), or CD19 (HIB19) or with mouse isotype control IgG1. Antibodies, except anti-CD4, were purchased from PharMingen, San Diego, Calif. Sections were washed in TBS and incubated for 30 minutes with rabbit antimouse Igs, washed, and incubated for a further 30 minutes with soluble complexes of alkaline phosphatase and antialkaline phosphatase antibodies. The intensity of staining was enhanced by reincubation for 15 minutes with the above reagents. As an additional control, parallel sections were incubated with the secondary antibodies alone. Staining was visualized with the New Fuchsin red substrate system. Endogenous alkaline phosphatase activity was inhibited with levamisole. The sections were washed with water and the cell nuclei were counterstained blue with hematoxylin (Merck, Darmstadt) before mounting in glycergel.

ELISPOT assay

MultiScreen-IP 96 well plates (Millipore, Eschborn) were coated overnight at 37°C with 70 μL per well of rat antihuman IL-5 (TRFK5), mouse antihuman IL-4 (MP4-25D2), both from PharMingen, or mouse antihuman IFN-γ (1-D1-K; Hölzel Diagnostika, Köln) at 10 μg/mL in PBS. Binding sites were blocked for 1.5 hour with PBS/3% BSA. Single-cell suspensions isolated from the spleen (15×10^4/well) or peritoneum ($5\text{-}15 \times 10^4$/well) were added to the wells. The cells were cultured at 37°C in 200 μL of RPMI 1640 medium supplemented with 2.5% AB + serum, 2 mmol/L L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin (Gibco, Paisley, UK) alone or in the presence of 20 μg/mL *D pteronyssinus* and/or 20 U/mL recombinant human IL-2 (a kind gift of Cetus Corporation, Emeryville, Calif) and/or 5×10^4 per well irradiated (30 gray) autologous PBMCs or 5 μg/mL PHA plus 10 ng/mL phorbol myristate acetate (PMA). From autologous PBMCs $CD14^+$, $CD19^+$, or $CD83^+$ cells were depleted or positively isolated by immunomagnetic beads coupled to the respective antibodies (Dynal, Hamburg). Efficient depletion was verified by fluorescence-activated cell sorter analysis. After 5 days the wells were washed, and 100 μL per well biotinylated rat antihuman IL-4 (2 μg/mL; 12.1, Hölzel), IL-5 (1 μg/mL; JES1-5A10, PharMingen), or IFN-γ (3 μg/mL; 7-B6-1, Hölzel) diluted in PBS/1% BSA/0.05% Tween 20 were added to the relevant wells and incubated for 1 hour at 37°C. After washing, the avidin-biotin complex (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif) was added and plates were incubated for 1 hour at 37°C. After washing, positively stained cells were detected with use of the 3-amino-9-ethylcarbazole substrate (Sigma) in 0.1 mol/L sodium acetate buffer, pH 5. All washes were performed with PBS/0.05% Tween 20. The number of immunostained cytokine spots per well was determined by video image analysis (Quantimet 600, Leica, Cambridge, UK).[12]

Reverse transcription–PCR

Cell pellets were homogenized in messenger RNA (mRNA) extraction buffer and mRNA prepared with the Quick-Prep Micro mRNA Purification Kit (Pharmacia Biotech, Freiburg). Reverse transcription (RT)–PCR was performed as described.[13] The primers huACT-1 (CCAGCCATGTACGTTGCT) and huACT-2 (CTTCTCCAGGGAGGAGCT) amplify a 327-bp fragment of human β-actin, IFN-γ-1 (CTCTTGGCTGTTACTGCCAG) and IFN-γ-2 (TCCTT-

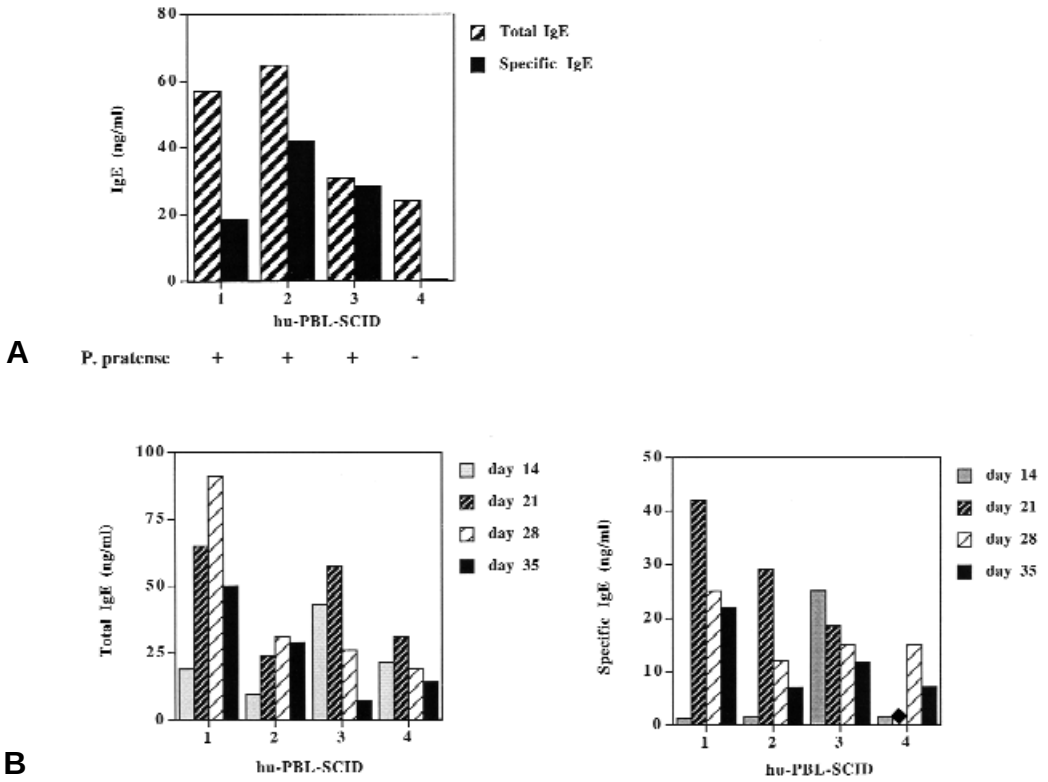

FIG 1. Antigen dependence and kinetics of human IgE production in hu-PBL-SCID mice. **A,** Production of detectable levels of specific IgE in hu-PBL-SCID sera is dependent on immunization with allergen. A group of 4 hu-PBL-SCID mice were generated by intraperitoneal transfer of 5×10^7 PBMCs from a single atopic donor. Three mice were immunized with *P pratense* at the time of cell transfer, and 7 days thereafter the fourth mouse received PBS alone. Total and *P pratense*–specific IgE levels in sera were determined 21 days after cell transfer. **B,** Kinetics of IgE production. Hu-PBL-SCID mice generated with 5×10^7 PBMCs from an atopic donor were bled weekly for 35 days after cell transfer. Total and *P pratense*–specific IgE levels in sera were determined by ELISA. *Solid diamond,* Insufficient sera available.

GATGGTCTCCACACT) amplify a 240-bp fragment of human IFN-γ, and IL-4-1 (GCAACTTTGTCCACGGACAC) and IL-4-2 (TCCAACGTACTCTGGTTGGC) amplify a 345-bp fragment of human IL-4 (MWG-Biotech, Ebersberg). Human IL-5 primers, which amplify a 271-bp fragment, were purchased from R&D systems (Wiesbaden, Germany). All primers were tested with human PBMCs and murine splenocytes to ensure that only human DNA fragments were amplified. The annealing temperature for IL-4 was $1 \times 58°C$, $2 \times 57°C$, $2 \times 56°C$, $30 \times 55°C$; for IL-5 it was $1 \times 61°C$, $2 \times 60°C$, $2 \times 59°C$, $30 \times 58°C$; and for IFN-γ it was $2 \times 53°C$, $2 \times 52°C$, $2 \times 51°C$, $25 \times 50°C$. β-actin complementary DNA (cDNA) was amplified in 30 cycles with a constant annealing temperature of 60°C.

RESULTS

IgE responses to allergen in hu-PBL-SCID mice

To verify that the hu-PBL-SCID model had been successfully established, the antibody response of hu-PBL-SCID mice was determined by measuring total and specific IgE levels in sera 21 days after transfer. Serum IgE levels in 8 groups of hu-PBL-SCID mice generated from individuals sensitized to aeroallergens of either *D pteronyssinus* or *P pratense* and immunized with the relevant allergen are presented in Table I. Whereas specific IgE levels in hu-PBL-SCID sera were related to levels in donor sera at the time of cell transfer, total IgE levels in hu-PBL-SCID sera were generally much lower. The production of detectable levels of allergen-specific human IgE in hu-PBL-SCID mice is dependent on immunization with the relevant allergen. In a representative group of hu-PBL-SCID mice, analysis of serum IgE levels show that, although hu-PBL-SCID mice immunized with allergen had significant levels of both total IgE and *P pratense*–specific IgE, no specific IgE was detectable in sera obtained from the hu-PBL-SCID mice injected with PBS (Fig 1, *A*). To investigate the kinetics of IgE production, hu-PBL-SCID mice were bled weekly after cell transfer. Substantial levels of total IgE and allergen-specific IgE in sera were observed between 14 and 35 days after cell transfer (Fig 1, *B*).

Migratory potential of human cells to SCID peripheral lymphoid organs

We confirmed the migration of human lymphocytes to peripheral organs by RT-PCR with use of human β-actin specific primers (data not shown). The extent of migration and the phenotype of these lymphocytes were deter-

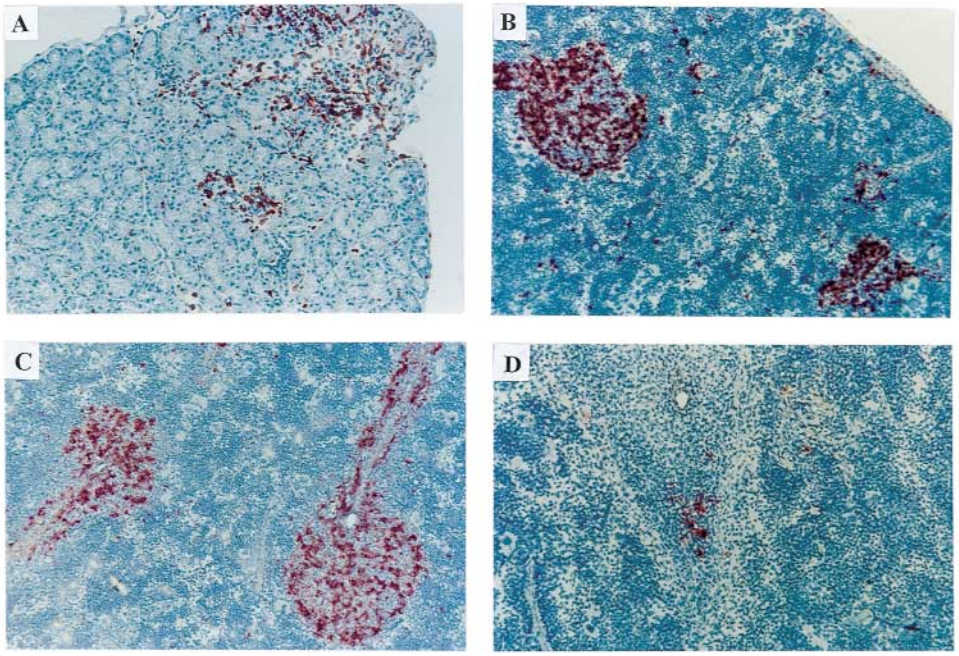

FIG 2. Immunohistochemical analysis of sections of hu-PBL-SCID tissue stained for infiltration of human lymphocytes. Cryosections of thymus **(A)** and spleen **(B-D)** were stained for infiltration of human $CD3^+$ (**A** and **B**) and $CD4^+$ **(C)** T lymphocytes as well as $CD19^+$ B cells **(D)** with use of specific antibodies. Positive cells, stained red, were detected with the alkaline phosphatase–antialkaline phosphatase complex. The tissues were counterstained blue with hematoxylin. Sections treated with isotype control antibodies did not show staining. (Original magnification: **A** and **D,** ×400; **B** and **C,** ×250.)

TABLE I. Total and allergen-specific IgE levels obtained from atopic donors and hu-SCID mice generated with PBMCs from these donors*

		Donor			hu-PBL-SCID		
		Total IgE	Specific IgE		Total IgE	Specific IgE	
Experiment No.	Allergen	(ng/mL)	EAST score	ng/mL	(ng/mL)	EAST score	ng/mL
1	*P pratense*	451	4.0	42	65, 57, 31	4.0, 3.3, 3.6	42, 18.5, 29
2	*P pratense*	437	3.6	29	257, 118, 106	3.6, 2.8, 3.5	29, 8, 25
3	*P pratense*	101	3.4	29	123, 44, 40, 23	2.9, 2.1, 2.3, 2.5	8, 3, 4, 5
4	Grass pollen	163	3.5	25	1317, 401, 360, 122, 113, 84, 38	3.8, 4, 3.2, 3.4, 3.6, 3.2, 2.9	35, 42, 15, 22, 29, 15, 8
5	*D pteronyssinus*	1449	3.2	15	629, 305, 233	3.6, 2.8, 2.6	29, 7, 6
6	*D pteronyssinus*	1173	4.0	42	682, 336, 292, 197	2.3, 3.1, 4.0, 4.0	4, 12, 42, 42
7	*D pteronyssinus*	1142	3.8	35	2333, 2196, 459, 134, 128	3.8, 3.7, 4.0, 3.9, 3.9	37, 33, 42, 40, 40
8	*D pteronyssinus*	872	3.1	12	78, 62, 49, 11, 8, ND, ND	3.1, 3.7, 3.8, 2.2, 2.3, 3.6 3.5	15, 34, 36, 3, 4, 29, 27

EAST, Enzyme allergosorbent test; *ND,* not done.
*IgE levels were determined in sera obtained from atopic individuals at the time of cell transfer and from hu-PBL-SCID mice 21 days after cell transfer.

mined by immunohistochemical analysis. The majority of human lymphocytes were T cells, primarily of the $CD4^+$ subset, located predominantly in the perivascular regions of the spleen and thymus (Fig 2). We failed to detect human lymphocytes in the lymph nodes (not shown). $CD19^+$ B cells were also located in the spleen (Fig 2). We did not find $CD14^+$ monocytes and $CD83^+$ dendritic cells (DC) in the spleen (not shown). Our results indicate that the hu-PBL-SCID mice generated exhibit similar characteristics to the hu-PBL-SCID mice reported in the literature.[14]

Human cytokine mRNA expression in hu-PBL-SCID mice

To investigate cytokine expression of transplanted human cells at the mRNA level, cells obtained from the

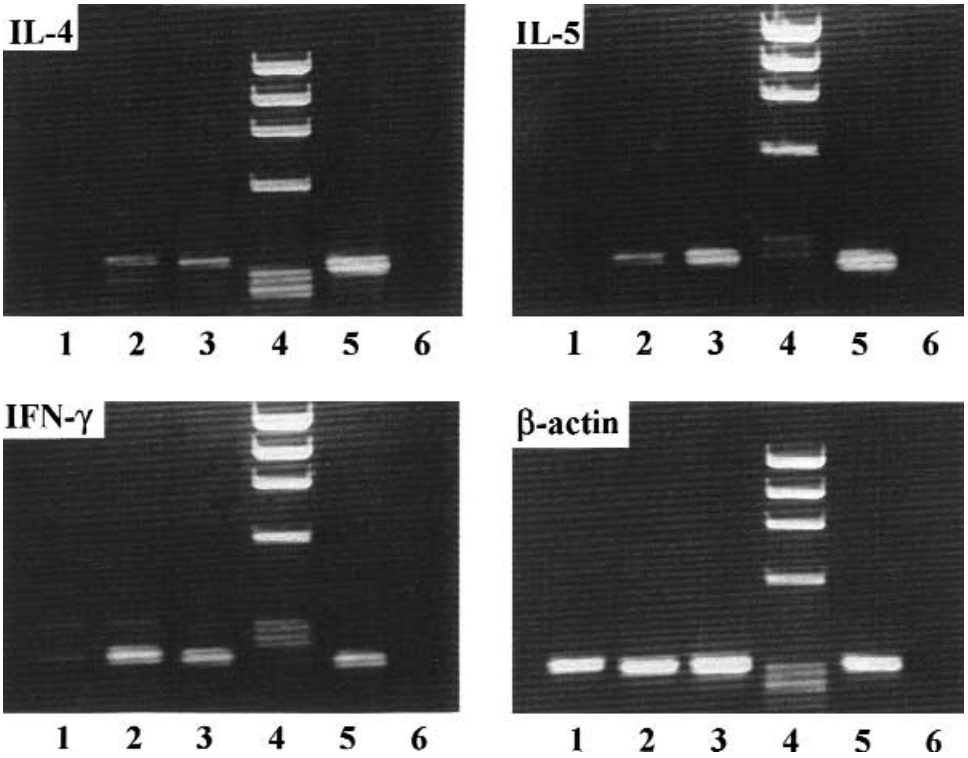

FIG 3. Expression of human IL-4, IL-5, and IFN-γ mRNA in spleens of hu-PBL-SCID mice. Pooled splenocytes of 9 hu-PBLSCID mice obtained 7 days after cell transfer were cultivated with 5 μg/mL PHA and 10 ng/mL PMA for 0 *(lane 1),* 6 *(lane 2),* and 24 hours *(lane 3).* RT-PCR was performed with primers specific for human IL-4, IL-5, and IFN-γ as well as β-actin to control for the quality of the RNA preparations. As a positive control *(lane 4)* we used IL-4, IL-5, and IFN-γ cDNA as well as PBMC-derived cDNA in the case of β-actin. cDNA prepared from mouse spleen cells served as a negative control *(lane 5).*

spleens of hu-PBL-SCID mice 7 days after cell transfer were cultured for 0, 6, or 24 hours in the presence of PHA plus PMA. Signals representing PCR products for IL-4 and IL-5 were only detected after stimulation of cells (Fig 3). In the case of IFN-γ, a very faint signal was observed with unstimulated cells, whereas strong signals were visualized after mitogenic stimulation of the cells. The low level of IFN-γ mRNA expression in unstimulated cultures of spleen cells obtained from hu-PBL-SCID mice indicates an absence of prominent inflammatory human graft-versus-host responses, which are associated with elevated IFN-γ and TNF-α production.

Functional activity of human lymphocytes isolated from the spleen and peritoneum of hu-PBL-SCID mice

To characterize the functional activity of allergen-reactive T cells in hu-PBL-SCID mice, cells isolated from the peritoneum and spleen 7 days after cell transfer were cultured for 5 days with *D pteronyssinus,* and the frequency of cytokine-producing cells was determined with the sensitive ELISPOT assay. The results, presented in Table II, were obtained from a representative group of 9 hu-PBL-SCID mice engrafted with 1×10^8 PBMCs isolated from an atopic individual sensitized to *D pteronyssinus.* No cytokine-producing cells were detected in cultures of peritoneal lavage cells stimulated with *D pteronyssinus* alone, although human T cells were present, with relatively high numbers of IL-4–, IL-5–, and IFN-γ–secreting cells detected after nonspecific stimulation with the mitogens PHA and PMA.

We determined whether the lack of cytokine production after stimulation with *D pteronyssinus* could be overcome by culturing peritoneal lavage cells with *D pteronyssinus* in the presence of exogenous IL-2. Under these conditions, 119 IL-4– and 30 IL-5–positive cells were detected compared with 41 IL-4– and 0 IL-5–secreting cells in cultures containing IL-2 alone, indicating that the increase in IL-4 and IL-5 production was antigen specific. The low response of cells isolated from hu-PBL-SCID mice may have arisen as a result of a lack of effective antigen presentation, caused either by low numbers of professional human APCs in the SCID mouse or by a loss of functional activity of the APCs present. To determine whether APC function in the hu-PBL-SCID system was defective, cultures of peritoneal lavage cells were stimulated with *D pteronyssinus* in the presence of exogenous irradiated autologous PBMCs as APCs. An additional increase in the cytokine response was observed, with 170 IL-4– and 67 IL-5–producing cells detected. No cytokines were detected in cultures containing media alone, indicating that the response was *D pteronyssinus* specific. Both the IL-4 and IL-5 response

TABLE II. The frequency of cytokine-producing cells present in the peritoneum and spleen of hu-PBL-SCID mice

Stimulus	No. of cytokine-producing cells per 1×10^6 cultured cells*		
	IL-4	IL-5	IFN-γ
PC	0	0	0
PC + *D pteronyssinus*†	3	0	0
PC + IL-2‡	41	0	144
PC + *D pteronyssinus* + IL-2	119	30	233
PC + APC§	0	0	0
PC + APC + *D pteronyssinus*	170	67	14
PC + APC + IL-2	26	8	352
PC + APC + *D pteronyssinus* + IL-2	837	933	330
PC + PHA‖ + PMA¶	656	1685	3711
SC	0	0	0
SC + *D pteronyssinus*	0	0	0
SC + IL-2	5	0	55
SC + *D pteronyssinus* + IL-2	0	0	18
SC + APC	0	0	0
SC + APC + *D pteronyssinus*	0	0	7
SC + APC + IL-2	13	2	53
SC + APC + *D pteronyssinus* + IL-2	44	27	53
SC + PHA + PMA	27	25	113
PBMC	0	0	3
PBMC + *D pteronyssinus*	40	113	123
PBMC + IL-2	47	67	1390
PBMC + *D pteronyssinus* + IL-2	180	223	1610
PBMC + PHA + PMA	1660	1000	4170

PC, Peritoneal cells; *APC*, antigen-presenting cells; *SC*, spleen cells.
*The frequency of cytokine-producing cells in the irradiated APC population was substracted (APC + *D pteronyssinus* + IL-2: 11 IL-4–producing cells, 7 IL-5–producing cells, 9 IFN-γ–producing cells; APC + *D pteronyssinus*: no cytokine-producing cells; APC + IL-2: no IL-4– or IL–5 producing cells, 2 IFN-γ–producing cells).
†20 μg/mL.
‡20 U/mL.
§Irradiated PBMCs (30 gray).
‖5 μg/mL.
¶10 ng/mL.

could be further enhanced in cultures stimulated with *D pteronyssinus* in the presence of autologous PBMCs and exogenous IL-2. This yielded, after the number of allergen-nonspecific cells induced in the presence of APC and IL-2 was subtracted, 811 IL-4– and 925 IL-5–secreting cells with specificity for *D pteronyssinus.*

Similar results were obtained with spleen cells (Table II). The low frequency of IL-4– and IL-5–secreting cells reflect the comparably low numbers of human lymphocytes present.

To identify the major APC, we stimulated cells isolated from the peritoneum of hu-PBL-SCID mice with *D pteronyssinus* in the presence of exogenous IL-2 with use of either whole PBMCs or populations depleted of $CD14^+$ monocytes, $CD19^+$ B cells, or $CD83^+$ DCs or positively selected for $CD14^+$ or $CD19^+$ cells. Because of the low numbers present, we were unable to use comparable numbers of positively selected $CD83^+$ cells. The results indicated that monocytes were the major APC

TABLE III. The role of different APC populations in determining the frequency of IL-5–producing cells isolated from the peritoneum of hu-PBL-SCID mice

Stimulus	No. of IL-5–producing cells per 1×10^6 cultured cells*
PC† + *D pteronyssinus*‡ + IL-2§	100
PC + APC‖ + *D pteronyssinus* + IL-2	1492
PC + CD14-depleted APC‖ + *D pteronyssinus* + IL-2	605
PC + CD19-depleted APC‖ + *D pteronyssinus* + IL-2	1900
PC + CD83-depleted APC‖ + *D pteronyssinus* + IL-2	1569
PC + CD14-positive APC‖ + *D pteronyssinus* + IL-2	1061
PC + CD19-positive APC‖ + *D pteronyssinus* + IL-2	177
PC + PHA¶ + PMA#	2885

PC, Peritoneal cells.
*The frequency of IL-5–producing cells in cultures containing medium instead of peritoneal cells was subtracted (≤54 IL-5–producing cells).
†13×10^4 per well.
‡20 μg/mL.
§20 U/mL.
‖5×10^6 per well.
¶5 μg/mL.
#10 ng/mL.

population. Depletion of this population resulted in a >60% reduction in the frequency of IL-5–producing cells (Table III). The low percentage of human $CD14^+$ monocytes recovered from the peritoneal lavage of hu-PBL-SCID mice would account for this loss in activity. Flow cytometric analysis revealed 0.8% $CD14^+$, 0% $CD83^+$, 6.1% $CD19^+$, and 49.6% $CD3^+$ cells in the peritoneum.

The data suggest that a large proportion of allergen-specific human T cells present in hu-PBL-SCID mice are in a state of anergy because the cytokine response can be restored when cells are cultured in the presence of *D pteronyssinus*–presenting APCs and IL-2. This may reflect clonal expansion of activated *D pteronyssinus*–specific effector T cells because enhanced IL-4 and IL-5 responses were also observed in donor PBMCs when stimulated with *D pteronyssinus* in the presence of IL-2 compared with *D pteronyssinus* alone. The data suggest that the lack of human professional APCs, particularly $CD14^+$ monocytes, in hu-PBL-SCID mice, is responsible for the reduction in functional activity of human allergen-specific T cells.

The frequency of IFN-γ–producing cells was largely independent of the presence of *D pteronyssinus* during culture of peritoneal and spleen cells (Table II). Stimulation of these cells in the presence of irradiated APCs and IL-2 resulted in comparable numbers of IFN-γ–secreting cells irrespective of the presence or absence of *D pteronyssinus*. These data suggest that the majority of the IFN-γ–producing cells were xenoreactive, being stimulated by antigens expressed on SCID mouse cells present in culture.

Under optimal culture conditions *D pteronyssinus*–specific cells isolated from hu-PBL-SCID mice express a T_H2-like cytokine profile, producing predominantly IL-4 and IL-5. Activated effector T cells present in the periph-

eral blood of the donor, when stimulated with *D pteronyssinus,* expressed a T_H0/T_H2 phenotype with 40 IL-4–, 113 IL-5–, and 123 IFN-γ–secreting cells detected in 1×10^6 cultured (Table II).

DISCUSSION

The aim of this study was to investigate the functional activity and cytokine profile of human allergen-specific T lymphocytes isolated from hu-PBL-SCID mice, generated by the transfer of PBMCs from atopic individuals. Optimal levels of allergen-specific IgE in hu-PBL-SCID sera were comparable to levels observed in donor sera at the time of cell transfer. Western blots revealed that allergen-reactive B-cell populations in hu-PBL-SCID mice were oligoclonal with binding activity observed to the major allergens of *P pratense* (data not shown). Production of detectable levels of specific IgE was dependent on immunizing with allergen, suggesting that IgE was synthesized after activation of B-cell populations in vivo and not as a result of the transfer of plasma cells in the injected cell population. Allergen-reactive $CD4^+$ T cells and the cytokines produced by them would therefore appear to be critical for the induction of IgE production in hu-PBL-SCID mice.

Allergen-reactive $CD4^+$ T cells isolated from atopic individuals express a T_H2/T_H0 cytokine profile.[15] IL-4 is an essential cytokine for the switch to ε germline transcription and IgE production.[16] Previous studies have demonstrated that IgE levels in hu-PBL-SCID mice can be greatly enhanced after injection of IL-4[4] and production of IgE abrogated by the addition of human soluble IL-4 receptor molecules.[7] Thus the decline in IgE levels in sera observed within 4 weeks after cell transfer suggests that after initial activation and clonal expansion of allergen-reactive T- and B-cell populations within the peritoneum there is a loss in $CD4^+$ T-helper cell activity. We were concerned that in the hu-PBL-SCID mouse the presence of xenoreactive human T cells expressing a T_H1 cytokine profile may generate a cytokine milieu that induces a shift in the cytokine profile of allergen-reactive T cells from a T_H2/T_H0 to a T_H1 response. However, on restimulation of human lymphocytes isolated from hu-PBL-SCID mice with allergen ex vivo, we detected only a very low frequency of IL-4–producing cells, which may be responsible for inducing IgE production. Substantial numbers of IL-4– and IL-5–producing cells were observed when peritoneal lavage cells were cultured in the presence of irradiated autologous PBMCs as a source of professional APCs. For optimal numbers of cytokine-secreting cells the combined presence of allergen, irradiated autologous PBMCs, and IL-2 was required. Under these conditions, allergen-reactive T cells isolated from the hu-PBL-SCID mouse were found to secrete a T_H2-like cytokine profile, producing high levels of IL-4 and IL-5 with very low amounts of IFN-γ. Our results suggest that as early as day 7 after cell transfer allergen-specific T cells present in hu-PBL-SCID mice are in an anergic state, possibly outnumbering functional T cells. However, the presence of the T-cell growth factor IL-2 may contribute to clonal expansion of allergen-specific effector T cells.

The induction of anergy in $CD4^+$ T cells occurs as a result of TCR occupancy in the absence of costimulation[17,18] and can be reversed by contact with IL-2 and IL-15.[19,20] In vitro the induction of anergy in allergen-reactive T_H2 cell populations, induced by culturing with antigen peptides in the absence of professional APCs, is accompanied by a loss in proliferation and cytokine production and a failure to induce IgG4 and IgE production by B cells.[21] The induction of anergy in allergen-reactive T cells is not an in vitro phenomenon because it has been associated with the loss in cytokine production in vivo after immunotherapy.[20] The loss in cytokine production in the hu-PBL-SCID mouse, which may have arisen after anergy induction in allergen-reactive T cells, may account for the reduced IgE response. By preventing anergy induction in the hu-PBL-SCID mouse, the extent and duration of the allergic response may be greatly increased.

In this study we have demonstrated that monocytes constitute the major APC in peripheral blood for effector or memory T cells. The role of $CD14^+$ monocytes present in the transferred PBMC population in the induction of IgE responses in hu-PBL-SCID mice has been previously shown.[7] The presence of monocytes in hu-PBL-SCID mice after cell transfer has been controversial.[14,22] Little is known about the extent to which monocytes and dendritic cells survive in the SCID mouse over time. We failed to detect $CD14^+$ or $CD83^+$ cells in spleen or in peritoneal lavage fluid obtained from hu-PBL-SCID mice as early as 7 days after PBMC transfer. This would account for our ability to substantially enhance the frequency of cytokine-producing cells by addition of irradiated autologous PBMCs or monocytes to cultures. This suggests that a loss of professional APCs was responsible for the loss in T-cell responsiveness. These results have important implications when considering the application of hu-PBL-SCID mice in immunotherapy. It may prove to be necessary to additionally transfer professional APCs, such as monocytes, to maintain a state of functional activity in the transplanted human T-cell population.

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