



SPECIFIC HUMAN IgE RESPONSE AFTER IMMUNIZATION WITH BIRCH POLLEN IN scid MICE TRANSPLANTED WITH HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS—A MODEL FOR HUMAN ALLERGY?

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Abstract—C.B-17 scid mice were transplanted ip with human peripheral blood mononuclear cells. Some transplanted mice were immunized ip with birch pollen. Levels of total and birch pollen-specific human IgE were measured in serum. Low levels of total human IgE were detected after 9 days. 20 days after transplantation 12 out of 12 mice transplanted with cells from a birch pollen allergic donor showed higher but variable levels of total human IgE, and a low level of birch pollen specific IgE was detected in one mouse that had not been immunized. Immunization with birch pollen did not influence the level of total human IgE. In a different experiment, mice transplanted with cells from a donor with a high level of total IgE, but without birch pollen allergy, developed high levels of total IgE 49 days after transplantation, whereas no birch pollen specific IgE was found (two immunized, two non-immunized mice). In contrast, two of four mice given cells from two donors with birch pollen allergy, but with lower levels of total IgE, produced birch pollen-specific IgE after immunization. Thus, measurable levels of total human IgE were spontaneously produced in most scid mice transplanted with human peripheral blood mononuclear cells. Detectable levels of birch pollen specific human IgE appeared to be produced in occasional non-immunized scid mice transplanted with cells from allergic donors. In mice given cells from some allergic donors, half the mice produced human birch pollen specific IgE after immunization.

INTRODUCTION

Mice with severe combined immunodeficiency (C.B-17 scid) lack functional T and B lymphocytes (Bosma *et al.*, 1983) and will accept transplanted human cells, because they cannot mount a specific rejection reaction. Foetal liver, thymus and lymph node cells (McCune *et al.*, 1988), as well as normal human peripheral blood mononuclear cells (Mosier, *et al.*, 1988) have been used to establish a functioning human immune system in scid mice (scid-hu mice). We have previously reported the successful use of scid-hu mice for the study of the human immune response to a vaccine preparation (Aaberge *et al.*, 1992). Scid-hu mice may prove useful in the study of the human immune response to micro-organisms, antigens and allergen preparations that for ethical and practical reasons cannot be tested in humans. Further, scid-hu mice may be a useful model for the study of the influence of the environment and the effects of toxic substances on human immune cell function. Finally, scid-hu mice may be regarded as an experimental model that is intermediate between *in vivo* and *in vitro* human systems, and may be of value in the evaluation of *in vitro* models. In the experiments reported here, we explored the use of the scid-hu model to study allergy-related human

immune responses. Measurable levels of human total IgE was obtained in most transplanted mice, and in some mice a human birch pollen specific IgE response could be demonstrated.

MATERIALS AND METHODS

Mice. C.B-17 male scid/scid mice, 6–8 wk old, were obtained from Gl. Bomholtgård Ltd (Ry, Denmark) under a licence from Fox Chase Cancer Center. The animals were housed as previously described (Aaberge *et al.*, 1992). No antibiotics were used. The mice were screened for spontaneous immunoglobulin production ('leakiness') by the breeder (limit <10 ng mouse IgG/ml serum).

Transplantation. Venous blood was obtained from normal and allergic, but otherwise healthy, adult volunteers. Peripheral blood mononuclear cells were prepared by density gradient separation as previously described (Aaberge *et al.*, 1992). $3\text{--}4 \times 10^7$ human cells from one donor were given ip in a volume of 1 ml to each mouse. Serum from each donor was analysed for total and birch pollen specific IgE before transplantation (Table 1).

Immunization and blood sampling. For immunization, 25 µg birch pollen grains (a gift from Pharmacia AB, Sweden) was given ip suspended in 0.5 ml

Table 1. Total and specific human IgE in human donors and scid mice transplanted with human peripheral blood mononuclear cells

Human donors			Recipient scid mice			
Donor no.	Total IgE (U/ml)	Birch pollen specific IgE (U/ml)	Days after transplantation	Immunized with birch pollen	Total human IgE*	Birch pollen specific IgE*
1	36	5.97	9	+	2/6	0/6
				—	6/6	0/6
2	72	16.00	20	+	6/6	0/6
				—	6/6	1/6
3	425	<0.35	49	+	ND	0/2
				—	2/2	0/2
4	31	14.14	49	+	ND	1/2
				—	ND	0/1
5	41	4.48	49	+	1/2	1/2
				—	ND	0/2

*Numerator indicates number of mice positive, denominator indicates number of mice examined. Because of small amounts of serum, not all mice could be examined for both total and birch pollen specific IgE (ND = not determined).

phosphate buffered saline 3 days or 3 and 15 days (experiments shown in Fig. 1) or 7 and 21 days (last experiment) after transplantation.

At the end of each experiment, the mice were exsanguinated by heart puncture under CO₂ anaesthesia. Serum was stored at -20°C until it was analysed.

Quantification of immunoglobulins. A normal human serum and sera from scid mice that had not received transplants were included as controls in all

immunoglobulin measurements. The concentration of human IgG in the mouse sera was measured by turbidimetry (detection limit 1 g/litre) (Behringwerke AG, Marburg, Germany). Total and birch pollen specific human IgE were measured with the Pharmacia CAP System. Mice were defined as positive with regard to total and birch pollen specific human IgE if measured serum levels were ≥ 2 U/ml and ≥ 0.35 U/ml, respectively.

Statistical analysis. Statistical analysis was performed with the Minitab statistical package for the PC (Minitab Inc., State College, PA USA). Student's *t*-test, one-way analysis of variance and the Pearson product moment test for correlation were used. *P* = 0.05 was used as the limit for statistical significance.

RESULTS

In the first set of experiments, scid mice received transplants of cells from two allergic donors. Total and birch pollen specific IgE of the donor for mice bled after 9 days (Donor 1) and the donor for the 20-day experiment (Donor 2) are given in Table 1. The mice showed no detectable levels of human IgG 9 days after transplantation, while 12 out of 12 mice had detectable levels of human IgG after 20 days, with concentrations ranging from 1.3 to 2.9 g/litre (data not shown). Low levels of human IgE (2–5 U/ml) were detected in eight out of 12 mice after 9 days and higher levels (4–146 U/ml) in 12 of 12 mice after 20 days (Fig. 1, Table 1). In the 20-day experiment, a marginally positive level of birch pollen specific IgE (1.29 U/ml) was detected in one mouse that had not been immunized (Table 1). There was no significant difference between immunized and non-immunized mice with regard to human IgG or total human IgE levels.

In a 49-day experiment, two immunized and two non-immunized mice that received transplants of cells from a donor with relatively high levels of total IgE but no manifest birch pollen allergy (Donor 3) showed no detectable birch pollen specific IgE (Table 1). In contrast, two out of four immunized mice that received transplants of cells from birch pollen allergic donors (Donors 4 and 5) with lower levels of total IgE produced significant levels of birch pollen specific IgE (18.2 and 0.87 kU/ml) (Table 1).

DISCUSSION

In the reported experiments, measurable levels of total IgE were found in eight out of 12 mice examined 9 days after transplantation, and in 15 out of 16 mice examined 20 or 49 days after transplantation. Immunization by ip injection with birch pollen had no statistically significant effect on total IgE levels. The variation between mice in total human IgE levels appeared to be larger than the variation in total human IgG. The results indicate that in the scid-hu

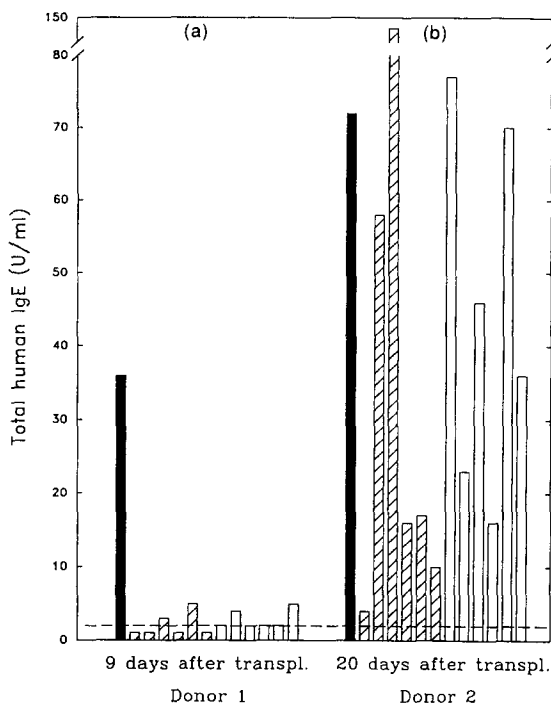


Fig. 1. Total human IgE in sera from mice transplanted with peripheral blood mononuclear cells, and in sera from the donors (■). ▨, values for mice that were immunized ip with 25 µg birch pollen (a) 3 days or (b) 3 and 15 days after transplantation; □, values for mice that were not immunized. The dotted line indicates the lower limit for a positive response (2 U/ml). Individual values for 12 mice in each experiment are given.

model, which is based on transplantation with human peripheral blood mononuclear cells, measurable levels of total human IgE can regularly be obtained even with cells from donors that have moderate levels of IgE. Preliminary analysis of material from later experiments supports this, and seems to indicate that the level of total IgE in recipient mice is dependent on the cell donor and show about the same variation between different mice as shown in Fig. 1b. Thus, the scid-hu model with peripheral blood mononuclear cells may be suitable for use in the study of factors that modulate total IgE production.

There are few reports on a specific human immune response in scid mice after transplantation with human peripheral blood mononuclear cells (Duchosal *et al.*, 1992; Mazingue *et al.*, 1991; Mosier *et al.*, 1988). We have previously obtained a specific human IgG response in about 70% of scid-hu mice immunized with a pneumococcal carbohydrate vaccine (Aaberge *et al.*, 1992). In the present experiments, immunization of 12 mice with birch pollen did not induce detectable levels of birch pollen specific IgE by day 9 or day 20. However, two out of four mice transplanted with cells from allergic donors and immunized with birch pollen showed increased levels of birch pollen specific IgE after 49 days (28 days after last immunization). In contrast, two immunized mice and two non-immunized mice with cells from a non-allergic donor with high levels of total IgE developed no specific IgE response. The reason why there was no detectable specific human IgE response in immunized mice in the nine and 20-day experiments is unclear. One explanation could be that the mice were bled too early, or the time of blood donation in relation to the allergen season may be of importance. In one non-immunized mouse from a birch pollen allergic donor, a low level of birch pollen specific IgE was detected. It is not surprising that occasional mice that received transplants of cells from donors with high levels of specific IgE (and therefore a high number of specific IgE-producing cells) produce detectable levels of specific IgE. This finding must be expected early after transplantation and for as long as the transplanted human cells remain activated without further specific antigen stimulation.

Importantly, these experiments show that not only a human IgE response, but also an allergen-specific IgE response, can be obtained in scid-hu mice transplanted with human peripheral blood mononuclear cells. Immunization ip with allergen appears to boost the specific IgE response, but further experiments with larger numbers of mice are necessary to confirm this. The findings are compatible with the notion that the presence of a specific allergy in the donor may be a requirement for a corresponding specific IgE response in the recipient scid mouse. As a whole, the results are encouraging with regard to the possibility of establishing the scid-hu mouse as a model to study human allergy-related immune responses.

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REFERENCES

- Aaberge I. S., Michaelsen T. E., Rolstad A. K., Groeng E.-C., Solberg P. and Løvik M. (1992) SCID-hu mice immunized with a pneumococcal vaccine produce specific human antibodies and show increased resistance to infection. *Infection and Immunity* **60**, 4146–4153.
- Bosma G. C., Custer R. P. and Bosma M. J. (1983) A severe combined immunodeficiency mutation in the mouse. *Nature* **301**, 527–530.
- Duchosal M. A., Eming S. A., Fisher P., Leturcq D., Barbas C. F. III, McConahey P. J., Caothien R. H., Thornton G. B., Dixon F. J. and Burton D. R. (1992) Immunization of hu-PBL-SCID mice and the rescue of human monoclonal Fab fragments through combinatorial libraries. *Nature* **335**, 258–262.
- McCune J. M., Namidawa R., Kaneshima H., Shultz L. D., Lieberman M. and Weissman I. L. (1988) The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* **241**, 1632–1639.
- Mazingue C., Cottrez F., Auriault C., Cesbron J.-Y. and Capron A. (1991) Obtention of a human primary humoral response against schistosome protective antigens in severe combined immunodeficiency mice after the transfer of human peripheral blood mononuclear cells. *European Journal of Immunology* **21**, 1763–1766.
- Mosier D. E., Gulizia R. J., Baird S. M. and Wilson D. B. (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* **335**, 256–259.