

## Human IgE production in hu-PBL-SCID mice injected with birch pollen and diesel exhaust particles

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### Abstract

Mice with severe combined immunodeficiency were transplanted with human peripheral blood lymphocytes (hu-PBL-SCID mice). The response to immunisation with birch pollen was used to study possible effects of diesel exhaust particles (DEP) and aluminium hydroxide (Al(OH)<sub>3</sub>) on human IgE production in this human *in vivo* model. The adjuvants were well tolerated, as determined by the number of human cells in the peritoneal cavity at the end of the experiments. Total and birch pollen-specific IgE was detected in 76 and 41% of the mice, respectively. In the present experiments where the mice were stimulated early with birch pollen, a doubling in percentage of hu-PBL-SCID mice with production of specific IgE was observed, as compared to later stimulation used in previous experiments. Although a tendency to higher total IgE levels was observed after treatment with DEP, no statistically significant adjuvant effect of DEP or Al(OH)<sub>3</sub> could be demonstrated. Electron microscopy analysis after immunogold labelling showed that the major birch pollen allergen Bet v I was released from the pollen grains and adsorbed to the surface of the DEP. Early stimulation with allergen appears to be important for optimal production of specific IgE in the hu-PBL-SCID model. However, our results show that further improvements are needed in order to demonstrate the expected effects from adjuvants and environmental pollutants. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Hu-PBL-SCID mice; Diesel exhaust particles; Aluminium hydroxide; IgE

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## 1. Introduction

Allergic diseases represent an increasing problem in the industrialised world. Since the prevalence of allergic diseases seems to be higher in urban than in rural areas (Ishizaki et al., 1987; Keil et al., 1996), the effect of diesel exhaust particles (DEP) has attracted attention. It has been shown that aromatic hydrocarbons from DEP have an adjuvant effect on IgE production in cell cultures (Takenaka et al., 1995). DEP has also been shown to enhance specific IgE production in vivo using mice as a model system, both by intraperitoneal, intranasal and intratracheal injection of allergen and DEP (Muranaka et al., 1986; Takafuji et al., 1987; Suzuki and Kanoh, 1993; Fujimaki et al., 1994, 1997; Løvik et al., 1997). In humans, DEP has been shown to increase both total and specific IgE levels in nasal lavage fluid (Diaz-Sanchez et al., 1994, 1997). Aluminium hydroxide ( $\text{Al}(\text{OH})_3$ ) is a commonly used adjuvant in vaccines, and has been shown to enhance IgE production in mice (Beck and Spiegelberg, 1989), probably by suppressing Th1 development via an IL-4-dependent mechanism (Brewer et al., 1996).

Mice with severe combined immunodeficiency (SCID) lack mature T- and B-cells, and can therefore easily be transplanted with human cells without the problem of immunological graft rejection (Bosma et al., 1983; Mosier et al., 1988). We have previously reported that human peripheral blood lymphocytes (PBL) showed a better engraftment and a better antibody response when transplanted into SCID mice than into RAG-2 (recombinase activating gene-2) knock-out mice (Steinsvik et al., 1995). Several workers have begun using SCID mice transplanted with human PBL (hu-PBL-SCID mice) as a model to study human allergy, and the production of total as well as specific IgE has been reported (Saxon et al., 1991; Pestel et al., 1994; Spiegelberg et al., 1994; Steinsvik et al., 1994; Chiang et al., 1995).

In the present experiments, we explored if early stimulation of the human donor cells would improve the production of specific IgE in hu-

PBL-SCID mice. As immunotoxicity studies are difficult to perform on humans there is a need for alternative human models. Therefore, we wanted to explore the potential of hu-PBL-SCID mice for such studies. In particular, we investigated whether human IgE production could be modulated by DEP and  $\text{Al}(\text{OH})_3$  in this experimental human in vivo model.

## 2. Materials and methods

### 2.1. Animals

Thirty-nine 6–8-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. The mice were housed and tested for spontaneous immunoglobulin production (leakiness), as previously described (Steinsvik et al., 1995). 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.

### 2.2. Birch pollen, diesel exhaust particles and aluminium hydroxide

Batch suspensions of birch pollen (Allergon Ab, Engelholm, Sweden) and diesel exhaust particles (DEP) (Standard Reference Material, SRM 1659, US National Institute of Standards and Technology (NIST), Gaithersburg, MD) were made at concentrations of 2 and 10 mg/ml, respectively. For addition to cultures for prestimulation, RPMI 1640 medium with glutamine, 10% FCS and 1% penicillin/streptomycin (RPMI complete) was used to make the batch suspensions, while Hanks Balanced Salt Solution (HBSS) was used for intraperitoneal (i.p.) injection into hu-PBL-SCID mice.  $\text{Al}(\text{OH})_3$  (Superfos Biosector A/S, Vedbaek, Denmark) was delivered at a concentration of 30 mg/ml. Each suspension was stirred in a Whirlmixer, then allowed to stand for at least 1 h, and then stirred again immediately before use.

### 2.3. Experimental procedure

Venous blood was obtained from one donor for three experiments. Experiments 1a, 1b and 2 were started in November, March and early June, respectively. In the identical experiments 1a and 1b (put together and for simplicity referred to as experiment 1), peripheral blood lymphocytes (PBL) were separated from the blood by Lymphoprep (Nycomed, Oslo, Norway) centrifugation, as described by Bøyum (1968). Separated cells were preincubated on plastic dishes (catalogue no. 3060, Costar, Cambridge, UK) in RPMI complete with addition of either medium only, birch pollen (25 µg/ml), or birch pollen (25 µg/ml) and DEP (50 or 500 µg/ml). After 24 h incubation (37°C, 5% CO<sub>2</sub>), the cells were counted in a Bürker chamber, and 15–41 × 10<sup>6</sup> viable human cells were transplanted i.p. to groups of four to six SCID mice. At days 8 and 21 after transplantation, the mice were injected i.p. with either birch pollen (25 µg), or birch pollen (25 µg) and DEP (50 or 500 µg).

In experiment 2, venous blood was obtained from the donor and separated, as described above. The cells were counted and 26 × 10<sup>6</sup> viable human cells were transplanted i.p. to three groups of four SCID mice. At days 1 and 8 after transplantation, the mice were injected i.p. with either birch pollen (25 µg), birch pollen (25 µg) and DEP (500 µg), or birch pollen (25 µg) and Al(OH)<sub>3</sub> (500 µg).

The mean levels of total and birch pollen-specific IgE in serum from the donor were 123 ng/ml and 27 U/ml, respectively. Serum from the donor was shown to be negative for IgG antibodies to Epstein-Barr virus (EBV) capsid antigen by the immunofluorescence technique EB-VCA IFA Kit II (Organon Teknika Corporation, Durham, NC), kindly performed by Dr Anne-Lise Bruu, Department of Virology, National Institute of Public Health, Oslo, Norway.

### 2.4. Collection of mouse blood samples and peritoneal lavage fluids

The mice were bled four times during the experiments with a method previously described

(Steinsvik et al., 1995). After sacrificing the mice (36–37 days after transplantation), peritoneal lavage was performed by i.p. injection of 2.5 ml heparinised HBSS. The trypan blue exclusion method was used to determine viability of cells from the peritoneal cavity (median value: 97%, range: 88–100%).

### 2.5. Flow cytometry

Cells from peritoneal lavage fluid were washed, resuspended in HBSS and incubated with a FITC labelled monoclonal antibody against human CD45 lymphocyte antigen (KC56 (T-200)-RD1, Coulter) for 30 min at 4°C, washed twice in PBS with 0.2% BSA, and finally resuspended in 250 µl PBS/BSA. The antibody was shown not to react with murine lymphocytes (data not shown). Fluorescence intensity was measured using the Coulter Epics®-Profile II flow cytometer, where scatter gates were set on the lymphocytes. The data were corrected for background staining with the appropriate isotype control (FITC-conjugated mouse IgG1, Coulter). Measurements of human CD45 lymphocytes below 2% were considered as background.

### 2.6. Determination of total and birch pollen-specific human IgE

Determination of total human IgE was performed with ELISA, as previously described (Steinsvik et al., 1995). Briefly, mouse anti-human IgE mAbs 4.15 and 7.12 (2 µg/ml, 50 µl/well; overnight incubation) were used for coating the microtiter plates (Dynatech Immulon II M129 B). After washing with Tris-T<sub>20</sub> (0.1 M Tris-HCl buffer, pH 7.4 with 0.05% Tween-20), non-specific binding sites were blocked with Tris-T<sub>20</sub>/1% BSA for 1 h. After further washing, the mouse sera were added (diluted 1:5 or 1:10, 50 µl/well; 3 h incubation). The plates were thereafter washed, and ALP-conjugated goat anti-human IgE (Sigma A-3525) was added in a dilution of 1:3500 (50 µl/well; 3 h incubation). Finally, colour reaction was obtained by adding 1 mg/ml 2-*p*-nitrophenylphosphate (Sigma 104–105) in a diethanolamine buffer, pH 9.8 (100 µl/well), and the

Table 1

Percentage and total number of human CD45-positive cells in peritoneal lavage fluid from hu-PBL-SCID mice 36–37 days after transplantation

| Experiment no. | Allergen/adjuvant                 | n  | Percentage |       | Cell no. ( $\times 10^4$ ) |        |
|----------------|-----------------------------------|----|------------|-------|----------------------------|--------|
|                |                                   |    | Median     | Range | Median                     | Range  |
| 1              | None                              | 4  | 58         | 42–62 | 90                         | 44–129 |
|                | Birch pollen                      | 8  | 54         | 7–85  | 63                         | 3–232  |
|                | + 500 $\mu$ g DEP                 | 7  | 59         | 11–84 | 80                         | 15–152 |
|                | + 50 $\mu$ g DEP                  | 7  | 26         | 6–84  | 41                         | 4–167  |
| 2              | Birch pollen                      | 3  | 75         | 62–80 | 145                        | 98–195 |
|                | + 500 $\mu$ g DEP                 | 4  | 61         | 20–85 | 66                         | 33–151 |
|                | + 500 $\mu$ g Al(OH) <sub>3</sub> | 4  | 48         | 3–63  | 38                         | 3–160  |
| Total          | –                                 | 37 | 56         | 3–85  | 79                         | 3–232  |

A<sub>405</sub> was measured after about 40 min in an automatic ELISA plate reader (Thermo<sub>max</sub> equipped with SoftMax software, Molecular Devices, Menlo Park, CA). Serum concentrations of human IgE were determined with a standard serum calibrated against the CAP-system<sup>®</sup>. The detection limit in this assay was 1.25 ng/ml. Controls for human IgE (Pharmacia Total IgE Control, Kabi Pharmacia, Uppsala, Sweden), mouse Ig and sera from non-transplanted SCID mice were included in the analysis. No cross-reactivity against the other Ig-classes was observed.

Birch pollen-specific human IgE analysis was performed with the CAP technique (Kabi) (Ewan and Coote, 1990). The sera were either undiluted or diluted (1:2, 1:3, 1:4, 1:5 or 1:20), dependent on the amount of serum obtained from the mice. The detection limit in this CAP technique was 0.35 U/ml.

### 2.7. Immunogold labelling and electron microscopy

Immunogold labelling and scanning electron microscopy of polycarbonate filter specimens of 25  $\mu$ g/ml birch pollen grains and 500  $\mu$ g/ml DEP in HBSS, were performed as previously described (Ormstad et al., 1995). Mouse monoclonal anti-Bet v I (clone M82 D3/C8, Smith Kline Beecham, Middlesex, England) was used as a primary antibody, and the probe used was goat anti-mouse IgG conjugated with 30 nm gold particles (Bio

Cell Research Laboratories, Cardiff, UK). The immunogold labelling was shown to be specific, as neither the primary antibody nor the gold-conjugated antibody was able to bind directly to the DEP. As a negative monoclonal antibody control we used an anti-human serum albumin antibody (clone ZMHSA, Zymed, San Francisco, CA).

### 2.8. Statistical analysis

Statistical analysis was performed using the SigmaStat<sup>®</sup> software (version 1.0, Jandel Scientific GmbH, Erkrath, Germany). The non-parametric Mann–Whitney U-test and Spearman's rank correlation test were used, with  $P = 0.05$  as the limit for statistical significance.

## 3. Results

### 3.1. Flow cytometry analysis

At the end of the experiments (36–37 days after transplantation), the percentage and total number of human CD45-positive cells was determined in peritoneal lavage fluid from the hu-PBL-SCID mice (Table 1). Neither with regard to the percentage of human CD45-positive cells nor with regard to the absolute cell number were any statistical significant differences found between the various groups. The median percent and total number of CD45-positive cells for all the trans-

Table 2

Number and percentage of hu-PBL-SCID mice positive for human IgE in the different groups (median and range of maximum serum IgE concentrations obtained in these mice during the experiments are shown)

| Experiment no. | Allergen /adjuvant           | Positive <sup>a</sup> |     | Maximum IgE (ng/ml) |           |
|----------------|------------------------------|-----------------------|-----|---------------------|-----------|
|                |                              | <i>n</i>              | %   | Median              | Range     |
| 1              | None                         | 4/4                   | 100 | 21.5                | 12.9–60.2 |
|                | Birch pollen                 | 6/8                   | 75  | 18.9                | 2.92–70.7 |
|                | + 500 µg DEP                 | 6/7                   | 86  | 44.6                | 13.7–620  |
|                | + 50 µg DEP                  | 5/7                   | 71  | 23.9                | 1.40–53.3 |
| 2              | Birch pollen                 | 2/3                   | 67  | 28.2                | 4.29–52.1 |
|                | + 500 µg DEP                 | 3/4                   | 75  | 26.8                | 2.33–54.1 |
|                | + 500 µg Al(OH) <sub>3</sub> | 2/4                   | 50  | 8.90                | 6.50–11.3 |
| Total          | –                            | 28/37                 | 76  | 26.3                | 1.40–620  |

<sup>a</sup> IgE concentrations > 1.25 ng/ml.

planted mice was 56% and  $79 \times 10^4$  cells, respectively. In two mice the peritoneal lavage fluid contained < 2% CD45-positive cells, which was considered as a negative result. These two mice had no measurable human serum immunoglobulin, and were therefore excluded from the study, since they most likely represented graft failure. The percentage of CD45-positive cells in peritoneal lavage fluids showed statistically significant correlations both with the maximum concentration of total and birch pollen-specific human IgE in serum ( $r = 0.65$  and  $0.55$ , respectively).

### 3.2. Total human IgE

Detectable amounts of human IgE were found in 76% of the mice (Table 2). The median value of maximum IgE in all the positive mice was 26.3 ng/ml with a range from 1.40 to 620 ng/ml. In experiment 1, the maximum IgE level (median value) in the group that received birch pollen and 500 µg DEP was twice as high as the level in the group receiving only birch pollen, while these two groups had similar median levels in experiment 2. Fig. 1 shows the kinetics of IgE production as median values for the various groups of mice. In both experiments, the group of mice stimulated with birch pollen and 500 µg DEP attained the highest median IgE level, although the differences were not statistically significant.

### 3.3. Birch pollen-specific human IgE

Only mice that had been stimulated with birch pollen produced detectable amounts of birch pollen-specific human IgE (Table 3). Among these mice, 45% had measurable levels of birch pollen-specific IgE, with a median value of 4.56 U/ml (range 0.42–1296 U/ml). Maximum birch pollen-specific IgE concentrations showed a statistically significant correlation with the maximum concentration of total IgE ( $r = 0.56$ ). Fig. 2 shows the kinetics of birch pollen-specific IgE production in individual hu-PBL-SCID mice. In experiment 1, one mouse that was stimulated with birch pollen and 500 µg DEP produced extremely elevated levels of specific IgE as compared with the other hu-PBL-SCID mice (maximum value: 1296 U/ml). No statistically significant differences between the groups were detected.

### 3.4. Immunogold labelling and electron microscopy

Analysis in the scanning electron microscope (SEM) showed that the diameter of the birch pollen grains in our preparation was typically 20–30 µm. Furthermore, DEP had the characteristic structure of clustered, spherical subunits (Fig. 3b). When analyzing the immunogold labelled specimens in the backscatter electron imaging (BEI) mode of SEM, the gold probe was visu-

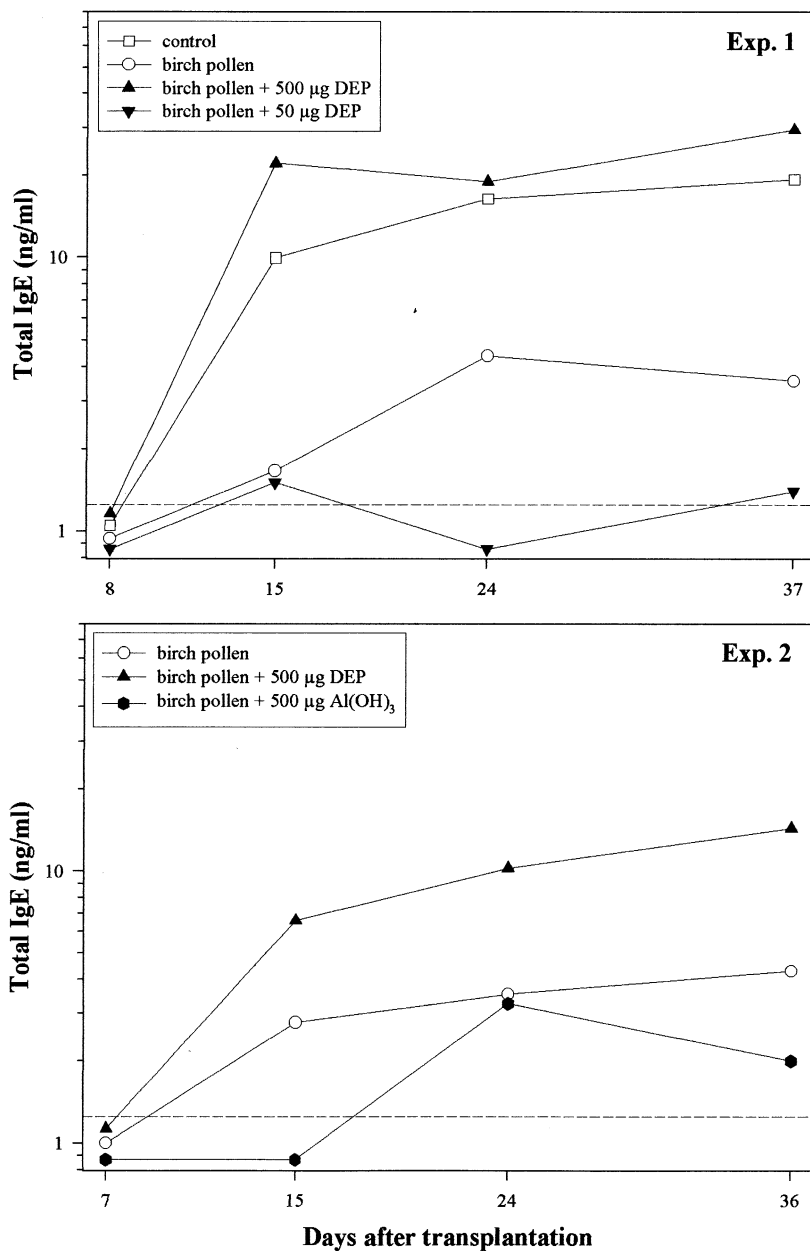


Fig. 1. Median values of human IgE in 4–8 hu-PBL-SCID mice in experiments 1 and 2 are shown. In experiment 1, the cells for transplantation were prestimulated as indicated, and transplanted mice were boosted i.p. with the same amount on days 8 and 21 after transplantation. In experiment 2, the mice were injected i.p., as indicated, at days 1 and 8 after transplantation. Dotted lines indicate the detection limit.

alised on the surface of the DEP (Fig. 3a). This shows that the birch pollen allergen Bet v I was released from the pollen grains in the humid

environment and adsorbed to the DEP. The gold probe was detected both on small and larger DEP aggregates.

Table 3

Number and percentage of hu-PBL-SCID mice positive for human birch-pollen specific IgE in the different groups (median and range of maximum serum IgE concentrations obtained in these mice during the experiments are shown)

| Experiment no. | Allergen/adjuvant            | Positive <sup>a</sup> |                 | Birch pollen-specific IgE (U/ml) |           |
|----------------|------------------------------|-----------------------|-----------------|----------------------------------|-----------|
|                |                              | <i>n</i>              | %               | Median                           | Range     |
| 1              | None                         | 0/4                   | —               | —                                | —         |
|                | Birch pollen                 | 3/8                   | 38              | 4.56                             | 1.28–38.8 |
|                | + 500 µg DEP                 | 3/7                   | 43              | 3.39                             | 0.77–1296 |
|                | + 50 µg DEP                  | 3/7                   | 43              | 4.98                             | 1.15–6.69 |
| 2              | Birch pollen                 | 3/3                   | 100             | 1.47                             | 1.45–13.8 |
|                | + 500 µg DEP                 | 2/4                   | 50              | 3.50                             | 0.42–6.58 |
|                | + 500 µg Al(OH) <sub>3</sub> | 1/4                   | 25              | 5.61                             | —         |
| Total          | —                            | 15/37                 | 41 <sup>b</sup> | 4.56                             | 0.42–1296 |

<sup>a</sup> IgE concentrations > 0.35 U/ml. <sup>b</sup> Among all the mice immunised with birch pollen, 45% had measurable levels of birch pollen-specific IgE.

#### 4. Discussion

At the end of the experiments (36–37 days after transplantation), a median level of human CD45-positive cells in peritoneal lavage fluid of 56% (Table 1) shows that the human cells were properly engrafted in the murine peritoneal cavity. Significant correlations were found between the percentage of human cells and IgE concentrations. However, we did not do further analysis of the anatomical location of the IgE-producing cells, which may differ with time after transplantation and immunoglobulin class (Aaberge et al., 1994), as antibodies used for detection of human B cells have shown high levels of non-specific binding to murine cells (Mosier et al., 1988, 1989). In the experiment in which Al(OH)<sub>3</sub> was used (experiment 2), we did not prestimulate the human cells prior to transplantation, as a preliminary experiment showed that prestimulation with Al(OH)<sub>3</sub> resulted in significantly lower levels of CD45-positive cells as compared to control groups (data not shown). In the present experiment, neither the percentage or total number of human CD45-positive cells (Table 1), nor the viability of the cells (data not shown) in the groups receiving DEP or Al(OH)<sub>3</sub> were significantly different from the controls. However, the group receiving Al(OH)<sub>3</sub> had

a relatively low level of CD45-positive cells in peritoneal lavage fluids (Table 1), and also had fewest responders with regard to both total (Table 2) and specific IgE production (Table 3). The total IgE level in this group had the lowest median value, and only one mouse responded with specific IgE production. Altogether, our experiments suggest that Al(OH)<sub>3</sub> may have an inhibitory effect on the development and function of human cells transplanted into SCID mice, whereas DEP in the amounts used was well tolerated.

In experiment 1, the maximum IgE concentration in the group that received birch pollen and 500 µg DEP was twice as high as the maximum concentration in the group only receiving birch pollen (Table 2). Although the difference did not reach statistical significance, the groups that received birch pollen and 500 µg DEP had the highest median total IgE level at all time points both in experiments 1 and 2 (Fig. 1). Altogether, this suggests that DEP have a promoting effect on the production of human IgE in this *in vivo* hu-PBL-SCID model. Because of the practical circumstances we have no data on mice given DEP or Al(OH)<sub>3</sub> alone. One mouse that received birch pollen and DEP produced extremely high levels of specific IgE, as compared to the other hu-PBL-SCID mice (maximum value: 1296 U/ml). The donor used was EBV-negative, and this

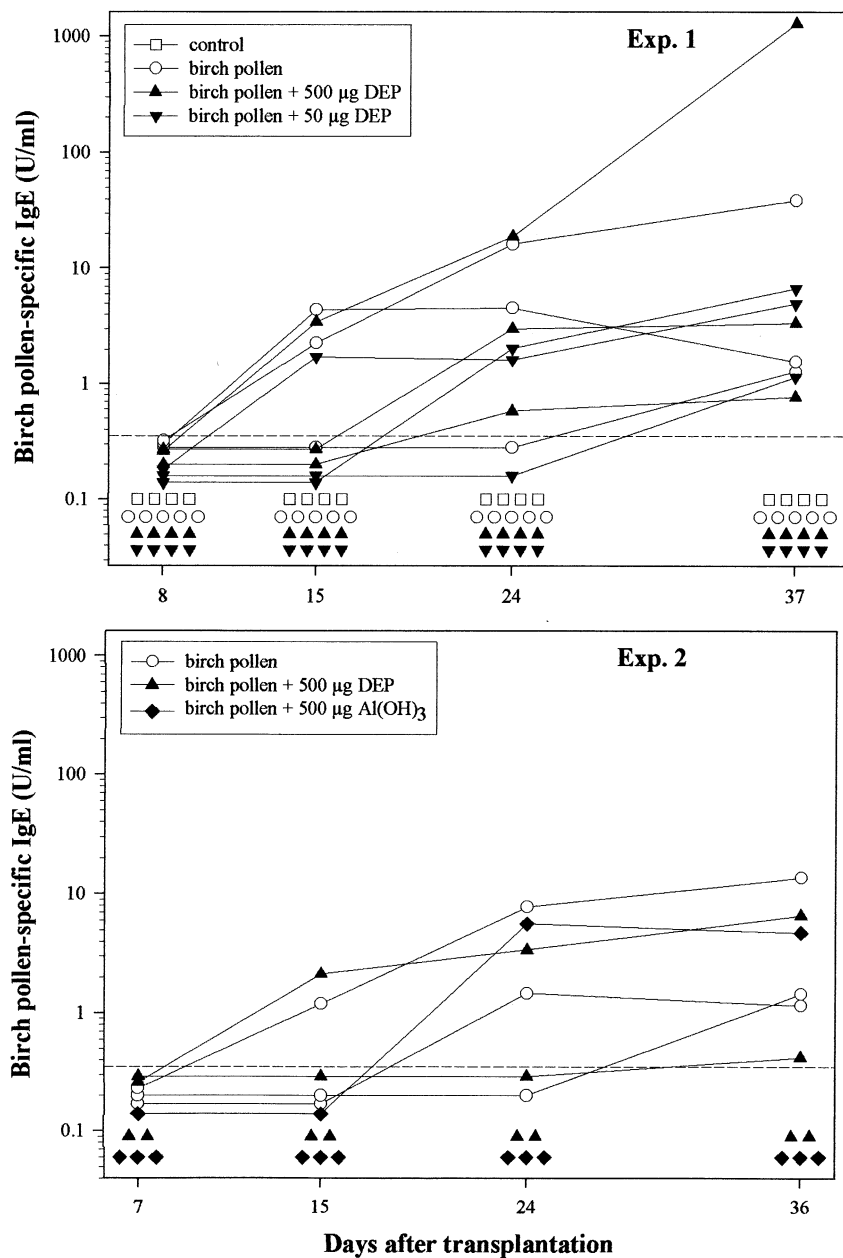


Fig. 2. Birch pollen-specific human IgE in individual hu-PBL-SCID mice in experiments 1 and 2 are shown. In experiment 1, the cells for transplantation were prestimulated as indicated, and transplanted mice were boosted i.p. with the same amount on days 8 and 21 after transplantation. In experiment 2, the mice were injected i.p., as indicated, at days 1 and 8 after transplantation. Dotted lines indicate the detection limit.

mouse did not suffer from any detectable tumor development. All the other mice immunised with birch pollen and DEP that produced specific IgE

had a still increasing level at the end of the experiments (Fig. 2). These results suggest that DEP also may act as an adjuvant on specific

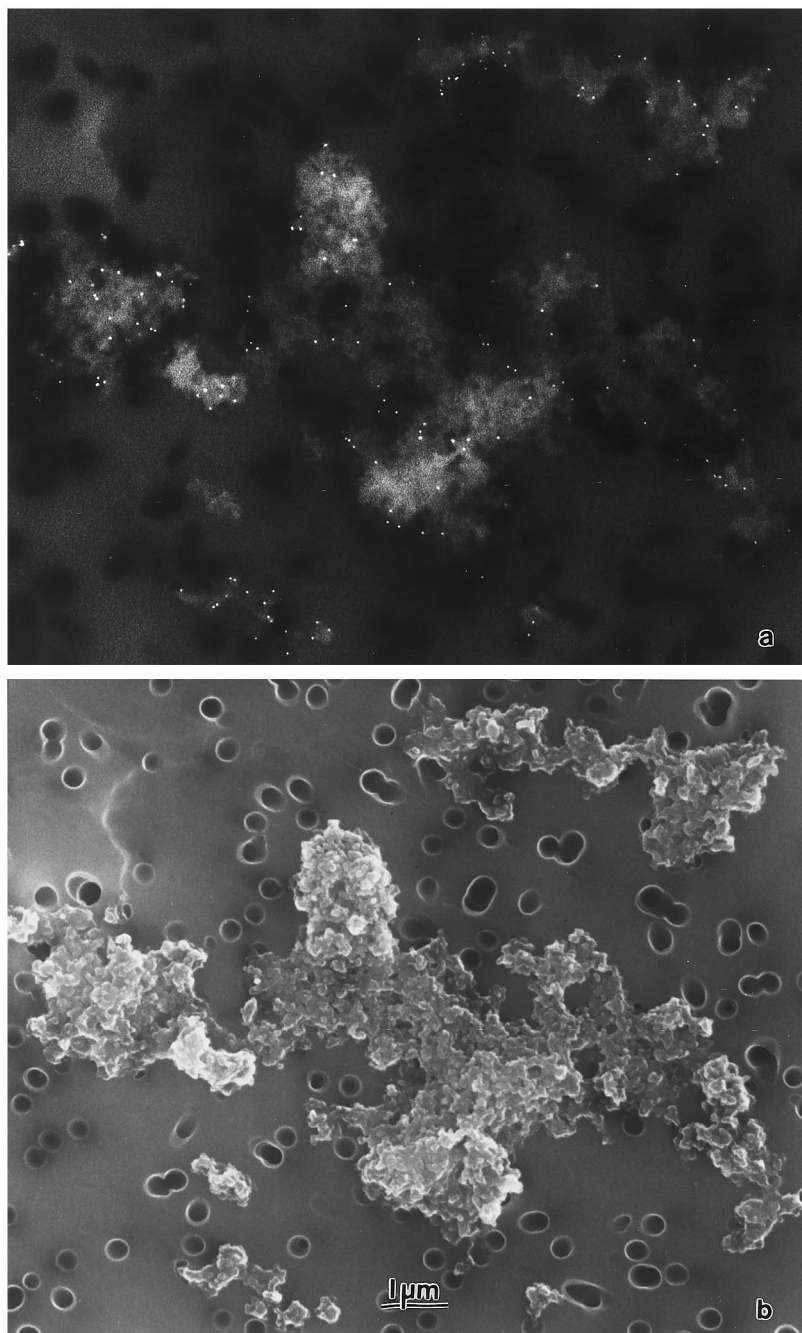


Fig. 3. Backscatter (a) and secondary (b) electron micrographs of a immunogold labelled solution with DEP (500  $\mu\text{g/ml}$ ) and birch pollen grains (25  $\mu\text{g/ml}$ ), showing that the major birch pollen allergen Bet v I was bound to DEP aggregates. The primary antibody was mouse IgG1 anti-Bet v I, and the probe used was goat anti mouse IgG conjugated with 30 nm gold particles. Magnification  $\times 10800$ .

human IgE production *in vivo*, but the effect was not strong enough to become statistically significant in our experiments. One explanation for this may be that the birch pollen grains themselves can act as an adjuvant, either because they are a persisting particle like the carbon core of DEP, or by releasing a low amount of birch pollen allergens for a prolonged period. If this was the case, the system was already strongly driven for IgE production without DEP or  $\text{Al}(\text{OH})_3$  so that an additional adjuvant effect would be hard to obtain. An adjuvant effect would perhaps be more easy to reveal with a non-particulate allergen.

Electron microscopy analysis of a suspension of birch pollen and DEP showed that birch pollen allergens were released from the pollen grains, and that the major birch pollen allergen Bet v I actually was adsorbed to the DEP. An adjuvant effect could therefore be explained by the DEP being able to carry and release allergens for a prolonged period. A positive correlation found between total and birch pollen-specific IgE in the groups receiving birch pollen and 500  $\mu\text{g}$  DEP ( $r = 0.80$ ) indicates that a putative adjuvant effect of DEP could result from a general stimulatory effect on the IgE production. Other mechanisms in addition to DEP's carrier effect could therefore be responsible for an adjuvant effect, and there is evidence that the production of IgE-promoting cytokines is enhanced by DEP (Fujimaki et al., 1994; Diaz-Sanchez et al., 1997).

In previous experiments, we found that birch pollen-specific human IgE production in transplanted SCID mice seemed to require the use of cell donors with the specific allergy, and that it was most readily obtained during or after a period of donor allergen exposure (Steinsvik et al., 1997). In these earlier experiments, both some birch pollen-immunised and some non-immunised hu-PBL-SCID mice produced specific IgE. In the present experiment 1, that was started out of the pollen season, only mice prestimulated with birch pollen produced specific human IgE. This is in accordance with our previous experiments started out of the pollen season (Steinsvik et al., 1997). In the present experiments, we stimulated the cells *in vitro* prior to transplantation (experiment 1), or immunised the mice just after transplantation (ex-

periment 2), and 45% (15/33) of the immunised mice produced birch pollen-specific human IgE. In our previous study (Steinsvik et al., 1997), where the mice were immunised with birch pollen later in the experiment (after 7 and 21 days), only 21% (10/48) of the immunised mice produced specific IgE. Thus, early immunisation with birch pollen resulted in a doubling of percentage of mice with specific IgE in our experiments. The importance of stimulation prior to or early after transplantation of cells into SCID mice also seems to be valid for other Ig-classes (Mosier et al., 1988; Duchosal et al., 1992; Pollock et al., 1994; Albert, 1997). The presence of human Ag-presenting cells in the peritoneal cavity may explain the success with early immunisation, as we have previously shown that transplanted cells migrate to other sites over time (Aaberge et al., 1994).

It might be argued that this animal model, where both transplantation of cells and immunisation with pollen and adjuvants are done intraperitoneally, is not comparable to a real life situation where pollen grains and DEP come into contact with the human body via breathing and inhalation into the lungs. In comparable experiments however, DEP has been shown to have an adjuvant effect on murine specific IgE production regardless of whether the mice were injected intraperitoneally, intranasally or intratracheally with pollen and DEP (Muranaka et al., 1986; Takafuji et al., 1987; Suzuki and Kanoh, 1993; Fujimaki et al., 1994, 1997). Also, because transplanted cells are able to migrate (Tary-Lehmann and Saxon, 1992; Steinsvik et al., 1995), the site of transplantation and immunisation of SCID mice is not likely to be critical for the physiological relevance of the experiments. Recently, we have observed that human PBL from allergics migrate to the lungs after *i.p.* transplantation into SCID mice (to be published).

In the hu-PBL-SCID model, a major problem is the large experimental variation (Pollock et al., 1994), which to some extent can be reduced by using human donors with known responder properties in repeated experiments (Steinsvik et al., 1997). In the case of seasonal allergies, starting the experiments in the allergy season will result in uncontrolled allergen exposure of the cell donor,

which we have documented affect the specific IgE response of transplanted SCID mice (Steinsvik et al., 1997). Starting the experiments out of the pollen season combined with the use of in vitro prestimulation or early in vivo immunisation with succeeding boosting, is likely to give a more controllable experimental system with more responders and minimised experimental variation. Our results shows that further optimisation of the hu-PBL-SCID model is needed in order to demonstrate in vivo effects of adjuvants and environmental pollutants.

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