

# Human IgE in SCID Mice Reconstituted with Peripheral Blood Mononuclear Cells from *Dermatophagoides pteronyssinus*-Sensitive Patients<sup>1</sup>

Joël Pestel,<sup>2\*</sup> Pascale Jeannin,\* Yves Delneste,\* Jean-Paul Dessaint,<sup>†</sup> Jean-Yves Cesbron,<sup>†</sup> André Capron,<sup>†</sup> Anne Tsicopoulos,\* and André-Bernard Tonnel\*

\*INSERM U416 and <sup>†</sup>INSERM U167, Pasteur Institute, Lille, France

SCID mice were reconstituted with purified peripheral blood mononuclear cells from patients sensitive to *Dermatophagoides pteronyssinus* (Dpt). After immunization with a low dose of the related allergen, these human (Hu)-SCID mice may develop a specific IgE response. By using an IgE-dependent platelet assay and the related allergen Dpt, the human IgE was demonstrated to be functional. Indeed a high correlation was obtained between both parameters. No rise in human IgE level was detected within 3 wk after the immunization with an unrelated Ag. When mice were reconstituted with peripheral blood cells from nonallergic donors and immunized with the Dpt allergen, no human IgE production was evidenced in the serum of SCID mice. However, in both allergic Hu-SCID mice (reconstituted with cells from allergic patients) or in nonallergic Hu-SCID mice (reconstituted with cells from healthy donors), an IgG response was detectable. Finally by radioallergosorbent test, a specific IgE Ab response was detected after the first allergen challenge only in allergic Hu-SCID mice. Thus, without ethical constraints, SCID mice represent a useful model for analyzing the IgE response occurring in allergic patients. *The Journal of Immunology*, 1994, 153: 3804.

**T**he severe combined immunodeficiency (SCID)<sup>3</sup> syndrome observed in C.B-17 mice has provided a model to analyze normal human immune function as well as human pathogenic mechanisms (e.g., autoimmune diseases) (1–7). Because of a mutation affecting the recombinase system, T and B cell progenitors are arrested early in their development (8, 9) and consequently, SCID mice are unable to reject allogeneic or xenogeneic transplants (10–12). Thus SCID mice tolerate normal human lymphoid tissues (including fetal liver, bone marrow, and thymus) and allow to some extent the differentiation of human lymphoid cells (13–17). Moreover, mature human lymphocytes isolated from peripheral blood survive for extended periods of time in SCID mice (18–19) and de-

velop a spontaneous production of human Ig (10, 20). This Ig production is usually pauciclonal and consists mainly (>85%) of the IgG isotype; some IgM, IgA, and IgD could be detected (20). Indeed human IgG serum levels increase in the SCID mouse recipient for 2 mo, but in some mice could remain high for at least 2 yr (10).

In contrast to SCID mice injected with normal human PBL, SCID mice reconstituted with PBL from patients with systemic lupus erythematosus (SLE) were shown to contain human immunoglobulins and autoantibodies characteristic of SLE in their sera (3). When human peripheral blood cells from donors vaccinated against tetanus toxoid are used for mice reconstitution, the immunized Hu-SCID mice develop an Ag-dependent Ab response against the related Ag (21, 22). The dose-dependent Ag-driven B cell activation led to the production of Abs with different avidities.

Thus SCID mice repopulated with human peripheral blood cells allow the induction of an *in vivo* secondary Ab response, and provide therefore a model for studying human memory cells, avoiding the ethical constraints of recall Ag administration in patients with allergic or inflammatory diseases. Indeed the humanized SCID mouse model seems to be useful for analyzing the regulatory mechanisms of human Ig formation under more physiologic conditions. Many *in vitro* studies emphasized the

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<sup>2</sup> Address correspondence and reprint requests to Dr. Joël Pestel, INSERM U 416, Institut Pasteur, BP 245, 59019-Lille, France.

<sup>3</sup> Abbreviations used in this paper: SCID, severe combined immunodeficient; SLE, systemic lupus erythematosus; Dpt, *Dermatophagoides pteronyssinus*; TT, tetanus toxoid; RAST, radioallergosorbent test; Hu-SCID, human-SCID.

T cell-dependent IgE secretion, however little is known of the in vivo conditions of the human IgE regulation. This study was undertaken to try to establish such an animal model of human IgE production.

In this report, SCID mice were reconstituted with purified peripheral mononuclear cells from donors who were nonsensitive or sensitive to house dust mite allergens (*Dermatophagoides pteronyssinus*) and after reconstitution, were immunized with the allergen. A specific IgE Ab response was detected after the first allergen challenge only in allergic Hu-SCID mice.

## Material and Methods

### Mice

C.B-17 SCID mice were bred and maintained under specific pathogen-free conditions without prophylactic antibiotic treatment at the Institut Pasteur de Lille. The SCID colony was derived from breeding pairs kindly provided by M. Lieberman (Stanford University, Stanford, CA). The absence of detectable Ig in mouse sera was checked by RIA before the transfer of human cells.

### Human peripheral blood mononuclear cells

Blood was collected from donors who were sensitive or nonsensitive to house dust mite. Allergic patients presented the usual features of house dust mite sensitization: skin-prick tests positive for *Dermatophagoides pteronyssinus* (*Dpt*) allergen (Stallergènes, Fresnes, France) (diameter  $\geq 10$  mm), and positive RAST (Pharmacia, Sollentuna, Sweden) (class 3 or 4). The serum IgE level of the allergic patients studied ranged from 785 to 3300 IU/ml. Healthy donors (RAST to *Dpt*  $< 0$ ) (without any allergic disorder or atopy) were tested as negative controls. Peripheral blood mononuclear cells were prepared by density centrifugation on Ficoll-Paque (Pharmacia, St. Quentin en Yvelines, France) of buffy coats from donors sensitive ( $n = 5$ ) or nonsensitive ( $n = 3$ ) to house dust mite. Mononuclear cells harvested at the interface were washed three times in sterile RPMI medium before transfer.

### Cell transfer and immunization

Mice were between 6 and 8 wk old at the time of cell transfer. Cells were injected i.p. in 400  $\mu$ l of RPMI into SCID mice via a 23-gauge needle. The number of cells injected in each experiment is indicated in the Results section. Two weeks after cell transfer, mice were immunized with a low dose of *Dpt* allergen (Stallergènes) (10 IR/ml) in aluminum hydroxide-Alu-Gel-S (Serva, Heidelberg, Germany). In some experiments, 3 wk after the first immunization, mice were boosted with the same amount of *Dpt* in the presence of Alu. *Hymenoptera* venom extract at a similar Ag concentration was used as a control. Of 61 reconstituted SCID mice, only two showed a low level of mouse IgG 90 days after their reconstitution. These leaky mice were not included in the present results.

### Human IgE and IgG measurements

After transplantation with human cells, mice were bled at the retro-orbital sinus under ether anesthesia. Total human IgG was measured by nephelometry, a technique requiring at least 300  $\mu$ l of diluted serum (usually at 1 in 10, i.e., 30  $\mu$ l of Hu-SCID mouse and 270  $\mu$ l of RPMI) for each Hu-SCID mouse. The sensitivity of the method allows the detection of low levels of IgG (1  $\mu$ g/ml). IgE was investigated by a two-site immunoradiometric method with the use of two different mouse mAbs specific for the  $\epsilon$ -chain (Immunotech International, Luminy, France). At least 20  $\mu$ l of serum was used in a duplicate test. The sensitivity of the method permits the detection of 0.1 IU/ml (0.24 ng/ml).

Specific IgE Ab against *Dpt* allergen were quantified by a RIA. Briefly, plastic tubes (Maxisorb Startube, Nunc, Denmark) were coated overnight with *Dpt* allergen in 0.1 M carbonate/bicarbonate buffer (pH 9.6) at 4°C and saturated with 1% BSA in 0.1 M PBS (pH 7.4) for 2 h at room temperature. After washing, the tubes were incubated for 2 h at

room temperature and overnight at 4°C with Hu-SCID mice serum diluted in PBS containing BSA (1%) and Tween (0.01%). After extensive washings, a radio-labeled anti-human Fc IgE (Southern Biotechnologies, Birmingham, UK) was added. After further overnight incubation, tubes were washed and the bound radioactivity was measured with a gamma counter (LKB, Uppsala, Sweden). Results are expressed as cpm. Background binding corresponds to the radioactivity measured in BSA-coated tubes instead of allergen-coated tubes. A human serum that contained 1733 IU of IgE/ml and corresponded to a RAST class 4 was used as a positive control; a human serum that contained 136 IU of IgE/ml and corresponded to a negative RAST was used as a negative control.

### Platelet stimulation

As it was difficult to passively sensitize human basophils with serum of Hu-SCID mice to measure the IgE-dependent assumed histamine release, the method (conventionally used in our laboratory) for evaluating the human platelet reactivity in response to an IgE Ab-mediated stimulation was used (23, 24). Platelets were sensitized with IgE-containing serum from Hu-SCID mice and incubated in the presence of the related allergen (*Dpt*). If specific IgE is present, platelets are stimulated and release cytosolic factors able to kill targets such as schistosomula. Platelet stimulation was estimated by measuring the percentage of dead larvae.

Venous blood from healthy donors collected in ACD (citric acid-trisodic citrate-dextrose) was centrifuged for 15 min at 120  $\times g$ . After collection and centrifugation of the platelet-rich plasma (200  $\times g$ ), the pellet was washed three times in saline supplemented with citric acid (36 mM), glucose (5 mM), calcium (2 mM), magnesium (1 mM), BSA (0.35%), and PGE1 (100 mM). The last pellet was resuspended and adjusted to the appropriate concentration before use.

Platelets ( $15 \times 10^7$ ) were suspended in Eagle's minimum essential medium and incubated in flat-bottom microplates (Nunc-Roskilde, Denmark) with 50 to 100 *Schistosoma mansoni* schistosomula, 10% serum from Hu-SCID mice (20  $\mu$ l), and *Dpt* allergen in a final volume of 200  $\mu$ l according to the method previously described (23, 24). Each Hu-SCID mice serum was tested in duplicate. The stock *Dpt* Ag preparation (corresponding to 100 IR units/ml) was used at the dilution of  $10^{-1}$  to  $10^{-4}$ . After 18 h at 37°C and 5% CO<sub>2</sub> in air, motionless and dark dead larvae were counted under an optical microscope and the percentage of cytotoxicity was calculated.

## Results

### Evidence of a human IgE production in SCID mice reconstituted with PBL from *Dpt*-sensitive patients

In a preliminary experiment, SCID mice were reconstituted with different numbers of human mononuclear cells from a patient sensitive to *Dpt* (from  $5 \times 10^6$  to  $25 \times 10^6$ ) and immunized with *Dpt*. Results indicated that  $10 \times 10^6$  cells were sufficient to induce a significant production of human IgE in mice reconstituted and immunized with *Dpt* (data not shown). One example of the response pattern obtained in these defined conditions is shown in Figure 1A. The pattern of response was different for each mouse tested. Though the human IgE response was consistently elevated at day 14 after immunization, the peak of the response was delayed in some mice at day 21. After boosting, mice may have another peak of human IgE production. Thus peripheral blood cells that have colonized SCID mice were able to respond twice to allergen injection, evoking a secondary IgE response.

Similar results were obtained in other reconstitution experiments conducted with the purified mononuclear cells from five different allergic donors sensitive to *Dpt* (five mice for each patient and  $10 \times 10^6$  cells injected to each mouse). Indeed the mean level of human IgE at day 14

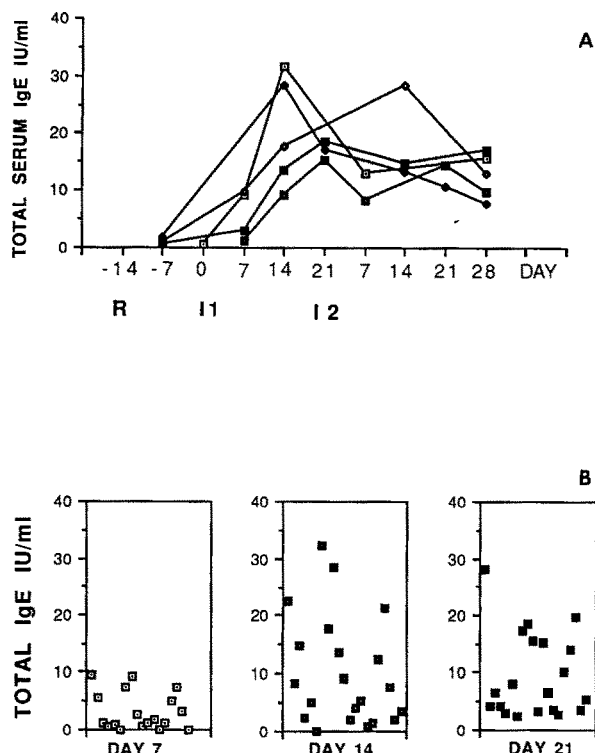


**FIGURE 1.** Evidence of IgE production in SCID mice reconstituted with purified mononuclear peripheral blood cells from patients sensitive to *Dpt*. **A**, a group of five SCID mice was reconstituted at day -14 (**R**) with mononuclear cells from one allergic donor sensitive to *Dpt* ( $10 \times 10^6$  cells/mouse). Two weeks after reconstitution, at day 0, a first injection (**I1**) of the related allergen *Dpt* was performed. In this group of mice, a second allergen injection (**I2**) was performed at day 21. After **I1** (day 0), mice were periodically bled at days 7, 14, and 21, and also after **I2** (day 21) at days 7, 14, 21, and 28. For each mouse the total human IgE level was measured. **B**, another group of 21 SCID mice was reconstituted at day -14 by i.p. injection of cells from five different allergic donors sensitive to *Dpt* ( $10 \times 10^6$  cells/mouse). After injection (**I1**) of the related allergen, at day 0, mice were periodically bled at day 7 ( $n = 18$ ), day 14 ( $n = 21$ ), and day 21 ( $n = 20$ ) and the total human IgE was measured. Each point represents the value obtained for one Hu-SCID mouse.

( $10.2 \pm 9.4$ ) was equal to that observed at day 21 ( $9.6 \pm 7.3$ ) but was statistically different from that obtained at day 7 ( $3.1 \pm 3.3$ ) (Fig. 1B).

In contrast, when SCID mice were either reconstituted with peripheral blood cells from different nonallergic donors and immunized with the *Dpt* allergen or reconstituted with peripheral blood cells from *Dpt*-sensitive patients and injected with another Ag (*Hymenoptera* venom), no human IgE was detected within 3 wk after the first immunization. After a second Ag challenge, only 2 of 20 mice were demonstrated to produce a very low level of human IgE (2 to 4 IU/ml) (data not shown). However, in SCID

mice reconstituted with cells from allergic patients as well as in SCID mice reconstituted with cells from healthy donors, an IgG response was detected along the time course of the experiment, particularly at day 14 and at day 21 (Fig. 2). These results suggest that after reconstitution with cells from patients sensitive to *Dpt*, an IgE response developed only when the specific allergen was injected.

#### *Functional activity of the human IgE produced in "allergic" mice*

The assumed functionality of the IgE produced was assessed by using a platelet-reactivity test. When a significant serum IgE level was detected in *Dpt*-allergic Hu-SCID mice, a significant platelet reactivity was observed also, suggesting the specificity of IgE present in the Hu-SCID mice against *Dpt* allergen (Fig. 3A). Thus the intensity of the platelet reactivity observed was demonstrated to be highly dependent on the IgE level observed in allergic Hu-SCID mice (Fig. 3B).

Indeed the response depends on the source of cells used for the reconstitution of mice and also on the Ag used for the immunization. When allergic Hu-SCID mice were immunized with an unrelated Ag such as *Hymenoptera* allergen or with *Aspergillus* Ag, no IgE production and no significant platelet cytotoxic response were observed (data not shown).

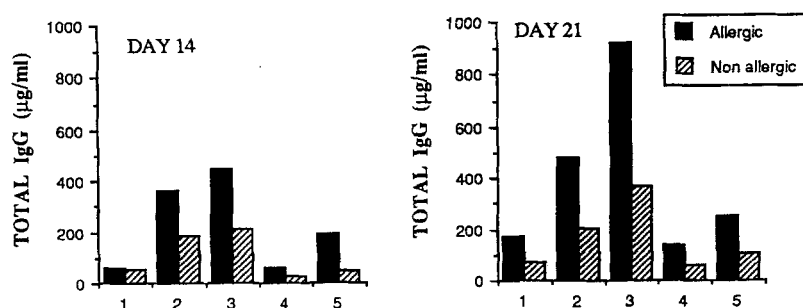
Moreover, when SCID mice were reconstituted with peripheral blood mononuclear cells from nonallergic donors and immunized with *Dpt* allergen, in all cases the IgE production was not greater than 2 IU/ml and the platelet reactivity remained below 10% (data not shown).

Finally, when the allergic Hu-SCID mice serum was depleted in IgE molecules by specific immunoadsorption, no *Dpt* allergen-dependent platelet reactivity was observed (Table I). Thus the IgE-dependent platelet reactivity seemed to be highly dependent on the Ab specificity of IgE produced in each reconstituted mouse.

#### *Evidence of Ag-specific IgE Abs in "allergic" Hu-SCID mice*

The presence of IgE Abs directed against the *Dpt* allergen was evidenced by a specific RIA (Fig. 4). By using *Dpt*-coated tubes, specific human IgE Abs were detected in allergic Hu-SCID sera at day 14 or at day 21 after immunization, particularly when human IgE was significantly elevated. In the absence of a significant human IgE serum level, no specific IgE Abs were detected (data not shown). Indeed no significant specific IgE was evidenced when sera from Hu-SCID mice reconstituted with *Dpt*-allergic cells and injected with an unrelated allergen were tested. Moreover, when positive allergic Hu-SCID serum was tested in BSA-coated tubes, the bound radioactivity remained low, similar to the background obtained with sera from nonallergic Hu-SCID mice (data not shown).

**FIGURE 2.** IgG serum level in SCID mice reconstituted with cells from patients sensitive to or nonsensitive to *Dpt*. The individual results obtained in two representative groups of five Hu-SCID mice were shown. One group was reconstituted with purified mononuclear cells from a patient sensitive to *Dpt* (■) ( $10 \times 10^6$  cells/mouse); the other group was reconstituted with purified mononuclear cells from a patient not sensitive to *Dpt* (▨) ( $10 \times 10^6$  cells/mouse). In both cases, Hu-SCID mice were immunized 2 wk later with *Dpt* allergen and the IgG response was evaluated at days 14 and 21 after allergen injection.



## Discussion

Several elegant reports demonstrated that a specific IgG-mediated immunity can be observed in SCID mice engrafted with human PBL, either if PBL were derived from a donor that has been recently immunized or, if the injected cells were previously incubated with Ag in vitro and if the recipient mice were immunized with the same Ag (3–6, 21–22). In this report we demonstrate that a specific IgE-mediated immunity can be transferred also in SCID mice. Indeed after reconstitution with PBL from patients sensitive to *Dpt*, Hu-SCID mice may develop a specific IgE Ab response only after immunization with the related allergen.

By using a similar protocol of cell injection, previous attempts to reconstitute SCID mice with human leukocytes led to the conclusion that human B cells may persist and develop in SCID mice (20–22). B cell disorders such as those observed in SLE (3) or induced by EBV (25, 26) were thus studied in SCID mice. In such SCID mice, reconstituted with  $20$  to  $50 \times 10^6$  human PBMC, only a restricted set of B cells grew and differentiated (25). A least 2 wk after the cell reconstitution, a spontaneous human IgG production was observed and persisted over a period of 2 mo as the result of an intensive IgG synthesis, despite the limited  $t_{1/2}$  (12 days) of this human isotype when injected in such immunodeficient mice (10). Other isotypes such IgM, IgA, and IgD have also been detected. Only rare reconstituted mice with cells from nonimmunodeficient donors were reported to produce measurable IgE (20).

When mice were reconstituted with purified mononuclear cells from patients sensitive to *Dpt*, the production of IgE could be detected only after a specific reimmunization. In our protocol, a moderate cell number ( $10 \times 10^6$ ) was used. Indeed the production of IgE was not enhanced by increasing the number of human cells injected. Moreover it has been reported that injection of higher number ( $35$  to  $60 \times 10^6$ ) of peripheral blood cells may induce an abnormal human B cell proliferative response and B cell lym-

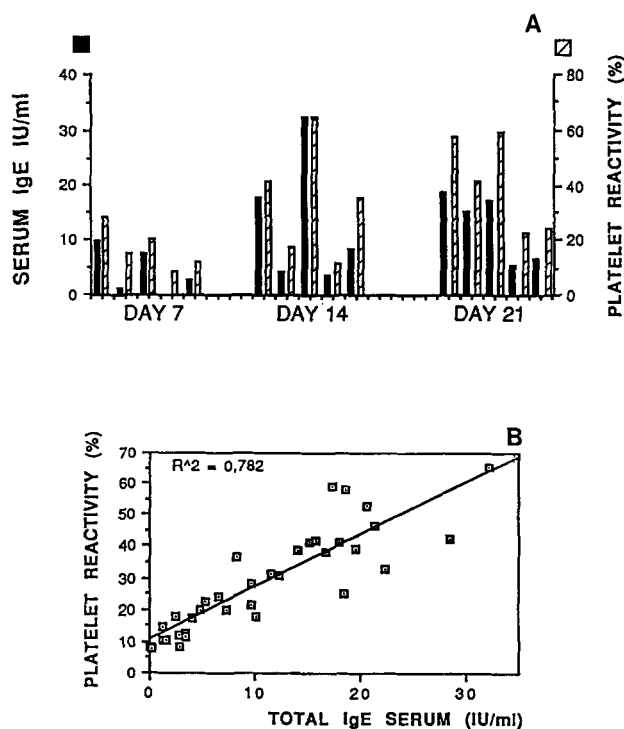


**FIGURE 3.** Relationship between the percentage of platelet reactivity and the amount of IgE present in the serum of "allergic" Hu-SCID mice. *A*, a group of five SCID mice was reconstituted with purified mononuclear cells from a patient sensitive to *Dpt* ( $10 \times 10^6$  cells/mouse) and immunized 2 wk later with *Dpt* allergen. At days 7, 14, and 21, the total human IgE serum level (■) was detected and the serum-associated platelet reactivity (▨) was simultaneously evaluated. Each column represents the value obtained for one mouse. *B*, a group of 15 mice was reconstituted with purified mononuclear cells from two patients sensitive to *Dpt* ( $10 \times 10^6$  cells/mouse) and immunized 2 wk later with *Dpt* allergen. At days 7, 14, and 21, the total human IgE serum level and the serum associated-platelet reactivity were simultaneously evaluated. A correlation between the values ( $n = 36$ ) obtained for each parameter was obtained.

Table 1. Effect of IgE-depleted serum from "allergic" Hu-SCID mice on the *in vitro* platelet reactivity<sup>a</sup>

| Platelets Incubated with: | Percentage of Reactivity |
|---------------------------|--------------------------|
| Hu-SCID serum 1           | 43.7 ± 1.4               |
| Hu-SCID serum 1-IgE       | 16.5 ± 1.6               |
| Hu-SCID serum 2           | 52.6 ± 1.9               |
| Hu-SCID serum 2-IgE       | 19.1 ± 2.1               |
| Allergic human serum      | 86.2 ± 2.1               |
| Allergic human serum-IgE  | 24.4 ± 2.8               |

<sup>a</sup> Native or IgE-depleted serum from Hu-SCID mice reconstituted with purified mononuclear cells from one allergic donor and immunized with the related Ag was incubated with normal FcεR-bearing human platelets and with the stimulating *Dpt* allergen. After a 16-h incubation the platelet reactivity was evaluated by measuring the cytotoxic effect on nonspecific targets (schistosomula). Results are expressed in percentage.

Two Hu-SCID sera were tested: one taken at day 14 (n°1) and the second taken at day 21 (n°2). A allergic human serum (from the cell donor sensitive to *Dpt*) was used as positive control. In the presence of a negative serum (from a donor nonsensitive to *Dpt*) the background value was 12.6 ± 1.8.

phomas (25). Recently, we also observed that according to the cell donor and to the number of cells injected, SCID-mice repopulated with allergic human cells may develop an IgE B cell proliferative disorder at least 7 to 9 wk after immunization; in this case serum human IgE level can reach 100 IU/ml. However, the fact that  $10 \times 10^6$  mononuclear cells was sufficient to achieve efficient reconstitution of SCID mice and render them able to respond to an allergen challenge indicates that immunization favors the expansion of IgE-producing B cells as well as IgG-producing B cells. As described for a specific IgG response to tetanus toxoid (TT) (21) as well for a specific IgG response to hepatitis core B Ag (22), the Ag stimulation might rescue B cells from apoptosis, a mechanism proposed for the selection of B cells under physiologic conditions. Indeed in SCID mice reconstituted with mononuclear cells from *Dpt*-sensitive patients, both a rise in IgE and IgG levels was observed, whereas in SCID mice repopulated with mononuclear cells from patients nonsensitive to *Dpt* only a IgG response was detected after injection of *Dpt* allergen.

The way by which the memory B cells were selected was uncertain. Indeed, in the absence of Ag, it is known that, after reconstitution, a decline of human cell number is observed in SCID mice; less than 10% of the injected cells is detectable 2 to 3 wk after reconstitution in the peritoneum (20, 27). After injection of high numbers of human PBL ( $10^8$ ), only a few human cells were detected in SCID spleens and lymph nodes shortly after *i.p.* injection. It has been proposed that early events of cell proliferation take place in the peritoneal cavity (27). The same observation was reported for T lymphocytes, which fall below the detection limit in the blood or in the peritoneum of the mice 1 or 2 mo after *i.p.* cell injection, but constitute up to 50% of all mononuclear cells in the lungs, liver, and spleen of recipient mice (18). Thus, in our reconstituted SCID mice, mature memory B and T lymphocytes could proliferate and participate in the generation of a specific IgE Ab response.

The number of T and B cells during the time course of the experiments has not been checked in detail. However, preliminary analysis of spleen and thymus by immunocytochemistry or by flow cytometry indicated the presence of human B and T cells in reconstituted SCID mice when IgE could still be detected in blood (data not shown). That human IgE production in Hu-SCID mice is a result of the recall response of memory cells is shown by the absence of IgE levels in mice reconstituted with PBL from healthy donors and in SCID mice reconstituted with cells from *Dpt*-allergic patients. The development of the IgE response was highly dependent on the related allergen. Indeed, SCID mice reconstituted with peripheral blood cells from *Dpt*-sensitive patients injected with another Ag such as *Hymenoptera* venom failed to exhibit a specific IgE response within 3 wk after the first immunization but if, after this period, the same SCID mice were immunized with *Dpt* allergen, they produced IgE (data not shown).

Two recent reports mentioned the ability to induce an IgE production by human B cell populations from reconstituted SCID mice. The first was after injection of at least  $50$  to  $100 \times 10^6$  PBL from healthy volunteers after leukopheresis (28); the second was after *in vitro* culture of CD19<sup>+</sup> B cells isolated from transplanted Hu-SCID thymus in the presence of cloned activated CD4<sup>+</sup>T cells and IL-4 (29). Both experimental conditions are different from our present protocol.

Several reports described the spontaneous production of human Ig in SCID mice reconstituted with human mononuclear cells. Some of the reports have focused on the ability to detect a specific Ab response (20–22). Two types of approaches were followed. One consisted of reimmunizing vaccinated healthy donors with TT (a thymus-dependent Ag) and to determine in human reconstituted-SCID mice the presence of anti-TT immune response. As might be expected, detectable anti-TT Abs were present only in SCID mice with high human IgG serum levels, but the intensity of response varied according to the Hu-SCID mice tested. A similar approach was developed to demonstrate the immune response to blood group A and D Ags.

The second approach consists of injection of peripheral blood cells from vaccinated donors into SCID mice to try to induce a specific Ab response to recall Ag by an *in vivo* immunization. Carlsson et al. (21) reported that after injection of 50 µg Ag 1 day after cell transfer, SCID mice repopulated with  $40 \times 10^6$  PBL from vaccinated healthy donors produced a specific anti-TT IgG response 2 wk later. This specific Ab IgG response was dose dependent and after two Ag-specific challenges, the intensity of the Ab response increased within the subsequent 4 to 6 wk. The mean titer of specific anti-tetanus Ab increased with time in responding and immunized animals, but not in control animals. This specific Ab IgG response was shown to be dependent on the donor.

In our experiments, the intensity of the IgE response was also dependent on several factors. First, the donor of

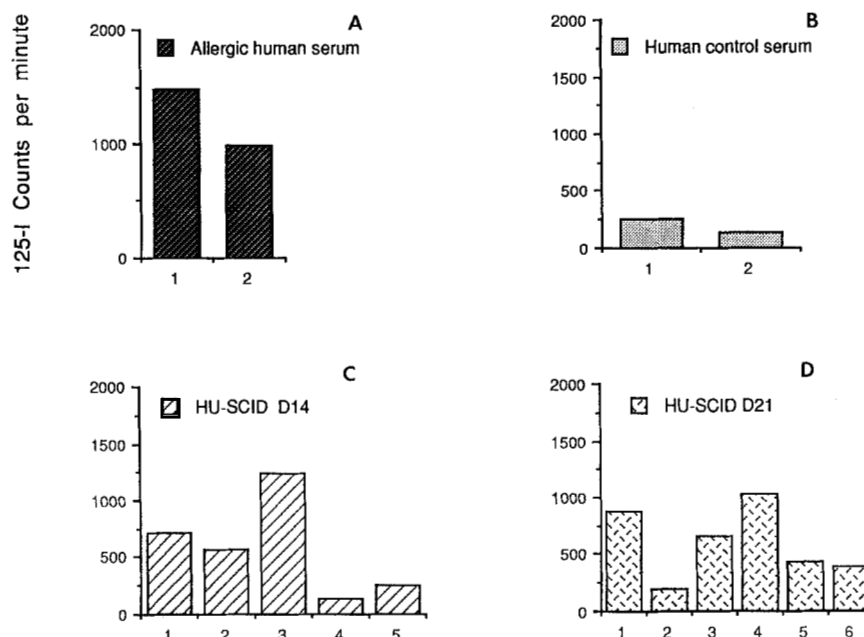


**FIGURE 4.** Presence of IgE specific to *Dpt* allergens in serum of "allergic" Hu-SCID mice. *Dpt* allergen-coated tubes were incubated with sera (taken at day 14 and at day 21 after allergen injection) from Hu-SCID mice reconstituted *Dpt*-sensitive patient (corresponding to sera previously evaluated for the IgE-dependent platelet reactivity; see Fig. 3A). After the addition of specific radiolabeled anti-IgE Abs and washings, the bound radioactivity was counted. **Panel A**, human positive control serum: (1) diluted at 1/5; (2) diluted at 1/10. **Panel B**, human negative control serum: (1) diluted at 1/5; (2) diluted at 1/10. **Panel C**, sera from Hu-SCID mice at day 14: (1) diluted at 1/4 (mouse No. 1); (2) diluted at 1/4 (mouse No. 3); (3) diluted at 1/2 (mouse No. 3); (4) diluted at 1/4 (mouse No. 4); (5) diluted at 1/4 (mouse No. 5). **Panel D**, sera from Hu-SCID mice at day 21: (1) diluted at 1/4 (mouse No. 1); (2) diluted at 1/10 (mouse No. 2); (3) diluted at 1/4 (mouse No. 2); (4) diluted at 1/4 (mouse No. 3); (5) undiluted (mouse No. 4); (6) undiluted (mouse No. 5).

cells must be sensitive to *Dpt*; when the donor was non-sensitive to *Dpt*, no IgE response was induced after allergen injection. Second, the intensity of response was not similar in each SCID mice within the 3 wk after immunization by allergen. This phenomenon was already mentioned in SCID mice in which the spontaneous and/or the specific immune response was analyzed (22). This usual variation in the ability of SCID mice to develop homogeneous human immunity was observed for the five patients sensitive to *Dpt*. Indeed, for each allergic patient tested, usually at least three of five mice exhibited a significant IgE level and *Dpt*-specific IgE within the 3 wk after specific immunization. Although the lower responses were obtained when SCID mice were reconstituted with peripheral blood cells from patients having lower specific IgE levels (as estimated by RAST), these preliminary results did not allow us to establish a strict correlation between the level of IgE in patients and the intensity of IgE response that developed in reconstituted SCID mice. Nonetheless, the main point of our study is that for the first time, to our knowledge, a specific IgE response is described in such a SCID mice model.

Taken together, the present results suggest that with the appropriate reconstitution and immunization of SCID mice, the SCID model could be useful in more precisely under-

standing the regulatory mechanisms that control the human IgE response as well as the allergy to house dust mite.

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