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Human antibody response to a pneumococcal vaccine in SCID-PBL-hu mice and simultaneously vaccinated human cell donors

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SUMMARY

Severe combined immunodeficient (SCID) mice were transplanted intraperitoneally with human peripheral blood lymphocytes (PBL) from nine healthy human donors (SCID-PBL-hu mice). None of the donors had ever received pneumococcal vaccine. Ten days after transplantation, 62 out of 111 transplanted mice and six of the nine donors were vaccinated with a 23-valent pneumococcal polysaccharide vaccine. For each donor, human IgG was detected in 91·7–100% of the SCID-PBL-hu mice, whereas specific human IgG antipneumococcal antibodies were demonstrated in 16·7–100% of the vaccinated SCID-PBL-hu mice. Most of the mice transplanted with cells from the same donor showed similar antibody response patterns in terms of kinetics and antibody levels. A significant antibody response was only obtained in mice that received cells from donors with relatively high antipneumococcal antibody levels at the time of transplantation, or donors that showed a substantial increase in antibody levels after vaccination. The immune response in the SCID-PBL-hu mice did not always reflect the ability of the respective donor to produce anti-pneumococcal antibodies. The donor dependency of the antipneumococcal antibody response has great practical importance for the use of the SCID-PBL-hu model. Donors should not be chosen randomly. By selecting donors whose cells have been found to result in successful engraftment, functional SCID-PBL-hu mice can be obtained for the study of human immune responses and function in an *in vivo* experimental model.

Keywords SCID SCID-PBL-hu pneumococcal vaccine anti-polysaccharide antibodies

INTRODUCTION

Severe combined immunodeficient (SCID) mice [1] lack a specific immune system and do not reject transplants. SCID mice may therefore be reconstituted with cells from the human immune system, and transplanted SCID mice acquire a certain degree of human immune function [2,3]. However, a primary human immune response to T-dependent antigens has been difficult to obtain in SCID mice transplanted with human peripheral blood lymphocytes (SCID-PBL-hu mice) [2,4,5], whereas an antibody response to such antigens can be induced when the donors have been immunized with the antigen before transplantation [2,4,6–8]. In contrast, a specific human anti-polysaccharide antibody response can be obtained after immunization of SCID-PBL-hu mice with polysaccharide antigens, which are T-independent antigens, when the mice are transplanted with cells from non-immunized donors [9–12]. The antibodies induced are functional because SCID-PBL-hu mice are protected after challenge with virulent bacteria [9,11]. The SCID-PBL-hu model can therefore be potentially useful for the study of immune regulation and function of anti-polysaccharide antibody responses. Because an antipneumococcal antibody response can be induced in SCID mice transplanted with cells from non-vaccinated donors, one could assume that the response after vaccination of transplanted mice would predict the vaccination response in the respective donor. In order to determine whether the human antipneumococcal antibody response in transplanted SCID mice does reflect the capacity for an antibody response in the individual donor, we have in this study transplanted groups of 11–16 mice with human PBL from nine donors. The donors and the transplanted SCID mice were vaccinated at the same time, and the responses induced in the vaccinated donors have been compared with the responses induced by vaccination of the corresponding transplanted mice.

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MATERIALS AND METHODS

Mice

Six to eight-week-old C·B-17 male SCID/SCID mice were obtained from Gl. Bomholtgård Ltd. (Ry, Denmark) under a licence from Fox Chase Cancer Centre. The mice were kept in sterilized microisolator cages containing six mice and were given

sterilized water with 2 mM HCl and irradiated pelleted food (Rat&Mouse no. 3 Breeding Diet; Special Diets Services, Witham, UK) *ad libitum*. The room had a 12 h/12 h light/dark cycle. The mice were rested 1–2 weeks before entering the experiments, and the experimental procedures were performed in a laminar air-flow hood. Each mouse was marked by ear punching. The mice were screened for 'leakiness' by analysing the level of mouse IgG antibodies [12], and only mice with concentrations $< 2\ \mu$g/ml were used in the experiments. The experiments were performed in conformity to the laws and regulations controlling experiments with live animals in Norway, and was approved by the local officer of the Experimental Animal Board under the Ministry of Agriculture.

Transplantation

Venous blood (450 ml) was obtained from nine healthy adult volunteers (employees at our Institute). PBL were separated by Lymphoprep (Nycomed, Oslo, Norway) centrifugation as described by Bøyum [13]. After centrifugation, PBL from the interface were washed twice in Hanks' balanced salt solution (HBSS). The cells were counted in a Bürker chamber, and $21{\cdot}4–50{\cdot}7 \times 10^6$ human cells were given intraperitoneally in a volume of 1 ml to the mice. The same number of cells from the same donor were given to 11–16 mice (regularly to 12 mice).

None of the donors had ever received pneumococcal vaccine at the time of blood donation. All the donors were positive for IgG antibodies to Epstein–Barr virus capsid antigen (EB-VCA IFA Kit II; Organon Teknika Corporation, Durham, NC; assay performed by Dr Anne-Lise Bruu, Department of Virology, National Institute of Public Health, Oslo, Norway).

Vaccination

A 23-valent pneumococcal polysaccharide vaccine (Pneumovax N; Merck, Sharp and Dohme b.v., Haarlem, The Netherlands) containing 25 μg of each of the pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F was used for vaccination of transplanted SCID mice as well as six of the human donors 10 days after the mice had been transplanted. For vaccination of the mice a single dose of $11{\cdot}5\ \mu$g antigen corresponding to $0{\cdot}5\ \mu$g of each of the constituent pneumococcal polysaccharides, was given intraperitoneally in a volume of 0·5 ml of NaCl with 0·25% phenol. For vaccination of the humans a single dose of the pneumococcal vaccine (0·5 ml, 25 μg of each of the constituent pneumococcal polysaccharides) was given subcutaneously in the deltoid muscle. All the donors who were vaccinated received pneumococcal vaccine in accordance with recommendations from our Institute, because they work with virulent pneumococci in diagnostic or research work.

Blood samples for antibody analysis

Blood samples from the mice were collected into heparinized capillary tubes (Vitrex, Herlev, Denmark) from the distal part of the lateral femoral vein, as previously described [9], or by heart puncture at the end of the experiments. Samples from the mice were obtained before transplantation, before immunization, and 2, 4, and 6 weeks after vaccination unless other time points are specified. Sera from all donors were obtained at the time of transplantation, and 2, 4, and 6 weeks after vaccination in the six donors who were vaccinated. Sera and plasma samples (referred to as sera) were stored at -20°C until analysed.

Quantification of human immunoglobulins

The concentration of human IgG in the mouse sera was measured with Turbiquant IgG, anti-human IgG from rabbit for Turbitimer (Behringwerke AG, Marburg, Germany). A normal human serum and a normal mouse serum were included as controls. The quantitative detection limit was 1 mg/ml. Sera with no detectable human IgG measured with Turbiquant were also analysed with a more sensitive ELISA technique for quantification of low levels of human IgG. Microtitre plates were coated for 2 h at room temperature, then overnight at 4°C with goat anti-human IgG (Jackson Immuno Research Labs Inc., West Grove, PA) in carbonate/bicarbonate buffer pH 9·6 (2 μg/ml). After washing five times with 0·1 M Tris/HCl buffer pH 7·4 with 0·05% Tween-20 (Tris–T_{20}), mouse sera diluted 1:100, 1:1000 and 1:10 000 in Tris–T_{20} with 1% bovine serum albumin (BSA) were added and incubated for 2 h at room temperature. Following further washing, alkaline phosphatase (ALP)-conjugated goat anti-human IgG (Jackson Immuno Research Labs) diluted 1:20 000 was added. After incubation for 30 min at room temperature with the substrate 2-*p*-nitrophenylphosphate (Sigma Chemical Co., St Louis, MO), the colour reaction was measured in an automatic ELISA plate reader ($\text{Thermo}_{\text{max}}$ equipped with SoftMax software; Molecular Devices Corporation, Menlo Park, CA). Serum concentrations were determined from Gammaglobulin 20 mg/ml (Kabi Pharmacia AB, Uppsala, Sweden). The detection limit with the dilutions used was $0{\cdot}6\ \mu$g/ml. Serum from a normal mouse and a normal SCID mouse were included and no cross-reactivity was observed.

Human IgM and IgA were quantified with ELISA technique as previously described [12]. The detection limits for human IgM and IgA were 1 and $0{\cdot}5\ \mu$g/ml, respectively.

Determination of human and mouse antipneumococcal antibodies

IgM, IgG and IgA antibodies to pneumococcal polysaccharide vaccine were determined by an ELISA technique as previously described [9]. For coating of microtest plates (Nunc, Roskilde, Denmark), the 23-valent pneumococcal vaccine was diluted to $2{\cdot}3\ \mu$g/ml in PBS pH 7·4. Sera were neutralized with pneumococcal polysaccharide (PnC) (kindly supplied by Dr Jørgen Henrichsen, Statens Seruminstitut, Copenhagen, Denmark) by diluting the sera 1:10 in PnC 10 μg/ml and incubating for 1 h at room temperature. Afterwards the neutralized sera were further diluted 1:10 in PBS with 0·05% Tween 20 (PBS–T) containing 0·5% BSA (final serum dilution 1:100). The amount of PnC used was found to neutralize the antibody activity to PnC in our standard serum (data not shown). Each serum was tested in duplicate, and all samples from each mouse were tested simultaneously. As conjugate for human antibodies, optimal dilutions of immunosorbent-purified biotin-labelled sheep anti-human IgM or IgG mixed with streptavidin and biotin-labelled alkaline phosphatase, or alkaline phosphatase-conjugated immunosorbent purified sheep anti-human IgA was used (kindly supplied by Dr Terje E. Michaelsen of our Department). As conjugate for mouse antibodies, alkaline phosphatase-conjugated goat anti-mouse IgM (μ-chain specific) (Sigma) was used. As substrate, 2-*p*-nitrophenylphosphate (Sigma) was added. Absorbance was measured at 405 nm in a spectrophotometer (Organon Teknika Reader Microelisa system) after incubation at room temperature until a standard serum had reached a predetermined value. As controls, a human post-vaccination serum from a healthy individual and a pool of sera from mice immunized with pneumococcal vaccine were included.

Statistical analysis and experimental design

Statistical analysis was performed with the Sigmastat software (version 1.0; Jandle Scientific GmbH, Erkrath, Germany). The Pearson product moment correlation coefficient test was used with $P = 0{\cdot}05$ as the limit for statistical significance. Two experiments of transplantation and immunization of SCID mice were included in this study. In Experiment 1, cells from donors 1–3 were used for transplantation of 39 SCID mice; 26 of the transplanted mice were immunized with the pneumococcal vaccine. In Experiment 1, half of the mice for each donor were given PCB-77 (3,3′,4,4′ tetrachlorobiphenyl) in order to study the effect of PCB on human immunoglobulin production. However, no tendency to any effect from PCB administration was found [14], and PCB administration has consequently been ignored in the presentation of the results in the present study. In Experiment 2, cells from donors 4–9 were used for transplantation of 72 SCID mice (cells from each donor were given to 12 mice). Half of the transplanted mice and all the six donors in Experiment 2 were immunized with the pneumococcal vaccine at the same time.

RESULTS

Human immunoglobulins in SCID-PBL-hu mice

Human IgG was detected in 91·7–100% of SCID mice after transplantation (Table 1). Median values of total IgG were 3·4 mg/ml and 1·6 mg/ml in Experiments 1 and 2, respectively (Table 2). Human IgM and IgA were detected in 95·8% and 90·3% of the sera from the transplanted SCID mice, respectively. Median levels of total human IgM and IgA were 0·03 mg/ml and 0·003 mg/ml, respectively (Table 2). Mice transplanted with cells from donor 6 and donor 7 showed relatively low levels of human IgG, IgM, and IgA after transplantation (Table 2). There were no differences in the levels of total human immunoglobulins, regardless of whether the transplanted mice had been immunized with pneumococcal vaccine or not.

Table 1. Transplanted SCID mice with detectable human IgG (all transplanted mice included) and transplanted SCID mice with specific human IgG antipneumococcal antibody response (only immunized transplanted mice included) after transplantation of human peripheral blood lymphocytes from nine donors

	Total human IgG		Human antipneumococcal IgG antibodies	
	Number	%	Number	%
Donor 1	12/12	100	7/8	87·5
Donor 2	16/16	100	2/10	20·0
Donor 3	11/11	100	8/8	100
Donor 4	12/12	100	6/6	100
Donor 5	12/12	100	2/6	33·3
Donor 6	12/12	100	2/6	33·3
Donor 7	11/12	91·7	1/6	16·7
Donor 8	11/12*	91·7	6/6*	100
Donor 9	11/12	91·7	2/6	33·3
Total	107/111	96·4	36/62	58·1

*The one SCID-PBL-hu mouse with no detectable human IgG was in the group of non-immunized mice.

Human antibodies to the pneumococcal vaccine

Specific human IgG antipneumococcal antibody response in sera from the immunized SCID mice was found in 16·7–100% of the mice transplanted with cells from the nine donors (Table 1). Most of the immunized mice transplanted with cells from donors 1, 3, 4, and 8 showed a significant antipneumococcal antibody response after vaccination. Mice transplanted with cells from donors 2, 5, 6, 7, and 9 showed low or no antipneumococcal antibody response after vaccination (Table 1, Fig. 1). One mouse transplanted with

Table 2. Total human IgG, IgM and IgA (mg/ml)* in SCID mice transplanted with human peripheral blood lymphocytes from nine donors (D1–D9) in two separate experiments (D1–D3 and D4–D9 in Experiments 1 and 2, respectively)

		IgG		IgM		IgA	
	n†	Median‡	Range	Median	Range	Median	Range
D1	12	5·1	0·5–9·2	ND§		ND	
D2	16	3·3	0·5–6·7	ND		ND	
D3	11	2·0	0·5–9·2	ND		ND	
D4	12	3·4	2·2–5·1	0·4	0·1–1·7	0·02	0·002–0·06
D5	12	2·8	1·1–5·6	0·1	0·01–0·8	0·02	0·005–0·03
D6	12	0·8	0·003–1·6	0·01	0·005–0·05	0·0009	0–0·007
D7	12	0·1	0–0·5	0·01	0·006–0·02	0·0007	0–0·001
D8	12	1·7	0–4·1	0·03	0·006–0·5	0·003	0–0·01
D9	12	1·6	0–2·9	0·06	0–1·0	0·003	0–0·04
Total							
D1–D3	39	3·4	0·5–9·2	ND		ND	
D4–D9	72	1·6	0–5·6	0·03	0–1·7	0·003	0–0·06

*Detection limit of human IgG, 0·0006 mg/ml; IgM, 0·001 mg/ml; IgA, 0·0005 mg/ml.

†n, Number of mice transplanted with cells from one donor.

‡For 11 mice values for human IgG were < 1, but $> 0{\cdot}5$ mg/ml, and exact quantitative values were not obtained. Values for these mice were set to 0·5 mg/ml in the statistical calculations. Exclusion of these mice gave slightly higher median IgG values for D1, D3, D6, and D9.

§ND, Not done.

cells from donor 7 showed antibodies to the vaccine only in the sample obtained 6 weeks after immunization (reanalysed with the same result). There was a weak, but statistically significant correlation between the human antipneumococcal antibody levels and total human IgG in the immunized SCID-PBL-hu mice ($r = 0{\cdot}33$, $P = 0{\cdot}01$). No antipneumococcal antibodies could be detected in any of the 49 non-immunized transplanted SCID mice (absorbance values $\leqslant 0{\cdot}03$ in most of the mice, nine mice showed values up to 0·1), except for one mouse which showed low level (absorbance value 0·25) (data not shown).

Variations in the antibody response pattern were observed between the groups of mice transplanted with cells from different donors, whereas there was a tendency for similar antibody response patterns in terms of magnitude and kinetics to be observed in mice transplanted with cells from the same donor (Fig. 1).

No human IgM or IgA antibodies to the pneumococcal vaccine could be detected in transplanted SCID mice. No mouse IgM antibodies to the vaccine could be detected in the SCID-PBL-hu mice, except for two mice which showed low levels (absorbance values $\leqslant 0{\cdot}21$) (data not shown).

Seven out of the nine donors had antibodies to the pneumococcal vaccine (absorbance values above 0·10) at the time when blood was donated for transplantation of the mice (Fig. 1, open triangles). After vaccination of donors 4–9, four of these six donors (donors 4, 7, 8 and 9) showed an increase in IgG antipneumococcal antibody levels (Fig. 1, open triangles). All the donors, except donor 7, showed an increase in IgM and IgA

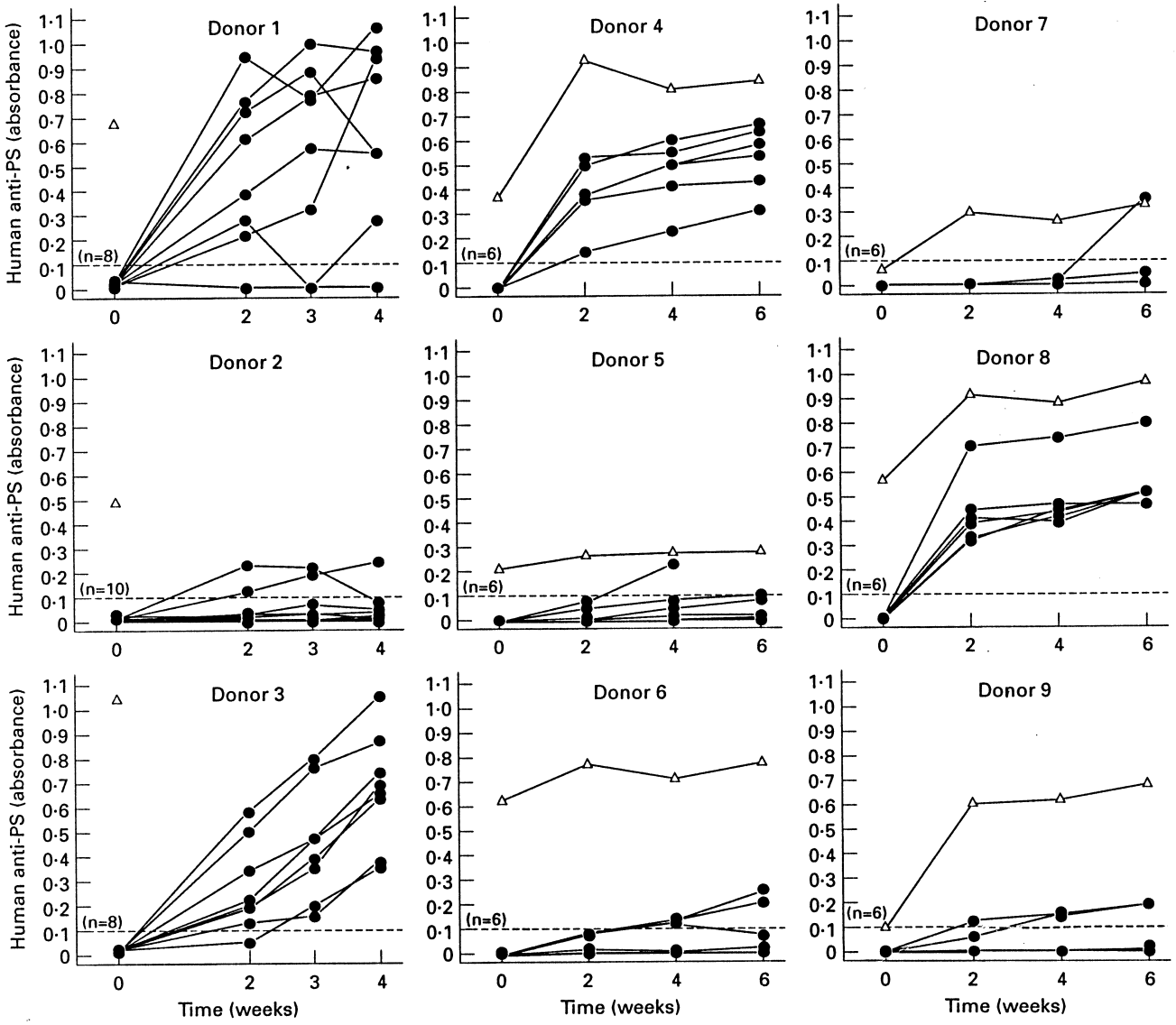

Fig. 1. Human IgG antibody levels to a 23-valent pneumococcal polysaccharide vaccine (human anti-PS) in SCID-PBL-hu mice and in the respective human donors (donors 1–9). All the SCID-PBL-hu mice and the human donors 4–9 were immunized with the pneumococcal vaccine at week 0 (10 days after transplantation of the mice). ●, Anti-PS in individual immunized SCID-PBL-hu mice; △, anti-PS in the respective human donors (donor values obtained at the time of transplantation of the mice are for simplicity drawn at time 0); – – – – –, cutoff level; *n*, number of mice. Transplantation and immunization of mice which received cells from donors 1–3 and transplantation and immunization of mice which received cells from donors 4–9 were performed in two separate experiments.

antibodies to the pneumococcal vaccine after vaccination (data not shown). There was a statistically significant correlation between the antipneumococcal antibody levels in the donors at the time of transplantation of the mice and post-vaccination antipneumococcal antibody levels obtained in the immunized SCID-PBL-hu mice ($r = 0{\cdot}68$, $P = 0{\cdot}04$). However, the correlation between post-vaccination antipneumococcal antibody values in the donors and post-vaccination values in the mice was not statistically significant ($r = 0{\cdot}72$, $P = 0{\cdot}11$). Only mice transplanted with cells from donors with relatively high 'background' antipneumococcal antibody levels or from donors that showed a substantial increase in antibody levels after vaccination, produced antipneumococcal antibodies.

Human IgG antibodies to the nine single pneumococcal serotypes, 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F, were also measured in sera from Experiment 2 (data not shown). Most of the donors had antibodies to all nine serotypes before vaccination, but prevaccination antibody levels to the various serotypes showed variation between donors. No mice transplanted with cells from donors without detectable antibodies at the time of transplantation to the serotype in question, obtained a serotype-specific human antibody response as determined with our panel of serotypes. Donors who showed an increase in antibody levels to the vaccine (Fig. 1) also tended to show increase in the amount of antibodies to most of the nine serotypes measured. Immunized SCID-PBL-hu mice showed antibodies to only some of the nine serotypes tested.

Human IgG antibodies to PnC were measured 6 weeks after vaccination in sera from transplanted mice and from the human donors in Experiment 2. All donors had high antibody levels to PnC (data not shown). Human antibodies to PnC were detected in the same mice that showed human antibodies to the pneumococcal vaccine. Mice transplanted with cells from donor 9 showed high antibody levels to PnC, although antibody levels to the pneumococcal vaccine were relatively low. There was a statistically significant correlation between human antibody levels to PnC in mice and antibody levels to PnC in donors ($r = 0{\cdot}94$, $P = 0{\cdot}005$).

DISCUSSION

SCID mice transplanted with human PBL regularly show human IgG in serum [2,3,4,6], and the presence of human immunoglobulins is used as an indicator for a functioning human immune system in the mice. In the study reported here, human IgG were detected in most of the transplanted mice. With regard to the concentration of immunoglobulins, there appeared to be some donor dependence, as evidenced by the data in Table 2. There were no differences in concentrations of total human IgG, IgM or IgA regardless of whether the SCID-PBL-hu mice had been immunized with the pneumococcal vaccine or not.

In our previous studies [9,12] transplantation of cells from some donors did not result in specific antipneumococcal antibody production in SCID mice, although the donors had antipneumococcal antibodies. In the present study, many mice were transplanted with cells from the same donor, which allowed us to study how the antibody response obtained in the recipient mice related to the antipneumococcal antibody level in the donor. The SCID mice were transplanted with cells from non-vaccinated donors, and the antipneumococcal antibody response induced after vaccination of both transplanted SCID mice and of the respective donors could therefore be compared.

The antipneumococcal antibody response induced in the SCID-PBL-hu mice was most probably a specific immune reaction, because antipneumococcal antibodies could not be measured in non-immunized mice. The induction of specific antibodies varied from 16·7% to 100% in the transplanted and immunized mice, dependent on which donor the cells had been obtained from. Further, cells from some donors resulted in strong antibody responses in the mice, whereas cells from other donors gave only low levels of antipneumococcal antibodies. This indicates that a specific immune response in transplanted mice is dependent on characteristics of the donor. Large variation in the specific antibody responses obtained within groups of mice transplanted with cells from the same donor has been reported [7,8]. In our studies there were also variations in the specific antibody levels within a group of mice, but similar patterns in terms of kinetics and magnitude of the antibody response were seen in mice transplanted with cells from the same donor (Fig.1). This is in agreement with observations reported by others [15].

Most of the mice with specific antipneumococcal antibodies had relatively high concentrations of human IgG, but there was only a weak correlation ($r = 0{\cdot}33$) between concentrations of human IgG and human antipneumococcal antibodies in the mice. Thus, the level of human IgG in SCID-PBL-hu mice did not reliably predict the ability to produce specific human antibodies. The best antipneumococcal antibody responses were induced in mice transplanted with cells from donors with high prevaccination antipneumococcal antibody levels or donors that responded well to vaccination. Thus, the expressed capacity of the donor to respond to pneumococcal antigens appears to be an important prerequisite for successful induction of specific immune responses in transplanted SCID mice (donors 4 and 8, Fig. 1). Apparently, with pneumococcal polysaccharide antigens no down-regulated capacities of the donor's immune response are revealed after transplantation to SCID-PBL-hu mice. However, whereas a certain level of antibodies in the donor appears necessary, the presence of antibodies in the donor is not sufficient for the prediction of a good response in transplanted mice (donors 2 and 6, Fig. 1). Clearly, other host factors than the capacity to produce specific antibodies, perhaps histocompatibility antigens or immunoglobulin allotype, also influence the function of transplanted human cells. Hesselton *et al.* have reported that no donor pattern was seen that could predict successful reconstitution in SCID-PBL-hu mice [6]. In this context, it is interesting that donor dependency appears to be even more pronounced in RAG-2 (129 mouse strain background) than in SCID (BALB/c background) mice [12].

Similar antipneumococcal antibody responses can be obtained in SCID mice when cells from the same donor are used in repeated experiments (unpublished observations). This is a further indication that successful engraftment in terms of induction of specific antibodies is not random, but is dependent on characteristics of the donor. By selecting donors whose cells have been found to function well in SCID mice, a good specific immune response to pneumococcal polysaccharide antigens can regularly be obtained in transplanted mice.

Human antipneumococcal antibodies can be induced in SCID mice transplanted with human cells from non-vaccinated donors, as we have demonstrated in this and in previous studies [9,12]. Pneumococcal polysaccharide antigens are T-independent antigens which may require less cooperation between different cell populations than T-dependent protein antigens to develop an immune response. Adults regularly have antibodies to many pneumococcal polysaccharide antigens which may have been induced by previous

contact with the antigen, for example by carrying the bacteria in the nasopharynx or by cross-reaction with enteric bacteria with similar capsular antigens. In our experiments, donors whose cells resulted in specific immune responses in the transplanted SCID mice had antipneumococcal antibodies in the serum. This may indicate that also for T-independent antigens some antigen priming may have had to occur earlier to obtain a response in SCID mice, in accordance with findings regarding the immune response to T-dependent antigens [2,4,6–8].

SCID mice transplanted with human cells may be useful for studying human immune responses to antigen preparations that for ethical or practical reasons cannot be tested in humans. With the human peripheral blood lymphocyte SCID model (SCID-PBL-hu), donors whose cells function well in SCID mice can be selected and used several times to produce functional SCID-PBL-hu mice for the study of human immune responses and function in an *in vivo* experimental model. The possibility of using donors with known properties several times is an important advantage of the SCID-PBL-hu model over the SCID-hu model, which uses transplantation of fetal tissues [16].

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