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Total and Birch Pollen-Specific Human IgE in Mice with Severe Combined Immunodeficiency Transplanted with Human Peripheral Blood Lymphocytes: Donor Dependence, Seasonal Variation and in vivo Half-Life

Key Words

Severe combined immunodeficiency
Peripheral blood lymphocytes, human
IgE, total
Birch pollen-specific IgE
Half-life

Abstract

Background: There is a need for animal in vivo models in the study of human allergy. The aim of the present experiments was to study production and catabolism of human IgE in mice with severe combined immunodeficiency transplanted with human peripheral blood lymphocytes (hu-PBL-SCID mice). **Methods:** Groups of SCID mice were transplanted intraperitoneally with hu-PBL from the same three donors in five experiments. Subgroups of transplanted mice were immunized with birch pollen. Production of human total and birch pollen-specific IgE in the hu-PBL-SCID mice was analyzed over a 7-week period. **Results:** Human IgE was detected in 93% of the hu-PBL-SCID mice, and the production showed reproducible donor-dependent kinetics. Production of birch pollen-specific human IgE, however, was seen only in mice transplanted with cells from birch pollen-allergic donors. A greater proportion of the mice produced specific IgE when the experiment was started in, or some months after a birch pollen season with high pollen counts. The half-lives of passively transferred human IgE were determined to be 24.0 and 23.4 h for total and birch pollen-specific IgE, respectively. **Conclusions:** This study demonstrates that human IgE production in hu-PBL-SCID mice is very reproducible when the same donor is used several times. Specific IgE production in recipient mice seems to require the use of cell donors with the actual specific allergy, and is most readily obtained during or after a period of donor allergen exposure. The short half-lives found indicate that hu-PBL-SCID mice have a high ongoing production of human IgE.

Introduction

Severe combined immunodeficiency (SCID) mice were first described in 1983 by Bosma et al. [1], and have been used in several fields of medical research. These mice do

not have functional T and B cells, and can therefore be transplanted with xenogeneic cells or tissues without problems with immune-mediated rejection. For studies on the human immune system, SCID mice are usually transplanted with either human fetal tissues or cells (SCID-hu mice) [2], or

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with human peripheral blood lymphocytes (hu-PBL-SCID mice) [3]. SCID-hu mice are able to support the maturation of primary lymphoid cells into mature cells producing low levels of human IgM and IgG [4], but the model has obvious ethical limitations and the access to transplantation material is restricted. The use of hu-PBL-SCID mice, on the other hand, has resulted in production of high levels of all immunoglobulin classes [3, 5], and cells for transplantation are easy to obtain. Recently, several articles have been published on the use of the hu-PBL-SCID mouse as a model to study human allergy, reporting the ability to produce total human IgE [5–16] as well as specific human IgE [9, 11–14].

In this article, we have investigated human IgE production in hu-PBL-SCID mice when cells from the same donor are used for repeated experiments. To induce production of specific IgE, we immunized the transplanted mice with birch pollen, which is an important outdoor allergen. In order to reveal the elimination rate of human IgE in SCID mice, we also determined serum half-life values of total and birch pollen specific human IgE.

Materials and Methods

Animals

Six to 8-week-old C.B-17 male scid/scid (SCID) mice were obtained from Gl. Bomholtgård, Ry, Denmark, under a license from Fox Chase Cancer Center. Murine serum IgG was used as a measure for leakiness, and only nonleaky mice were chosen for the experiments. Mice in experiments 1–4 were screened for leakiness by the breeder, while mice in experiment 5 were tested after arrival by ELISA as described earlier [16]. The mice were kept in sterilized microisolator cages containing 6 mice, and provided with sterilized water with 2 mM HCl and irradiated pelleted food (Rat & Mouse No. 3 Breeding Diet, Special Diets Services, Essex, UK) ad libitum. The experimental procedures were performed in a laminar airflow hood. The room had a 12-hour/12-hour light/dark cycle. The mice were rested for 1–2 weeks before entering the experiment, and each mouse was marked by ear punching. The experiments were performed in conformity with the laws and regulations controlling experiments with animals in Norway, and were approved by the local officer of the Experimental Animal Board under the Ministry of Agriculture.

Experimental Design

Venous blood was obtained from 3 healthy male adults, 2 of whom were allergic to birch pollen. PBL were separated by Lymphoprep (Nycomed, Oslo, Norway) centrifugation as described by Bøyum [17]. The cells were counted in a Bürker chamber, and the mice were given $25\text{--}60 \times 10^6$ viable human cells intraperitoneally (i.p.). Cells from each donor were given to separate groups of 4–14 mice. At days 7 and 21 after transplantation, subgroups of the mice were injected i.p. with a dose of 25 µg birch pollen (a gift from Pharmacia, Sweden) in 0.5 ml of Hanks' balanced salt solution (HBSS). Blood samples were obtained every 2 weeks over a period of 48–50 days, and at the end of the experiments, peritoneal lavage was performed.

Serum from each donor was analyzed for IgG antibodies to Epstein-Barr virus (EBV) capsid antigen by the immunofluorescence technique EB-VCA IFA Kit II (Organon Teknika, Durham, N.C., USA), kindly performed by Anne-Lise Bruu (Department of Virology, National Institute of Public Health, Oslo, Norway).

Collection of Mouse Blood Samples and Peritoneal Lavage Fluids

Blood samples were obtained from the lateral femoral vein. After removing hair from the area, the vein was punctured with a needle (21 gauge, Terumo Europe n.v., Leuven, Belgium), and 100- to 200-µl blood samples were collected into heparinized capillary tubes (Vitrex, Herlev, Denmark). At the end of the experiments, the mice were anesthetized with CO₂, and blood was obtained by heart puncture. Serum or plasma from all blood samples (for reasons of simplicity referred to as serum) was stored at –20°C until analyzed. After sacrificing the mice, peritoneal lavage was performed by i.p. injection of 2.5 ml heparinized HBSS. The trypan blue exclusion method was used to determine the viability of the cells, which was generally above 90%.

Flow Cytometry

Cells from peritoneal lavage fluid were washed and resuspended in HBSS and incubated with MoAb against human CD45 lymphocyte antigen [KC56 (T-200)-RDI, Coulter] for 30 min at 4°C, washed twice with PBS with 0.2% BSA, and finally resuspended in 250 µl PBS/BSA. The antibody had been shown to be specific for human lymphocytes. Fluorescence intensity was measured using the Coulter Epics®-Profile II flow cytometer with scatter gates set on the lymphocytes. The data were corrected for background staining with the appropriate isotype control (FITC-conjugated mouse IgG1, Coulter).

Determination of Human Immunoglobulins

Human IgG concentrations were measured by turbidimetry, as previously described [16]. The quantitative detection limit for these measurements was 1 mg/ml, and qualitative results below this limit were referred to as less than 1 mg/ml (<1 mg/ml). Determination of total human IgE was performed with ELISA, as previously described [16]. The detection limit with the dilutions used was 1.25 ng/ml. No cross-reactivity against the other Ig classes was observed. Birch pollen-specific human IgE analysis was performed by the CAP technique (Kabi Pharmacia Diagnostics, Uppsala, Sweden) [18]. Due to limited amounts of serum, birch pollen-specific human IgE in the mouse sera was measured only at the end of the experiments. The detection limit in this CAP technique was 0.35 U/ml.

Half-Life Calculations

Half-life values were calculated by the least-square regression method ($T_{1/2} = \ln 2/k$) using the slope (k) of a line fitting to the data points [19]. The statistical determination of the k values was done using the SigmaPlot® software (version 2.01, Jandel Scientific, Erkrath, Germany).

Statistical Analysis

Statistical analysis was performed using the SigmaStat® software (version 1.01, Jandel Scientific, Erkrath, Germany). The nonparametric Mann-Whitney U test and Spearman's rank correlation test were used with $p = 0.05$ as the limit for statistical significance.

Results

Total IgG

Analysis of total human IgG in hu-PBL-SCID mice was performed to verify successful engraftment and function of human B cells. At the end of the experiments, 93% of the transplanted mice (125/134) had detectable human IgG. The median value of human IgG in these mice was 3.54 mg/ml, and the concentration varied from <1 to 11.80 mg/ml (table 1).

Flow Cytometry Analysis

Human CD45-positive cells in peritoneal lavage fluid from transplanted SCID mice were determined at the end of experiments 2, 3, 4 and 5 (table 2). In each experiment, and with cells from each donor, the groups of recipient mice showed median values of human CD45-positive cells above 60% of total gated peritoneal lymphocytes [data not shown]. As seen from table 2, the median values of CD45-positive cells from mice transplanted with cells from different donors were quite similar, and the median value for all the transplanted mice was 70% (range 3–94).

Total IgE

Individual IgE values in immunized and nonimmunized mice transplanted with cells from the same donor were compared [data not shown]. Because no significant difference was found in total IgE levels between immunized and nonimmunized mice, the values from these mice were put together and median IgE values for mice transplanted with cells from each donor were calculated (table 3). Ninety-three percent of the hu-PBL-SCID mice had detectable levels of human IgE, although the maximum IgE values in individual mice differed. Total IgE concentrations in the donors at each donation were found to correlate with maximum IgE concentrations in recipient mice ($r = 0.64$, $p = 0.009$). Figure 1 shows the kinetics of IgE production (median values) in the groups of mice transplanted with cells from the three donors in the five experiments. Maximum serum values appeared at different time points dependent on the cell donor. Most mice transplanted with cells from donor 1 or donor 2 reached a maximum in IgE production after 21 days, although differing in IgE levels. Mice transplanted with cells from donor 3 had still increasing IgE levels at the end of the experiments. These trends were, as we can see from figure 1, reproducible in the five different experiments.

Birch Pollen-Specific IgE

None of the mice transplanted with cells from the non-birch-pollen-allergic donor (1) produced birch pollen-spe-

Table 1. Number and percentage of hu-PBL-SCID mice positive for human IgG, and median and range of serum IgG concentrations in these mice 48–50 days after transplantation

Donor	Experiment	n	Positive ^a %	IgG, mg/ml	
				median	range
1	1	4/4	100	6.41	3.11–11.20
	2	5/6	83	5.08	2.55–7.34
	3	10/10	100	4.07	<1–6.10
	4	12/13	92	4.71	2.36–11.80
	5	11/12	92	3.92	2.58–9.62
2	1	4/4	100	5.09	4.81–5.76
	2	4/6	67	7.17	2.16–9.55
	3	8/9	89	3.72	2.96–6.69
	4	12/12	100	3.40	<1–6.93
	5	11/12	92	4.47	2.23–6.74
3	1	2/4	50	1.84	1.75–1.93
	2	6/6	100	3.39	2.06–8.73
	3	12/12	100	2.81	1.35–3.94
	4	12/12	100	1.55	<1–4.00
	5	12/12	100	2.34	1.09–4.23
All donors	1–5	125/134 ^b	93	3.54	<1–11.80

^a IgG concentrations ≥ 1 mg/ml.

^b Human IgG was determined in 134 of 136 transplanted mice.

Table 2. Percentage and total number of CD45-positive cells in peritoneal lavage fluid in hu-PBL-SCID mice 48–50 days after transplantation

Experiment	Donor	n	Percentage		Cell number ($\times 10^4$)	
			median	range	median	range
2–5	1	37	71	3–94	59	0.18–240
	2	36	73	4–92	35	3.10–593
	3	38	65	10–91	63	7.08–305
2–5	1–3	111	70	3–94	48	0.18–593

cific IgE (table 4). In contrast, production of birch pollen-specific IgE could be detected when mice were transplanted with cells from the birch pollen-allergic donors (2 and 3). As seen from table 4, birch pollen-specific IgE could also be detected in some nonimmunized mice. Among all the mice transplanted with cells from donors 2 and 3, 5 of 41 nonimmunized mice and 10 of 48 immunized mice produced specific birch pollen IgE. The mice that were positive

Table 3. Number and percentage of hu-PBL-SCID mice positive for human IgE as well as median and range of maximum serum IgE concentrations obtained in these mice during the experiments, and total serum IgE in the donors

Donor number	Experiment	IgE in donors ^a ng/ml	n	Positive ^b %	Maximum IgE in mice, ng/ml	
					median ^a	range
1	1	836	4/4	100	217	92.3–243
	2	694	6/6	100	358	312–419
	3	808	10/10	100	46.3	9.29–93.5
	4	708	12/14	86	87.5	23.8–133
	5	766	12/12	100	148	2.99–326
2	1	191	4/4	100	112	66.7–260
	2	254	4/6	67	85.1	28.7–117
	3	317	9/10	90	37.1	7.29–182
	4	312	10/12	83	19.7	4.78–98.8
	5	228	11/12	92	44.0	7.83–196
3	1	93.1	2/4	50	34.2	25.2–43.3
	2	84.4	6/6	100	73.8	10.25–263
	3	100	12/12	100	34.3	5.43–355
	4	84.9	12/12	100	11.4	2.09–51.5
	5	69.7	12/12	100	12.4	1.31–190
All donors	1–5	–	126/136	93	49.8	1.31–419

^a Correlation coefficient: $r = 0.64$, $p = 0.009$ (Spearman's rank correlation test).

^b IgE concentrations > 1.25 ng/ml.

for birch pollen-specific IgE all had detectable levels of total IgE, although no statistically significant correlation between total and specific IgE was found.

Determination of Half-Lives

To determine serum half-lives of human total and birch pollen-specific IgE in the SCID mouse, 23 SCID mice were injected intravenously (i.v.) with 0.5 ml of a serum pool from five human donors of both sexes (total IgE = 1,655 ng/ml, birch pollen-specific IgE = 80.65 U/ml). The median weight of these mice was 26 g (range 23–29 g). At different time points after injection, groups of 2 or 3 mice were sacrificed and heart-punctured. The values of total and birch pollen-specific IgE in the mice sera were plotted against time in a semi-log scale (fig. 2). Serum half-life values were calculated from the decay curve by the least-square regression method, and were found to be 24.0 h for total IgE and 23.4 h for birch pollen-specific IgE with the serum pool used.

Discussion

IgG concentrations measured in mice sera were comparable with previous reports from us and others [7, 12, 13, 16, 20]. Donors 1 and 2 were EBV-positive, while donor 3 was

EBV-negative. Some investigators have reported that EBV positivity of cell donors correlates with high Ig levels in the recipient mice [21], but no such correlation was found in our experiments. Signs of lymphoproliferative disease were not observed in the transplanted mice.

The success of engraftment was also supported by the finding in flow cytometry of a relatively high percentage (median = 70%) of human CD45-positive cells in peritoneal lavage fluid 48–50 days after transplantation (table 2). We have previously reported a median value of 45% human CD45-positive cells in peritoneal lavage fluid from hu-PBL-SCID mice somewhat later (56–69 days) after transplantation [16]. The higher percentage found in the present study may be due to a beginning decrease of human CD45-positive cells in peritoneal lavage fluid from hu-PBL-SCID mice after 50–70 days [22].

Pestel et al. [11] transplanted SCID mice with hu-PBL from both nonallergic donors and *Dpt*-sensitive patients. They found that in order to obtain a human IgE response, the mice both had to be transplanted with cells from the *Dpt*-sensitive patients and injected with the specific allergen. In the present report however, the transplanted SCID mice did not require immunization with an allergen in order to produce human IgE. High levels of total IgE were detected in both immunized and nonimmunized mice. This is in agree-

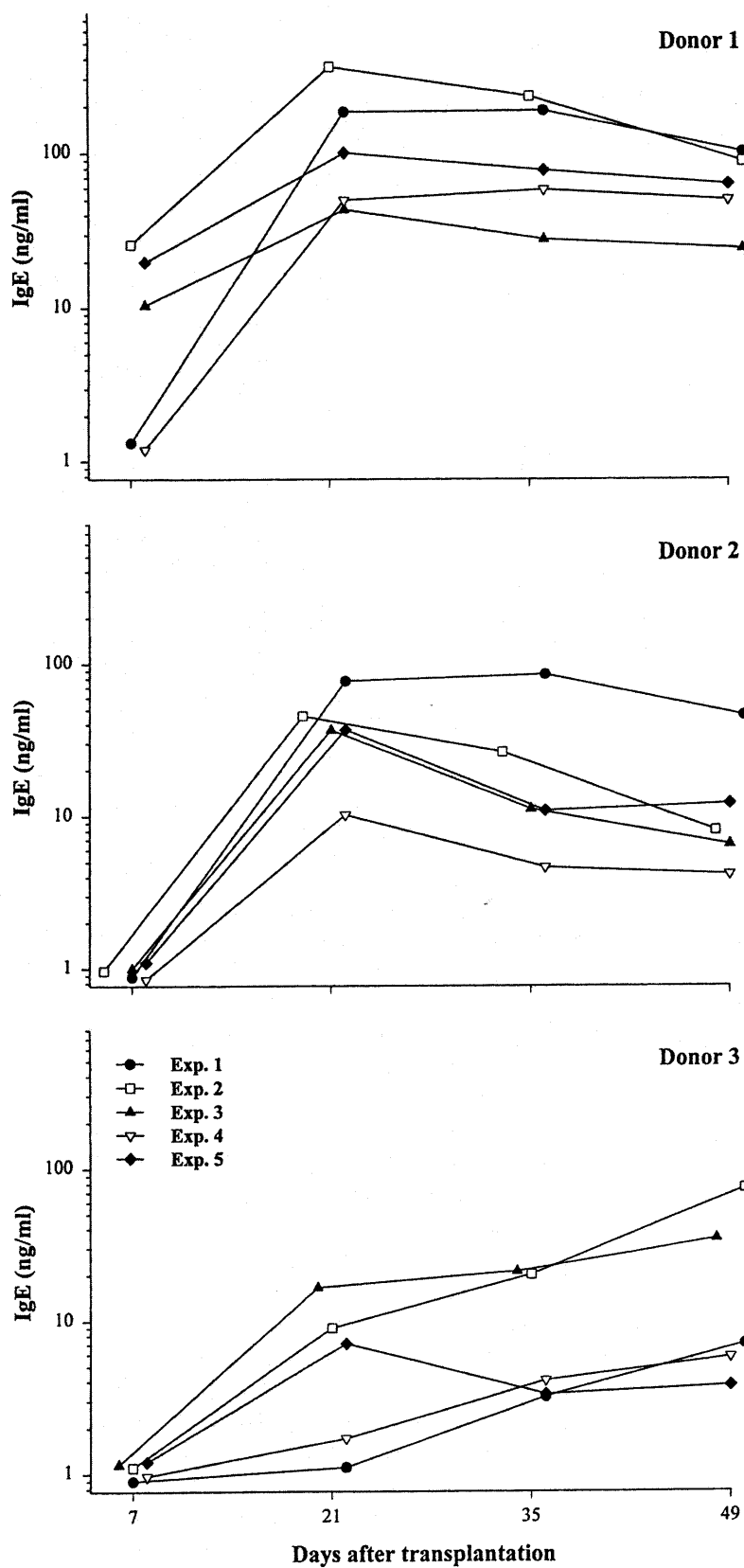


Fig. 1. Median values of human IgE in 4–14 SCID mice transplanted with cells from the same donor. Data from five experiments are shown.

Table 4. hu-PBL-SCID mice positive for birch pollen-specific human IgE 48–50 days after transplantation, and birch pollen-specific IgE concentrations in the donors

Experiment ^a	Donor ^b		Nonimmunized mice		Immunized mice	
	n	birch pollen IgE, U/ml	n	%	n	%
1 (September 1992)	1	<0.35	0/2	0	0/2	0
	2	15.80	0/1	0	1/2	50
	3	4.64	0/2	0	1/2	50
2 (December 1992)	1	<0.35	0/1	0	0/3	0
	2	13.97	0/2	0	0/4	0
	3	4.42	0/2	0	1/4	25
3 (May 1993)	1	<0.35	0/5	0	0/5	0
	2	19.80	0/5	0	1/5	20
	3	12.90	3/5	60	5/7	71
4 (January 1994)	1	<0.35	0/6	0	0/6	0
	2	13.97	0/6	0	0/6	0
	3	8.91	0/6	0	0/6	0
5 (June 1994)	1	<0.35	0/6	0	0/6	0
	2	14.10	0/6	0	0/6	0
	3	5.47	2/6	33	1/6	17
1–5	1	–	0/20	0	0/22	0
	2 and 3	–	5/41	12	10/48	21

Serum values of hu-PBL-SCID mice positive for birch pollen-specific human IgE ranged from 0.38 to 18.2 U/ml (median: 0.87 U/ml). Immunized mice were given 25 µg birch pollen i.p. on days 7 and 21.

^a The season of birch pollen in Oslo usually starts in the last week of April and is finished by the end of May. Birch pollen counts in Oslo for the pollen seasons in 1992, 1993 and 1994 were reported to be 1,375, 14,692 and 396 pollen grains/m³, respectively [Hallvard Ramfjord, Institute of Botany, NTNU, Trondheim, pers. commun.].

^b Only donors 2 and 3 were allergic to birch pollen.

ment with other reports from us and other investigators [5–10, 12, 13, 16]. The few mice that were negative for human IgE had low or no human IgG antibodies, as well as low numbers of human CD45-positive cells in peritoneal lavage fluid. It is therefore likely that these mice were not properly engrafted. In the successfully engrafted SCID mice, the kinetics of human IgE production was dependent on the donor (fig. 1). These results are in accordance with the kinetic patterns in IgE production reported by Kilchherr et al. [8], who observed two different time course patterns. The first pattern was characterized by an initial peak in IgE production followed by a decline and eventually, a rise after several weeks. The second pattern showed a slow increase in IgE with no characteristic peak. The mice transplanted with

cells from donors 1 and 2 in the present report had IgE kinetics that correspond to pattern 1, and mice with cells from donor 3 had kinetics that correspond to pattern 2. Previous publications from us and others also agree with these observations [6, 10, 16]. The different IgE patterns may result from differences in activation state in the B cell populations, the cells leading to the second pattern requiring activation and switching before IgE production. We found a correlation between the donors' IgE concentrations at each donation and maximum IgE concentrations in recipient mice. This is in agreement with some reports [6, 7], but disagrees with others [8, 10, 11]. The reason for this discrepancy is not known, but the number of donors used as well as the range of the donors' IgE levels may influence the statistical likelihood of revealing such a correlation. We have previously shown that hu-PBL-SCID mice transplanted with cells from the same donor show similar IgG antibody response patterns in terms of kinetics and antibody level after vaccination with a pneumococcal polysaccharide vaccine [16, 23].

In a preliminary report, we for the first time showed a production of specific human IgE antibodies in hu-PBL-SCID mice [9]. Those results indicated that a specific human IgE response in transplanted SCID mice could only be obtained by using cells from a donor with the actual specific allergy. In the present report, we confirm these findings (table 4). Three other groups have reported specific human IgE production in hu-PBL-SCID mice [11–14]. These papers all differ from the present report, and in two of them the actual allergen was injected together with an adjuvant [11, 12, 14].

The fact that there was no significant correlation between total and specific IgE in the present report indicates that the specific IgE production was not simply a result of a general rise in total IgE. The results also showed that the season for transplantation is of importance when dealing with seasonal allergens, as a greater proportion of the immunized mice produced specific antibodies when the experiment was started in or some months after a birch pollen season with high pollen counts (table 4). In addition, 5 of 11 nonimmunized mice transplanted with cells from donor 3 produced birch pollen-specific IgE (experiments 3 and 5). The most likely explanation for this observation is that these transplanted cells were activated by the donors' exposure to birch pollen before blood donation, thus being already stimulated for production of birch pollen-specific IgE. Epidemiological studies in humans have shown that seasonal exposure to allergens increases the levels of total and allergen-specific IgE [24], as well as in vitro release of IgE and IL-4 [25, 26]. These observations correlate well with the IgE levels in sera from donors 2 and 3. The highest levels in both total and birch pollen-specific IgE (tables 3 and 4, respec-

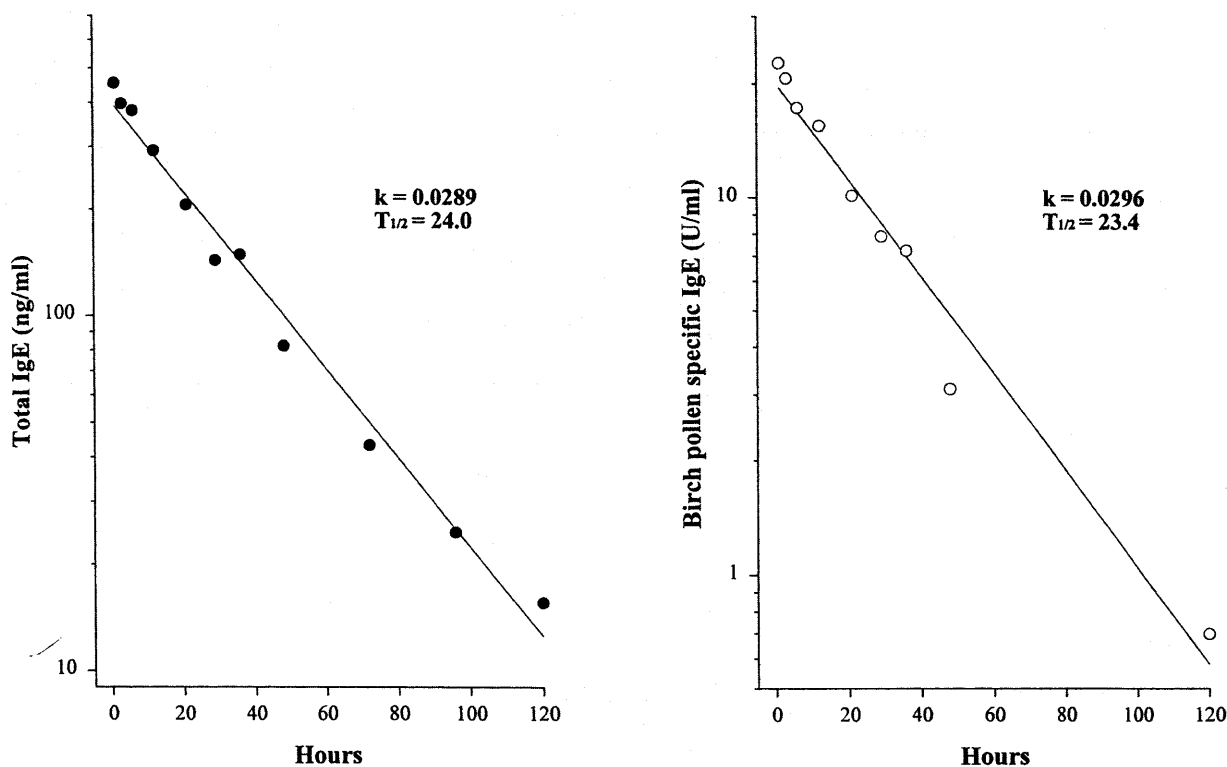


Fig. 2. Total and birch pollen-specific human IgE in sera from mice injected i.v. with 0.5 ml of pooled human sera (total IgE = 1,655 ng/ml, birch pollen-specific IgE = 80.65 U/ml). Each point represents pooled values from 2 or 3 mouse sera. The calculated values of the elimination constant (k) and half-lives ($T_{1/2}$) are shown.

tively) in these two birch pollen-allergic donors were measured in experiment 3, which was started in a pollen season with extremely high birch pollen counts (see footnote of table 4). No nonimmunized mice transplanted with cells from donor 2 produced birch pollen-specific IgE. Although this donor's high specific IgE level indicates that birch pollen IgE-producing cells were activated, these cells do not necessarily have to circulate in the blood, and may therefore not have been transferred to the recipient mice. The finding that 5 of 41 nonimmunized and 10 of 48 immunized mice produced birch pollen-specific human IgE may suggest some effect of immunization, but the difference is not statistically significant.

The serum half-life of total human IgE in SCID mice (24.0 h) was found to be short compared to the serum half-life of IgE in humans (2.3 days [27]), a result that is in accordance with the observation that the Ig catabolism correlates inversely with the size of the species [28]. Serum half-life of murine IgE in normal mice is reported to be 12 h [29]. The half-lives of total and birch pollen-specific human IgE

were quite similar (24.0 and 23.4 h, respectively). This is in agreement with the previous observation that the half-lives of human IgG1 and IgG3 MoAbs in SCID mice were unrelated to their specificity [30]. Our results disagree with the work of Kilchherr et al. [8] who reported a considerably longer serum half-life of total human IgE in SCID mice (88 h). However, these researchers used a single human IgE myeloma protein for i.v. injection, which may be less physiologic than a polyclonal serum pool like the one we used. Together with the fact that the hu-PBL-SCID mice produced high levels of human IgE 49 days after transplantation the short half-life indicates that there must be a high ongoing production of human IgE.

It has been reported that the catabolic rate of IgE in humans varies inversely with the serum concentration [31]. A repeat experiment using a single human serum with lower total IgE concentration (756 ng/ml) revealed a half-life of 16.4 h [data not shown], which indicates that the association between serum concentration and half-life of human IgE in SCID mice is similar to that in humans. This observation

further supports the relevance of this animal model for in vivo studies of human allergy.

To our knowledge, this is the first report that investigated the repeated use of cells from the same donor for transplantation into SCID mice. A general problem with the hu-PBL-SCID model is the large experimental variation [32]. We have previously reported that transplantation of hu-PBL to RAG-2-deficient mice, presumed to be phenotypically more homogenous, is associated with a greater variation in immunoglobulin production than transplantation into SCID mice [16]. Our present results (see fig. 1) show that by using the same donors several times one can obtain data that are more comparable than data obtained using different donors. Our findings demonstrate the advantage of using the same

donors for comparison of results in parallel groups as well as in experiments performed over time. The relative stability of the individual donor-dependent patterns over time is a very valuable property of this hu-PBL-SCID model.

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