*Parasite Immunology*, 2002, **24**, 1–13

# Human *Trichuris*-specific antibody responses in vaccinated hu-PBL-SCID mice

MATTHEW D. TAYLOR[1] & KATHRYN J. ELSE[2]

[1]Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh and [2]School of Biological Sciences, University of Manchester, Manchester, UK

## SUMMARY

Trichuris trichiura *is a highly prevalent intestinal helminth of humans with a well-characterized animal model,* Trichuris muris *in the mouse. Relating the murine work back to the human infection has been difficult, however, as many of the questions addressed in the mouse cannot be asked in humans. The ability to reconstitute a mouse with a human immune system could help bridge this gap, allowing a human immune response to be studied under a controllable laboratory environment. In this study, we demonstrate that severe combined immunodeficient mice engrafted with naïve human peripheral blood lymphocytes are capable of mounting a* Trichuris *specific human antibody response after vaccination with* T. muris *antigens. The phenotype of the response depended on the vaccinating antigen with excretory/secretory antigens eliciting a human immunoglobulin (Ig)G2 response, and whole worm homogenate stimulating IgG1 and IgG2 responses. Vaccination with homogenate also enhanced a human IgG response against a 66-kDa component of* T. muris *homogenate in a donor-dependent manner. This work shows the potential of using the humanized mouse model for studying the immune responses of humans living in* T. trichiura *endemic areas.*

***Keywords*** *severe combined immunodeficient*, *B cell*, *antibody*, Trichuris, *hu-PBL-SCID*, *vaccination*

*Correspondence*: Matthew Taylor, Institute of Cell, Animal and Population Biology, Ashworth Laboratories, Kings Buildings, West Mains Road, University of Edinburgh EH9 3JT, UK (e-mail: matthew.taylor@ed.ac.uk).
*Received*: 9 July 2001
*Accepted for publication*: 30 August 2001

## INTRODUCTION

In 1988, a novel approach was pioneered by three groups to try and bridge the gap between the logistical and ethical problems of studying human immunology, and the lack of animal models for certain human diseases (1–3). The model involved reconstituting B and T cell deficient severe combined immunodeficient (SCID) mice with different sources of human immune cells, allowing a functional human immune system to develop within a murine host. The aim was to develop an *in vivo* mouse model to perform studies on human cells that could not be accomplished *in vivo* in humans. Subsequently, humanized SCID mice have been used to study human immune responses against a variety of infectious and autoimmune diseases, and also responses to vaccination (4–7). Of particular interest to us was the route described by Mosier *et al*. (3) in which human peripheral blood lymphocytes (PBL) were used to reconstitute the SCID mice (hu-PBL-SCID). This provides the most practical approach for applying the humanized mouse model to the study of human parasitic helminths, where the ability to sample human tissues during a field study is limited. The feasibility of the hu-PBL-SCID model in this respect has already been illustrated by Mazingue *et al*. who reconstituted SCID mice with PBL from a healthy donor and a *Schistosoma mansoni* infected donor (8). *S. mansoni* specific human antibody responses were generated in both groups of mice after vaccination with *S. mansoni* antigens.

*Trichuris trichiura* is a highly prevalent intestinal helminth infecting humans. One of the advantages of studying this parasite is the existence of a good laboratory model, *T. muris* infection of the mouse. *Trichuris muris* occupies the same environmental niche as *T. trichiura*, has similar morphology, and shares a high degree of antigenic cross-reactivity (9). The murine immune responses against *T. muris* have been well characterized with resistance being dependent on the development of a type 2 T cell response, and susceptibility being linked to the development of a type 1 T cell response (10–12). A role for B cells has also been recently

defined in the priming of a protective response, and a role for antibody in protection against challenge infections (13). Despite progress in the murine model, there is a limited amount of information known regarding *T. trichiura* infection in humans, mainly related to the difficulties of performing field studies. The serum antibody responses of humans have been characterized and rectal biopsies have been performed (14–18), but these can neither confirm nor deny the results found in the mouse. Hu-PBL-SCID mice could therefore provide a valuable tool for elucidating the human immune responses against *T. trichiura*. Reconstituting SCID mice with PBL from humans living in *T. trichiura* endemic areas would allow us to combine the study of *T. trichiura* specific human immune responses that have been generated under natural infection conditions, with the advantages of a controllable laboratory model. Because *T. muris* is so closely related to *T. trichiura*, this could be in the form of an infection model to study human immune responses against live *T. muris* infection. Work from our laboratory has shown that SCID mice reconstituted with human PBL from naïve Western donors are capable of mounting an antibody response against live *T. muris* infection (19). The complementary approach discussed in this study is that of a vaccination model to study human antibody responses against specific antigens of *T. trichiura*. The aim of the vaccination model would be to help determine which antigens are immunogenic, which antigens are important in stimulating a protective response, and whether distinct antigens produce specific types of immune responses. A future use could be to apply this model to the development of vaccination strategies for promoting a desired type of immune response against a chosen antigen.

Using *T. muris* antigens, we describe the development of a hu-PBL-SCID mouse vaccination model to be used to study human antibody responses against antigens of *T. trichiura*. We show that hu-PBL-SCID mice reconstituted with PBL from Western donors, naïve in regard to *T. trichiura* and *T. muris*, were capable of mounting a *T. muris* specific human immunoglobulin (Ig)G response after vaccination with *T. muris* antigens. The phenotype of the antibody response was dependent upon the antigenic mixture used for vaccination, and showed donor-dependent differences. This work demonstrates the potential for using the hu-PBL-SCID mouse model to dissect out human immune responses against antigens of *T. trichiura*.

## MATERIALS AND METHODS

### Mice

C.B-17 *scid*/*scid* (SCID) mice were bred and maintained in microisolator cages in the animal facility at the University of Manchester. The original breeding pairs were obtained from Charles River (Margate, Kent, UK). SCID mice were fed autoclaved food and water and all manipulations were performed under laminar flow. Male SCID mice were used when aged 4–12 weeks.

### Parasite antigens

The Edinburgh strain of *T. muris* was maintained through passage in SCID mice. *T. muris* Excretory/Secretory (E/S) antigens were prepared as described by Artis *et al*. (20). In brief, adult worms were cultured *in vitro* at 37°C for 4 h or overnight in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 500 IU/ml penicillin and 500 µg/ml streptomycin (Gibco BRL). The supernatants were concentrated with Centriprep Concentrators (Amicom, Stonehouse, Gloucestershire, UK), and dialysed in PBS. *T. muris* homogenate was prepared by homogenizing whole adult worms in PBS and collecting the soluble protein fraction, which was then treated as for *T. muris* E/S Ag but without dialysis.

### Reconstitution of SCID mice

Human venous blood was collected with heparin (10 IU/ml of blood, CP Pharmaceuticals Ltd, Wrexham, UK) from normal volunteers. Two to 3 ml of blood was collected in the absence of heparin for isolation of human serum. Human PBL were separated on a ficol gradient using lymphoprep 1077 (Gibco BRL), washed twice with Hank's balanced salt solution (HBSS) (Gibco BRL) supplemented with 2% v/v foetal bovine serum (FBS) (Gibco BRL), penicillin and streptomycin, and washed a final time in HBSS alone. Each SCID mouse received $2 \times 10^7$ human PBL i.p. in 200 µl of HBSS. Non-reconstituted SCID controls received 200 µl HBSS alone. For freezing, the human PBL were resuspended at a final concentration of $5 \times 10^6$ cells per ml in RPMI-1640 medium with 20% v/v FBS and 20% DMSO (Sigma-Aldrich, Poole, Dorset, UK). PBL were frozen overnight at –70°C in a Mr Frosty (Fisher Scientific, Loughborough, Leicestershire, UK) filled with isopropanol (BDH Laboratory Supplies, Poole, UK), before being placed in liquid nitrogen. For thawing, the human PBL were rapidly defrosted and mixed gradually into an equal volume 50% RPMI medium/50% FBS. The PBL were then washed in HBSS and injected into SCID mice as above. The recovery of human cells after freezing was approximately 81%.

### Vaccinations

Vaccinations were performed s.c. with 0·5 mg of the relevant *T. muris* Ag in adjuvant on days 1 and 15 post reconstitution (p.r). Mock-vaccinations were performed with PBS mixed with adjuvant. To prepare vaccinations in Incomplete Freunds Adjuvant (IFA), *T. muris* Ag at a concentration of 5 mg/ml was


 

mixed with an equal volume of IFA (Sigma-Aldrich). To prepare vaccinations in alum, *T. muris* Ag was adjusted to 1 mg/ml in PBS and 0·45 ml of 1 M sodium hydrogencarbonate (BDH Laboratory Supplies) added dropwise per ml of antigen solution. A volume of 0·2 M aluminium potassium sulphate (BDH Laboratory Supplies) equal to that of the original antigen solution was then added slowly with stirring. The solution was mixed for 30 min at room temperature. The precipitate was washed three times in PBS for 10 min at 720 *g* before adjusting to a final protein concentration of 2·5 mg/ml.

### Preparation of mouse tissue

During the course of the experiment, mice were tail bled at approximately weekly intervals, and were exanguinated at the final autopsy. Blood was allowed to clot at room temperature to purify the serum. Peritoneal lavage was performed using 5 ml RPMI-1640 with 2% v/v FBS and penicillin-streptomycin. The recovered cells were washed three times in PBS supplemented with Dulbeccos A + B salts (GibcoBRL), 2% FBS and 0·1% w/v sodium azide (Sigma-Aldrich), before being resuspended at $5 \times 10^6$ cells per ml for flow cytometric staining. Mouse spleens were dissociated using 100 μm Falcon Cell Strainers (Becton-Dickinson, Oxford, UK). Red blood cells were lysed using 0·85% w/v sterile filtered ammonium chloride (BDH Laboratory Supplies) and the remaining spleen cells treated for flow cytometric analysis as above. Bone marrow cells were isolated from the femur, and treated as for the dissociated splenocytes. Inguinal and popliteal lymph nodes were pooled within each vaccination group and treated as for the spleen cells, but without lysis of the red blood cells.

### Flow cytometric analysis

$1 \times 10^6$ cells were stained with fluorescein isothiocyanate (FITC) conjugated mouse anti-human CD19 (Becton-Dickinson), phycoerythrin (PE) conjugated mouse anti-human HLA-ABC (Pharmingen, BD Biosciences, Oxford, UK) and PE conjugated mouse anti-human CD3 (Becton-Dickinson). FITC and PE conjugated isotype matched control antibodies were bought from Becton-Dickinson. The cells were acquired on a FACScan using the Cellquest software (Becton-Dickinson). The settings for acquisition were defined on human PBL isolated from the relevant donor.

### Antibody ELISA

Standard sandwich ELISA were carried out on 96-well Immulon 4 HBX flat bottomed microtiter plates (Dynex, Billingshurst, West Sussex, UK). Washes were performed in PBS with 0·05% v/v polyoxyethylene-sorbitan monolaurate (Sigma-Aldrich) (PBS/T). 2,2′ azino-bis(3-ehtylbenzthiazoline-6-sulphonic acid) (ABTS, Sigma-Aldrich) was used as substrate. Plates were read using a MR7000 plate reader (Dynatech, West Sussex, UK), and analysed with the Revelation software (Dynex). Total human IgM and IgG, ELISA plates were coated with goat anti-human IgG or goat anti-human IgM (Tago Immunochemicals, Serotec Ltd, Oxford, UK) in carbonate/bicarbonate buffer (0·015 M sodium carbonate/0·035 M sodium hydrogen carbonate, Sigma-Aldrich). PBS/T with 3% w/v bovine serum albumin (BSA, Sigma-Aldrich) was used as diluent and blocking reagent. IgG and IgM concentrations were standardized against human IgG and human IgM (DAKO Ltd, Buckinghamshire, UK). Detection was performed using HRP conjugated goat anti-human IgG, or HRP conjugated goat anti-human $IgM_k$ (BioSource International, Hertfordshire, UK). For detection of *T. muris* specific antibodies, ELISA plates were coated with *T. muris* homogenate or E/S Ag at 5 μg/ml in carbonate/bicarbonate buffer. Blocking and dilutions were performed in PBS/T with 5% w/v BSA. Human IgG isotypes were detected using mouse anti-human IgG1 (NL16), IgG2 (GOM1), IgG3 (ZG4) and IgG4 (RJ4) (Bionostics, Bedfordshire, UK). Biotinylated rat anti-mouse IgG (Serotec Ltd) followed by 0·5 IU/ml streptavidin peroxidase (Boehringer Mannheim, East Sussex, UK) were used to detect bound antibody. To maximize sensitivity, the samples and anti-human IgG isotype antibodies were incubated overnight at 4°C. To standardize the assay all plates were read 20 min after addition of substrate. Total murine Ig levels of the SCID mice were measured before reconstitution and at the time of final autopsy to check for mice with a 'leaky' phenotype. Plates were coated with goat anti-mouse Ig (DAKO Ltd) diluted in carbonate/bicarbonate buffer. Blocking and dilutions were performed in PBS/T with 3% w/v BSA. Ig concentrations were standardized against mouse $IgM_k$ monoclonal antibody (Pharmingen). HRP conjugated rabbit anti-mouse Ig (DAKO Ltd) was used for detection. In each experiment, 14·3–38·5% of the SCID mice had detectable levels of murine Ig (leaky phenotype) as defined by greater than 5 μg/ml serum Ig concentrations. To verify the murine Ig was nonfunctional, the *T. muris* specific ELISA was performed in the absence of the anti-human isotype antibodies to measure the levels of *T. muris* specific murine IgG. The presence of murine Ig neither had an effect on the level of human cell engraftment, nor on the ability of the hu-PBL-SCID mice to respond to vaccination. *T. muris* specific murine IgG was not detected in any mouse in any experiment.

### Western immunoblots

32·5 μg/well of *T. muris* homogenate was separated according to size on 12% SDS-PAGE reducing gels and transferred

**Table 1** Incidence and total human IgG serum concentrations in hu-PBL-SCID mice

| Donor | Days p.r. | Incidence | Range[1] (μg/ml) | Mean ± SD (μg/ml) | Median (μg/ml) | Donor's IgG[2] (μg/ml) |
|---|---|---|---|---|---|---|
| D1 | 57 | 10/11 | 61–2538 | 856 ± 867 | 562 | 15 600 |
| D1 | 52 | 11/13 | 311–3885 | 1577 ± 1343 | 1415 | 11 600 |
| D1 | 50 | 9/10 | 466–6985 | 2290 ± 2012 | 1813 | 11 900 |
| D1[3] | 47 | 8/8 | 150–5186 | 1398 ± 1740 | 625 | 19 700 |
| D2 | 55 | 11/11 | 33–3484 | 1564 ± 1094 | 1128 | 17 000 |
| D3 | 55 | 10/10 | 345–6760 | 4137 ± 2155 | 4416 | 21 200 |

[1]hu-PBL-SCID mice positive for human IgG. [2]Serum concentration at time of sampling PBL. [3]PBL were freeze/thawed in liquid nitrogen prior to reconstitution.

**Table 2** Incidence and total human IgM serum concentrations of hu-PBL-SCID mice

| Donor | Days p.r. | Incidence | Range[1] (μg/ml) | Mean ± SD (μg/ml) | Median (μg/ml) | Donor's IgM[2] (μg/ml) |
|---|---|---|---|---|---|---|
| D1 | 57 | 9/11 | 1–80 | 19·5 ± 28·9 | 5 | 741 |
| D1 | 52 | 11/13 | 4–445 | 56 ± 124 | 13 | 826 |
| D1 | 50 | 9/10 | 10–1154 | 213 ± 376 | 50·5 | 678 |
| D1[3] | 47 | 8/8 | 2–242 | 40 ± 82 | 8 | 915 |
| D2 | 55 | 9/11 | 1–14 | 6 ± 5 | 4 | 617 |
| D3 | 55 | 10/10 | 5–6651 | 1528 ± 2114 | 736 | 425 |

[1]hu-PBL-SCID mice positive for human IgM. [2]Serum concentration at time of sampling PBL. [3]PBL were freeze/thawed in liquid nitrogen prior to reconstitution.

to HYbond ECL nitrocellulose membranes (Amersham-Pharmacia Biotech, Buckinghamshire, UK) using Bio-Rad (Hertfordshire, UK) mini-gel and wet cell transfer equipment. Band sizes were equated to SDS-PAGE broad range molecular weight standards (Bio-Rad). The membranes were blocked overnight in PBS with 5% w/v BSA, and washes were performed in PBS/T. Serum samples were diluted 1 : 10 in PBS with 2% w/v BSA, diluent alone was used as a negative control. Detection of bound human IgG was performed with HRP conjugated goat anti-human IgG, and visualized using an ECL detection system (Amersham-Pharmacia-Biotech) on Kodak BIOMAX light-1 film (Sigma-Aldrich).

### Statistical analysis

Non-parametric correlation was performed using Spearman's rank correlation.

## RESULTS

### Engraftment of human PBL within SCID mice

To engraft SCID mice with human cells, each mouse was injected i.p. with $2 \times 10^7$ naïve human PBL. The hu-PBL-SCID mice were then vaccinated s.c. with 0·5 mg *T. muris* antigen in adjuvant (vaccinated), or with adjuvant alone (mock-vaccinated) on days 1 and 15 p.r. One group of SCID mice was vaccinated with parasite antigen in adjuvant, but was not engrafted with human cells (non-reconstituted SCID controls). To monitor the level of human cell engraftment throughout the course of the experiment, we measured the serum concentrations of total human IgG and IgM at approximately weekly intervals. At the final time point, the mice were autopsied and flow cytometric analysis used to look for the presence of human cells within various tissues and organs. Neither human cells, nor human Ig were detected in the non-reconstituted SCID controls.

The incidence of human cell engraftment was high with greater than 80% of hu-PBL-SCID mice having detectable serum concentrations of total human IgG and IgM in each experiment, with IgG being the predominant isotype (Tables 1 and 2). Similar to previous reports, the serum concentrations of both total human IgG and IgM were variable, even between SCID mice reconstituted with PBL from the same donor in the same experiment (7,21,22). Vaccination with antigen had no effect on the serum concentrations of total human IgM or IgG compared to the mock-vaccinated controls. The majority of hu-PBL-SCID mice had serum concentrations of total human IgM of less than 100 μg/ml (30/37 for donor 1, 11/11 for donor 2, 3/10 for donor 3). A few hu-PBL-SCID mice reconstituted with PBL from donor 1 (4/37), and the majority of those reconstituted with PBL

**Table 3** Incidence of hu-PBL-SCID mice with detectable levels of HLA-ABC$^+$, CD19$^+$ and/or CD3$^+$ human cells

| Donor | Days p.r. | Peritoneal lavage HLA-ABC$^+$ | Bone marrow HLA-ABC$^+$ | Spleen HLA-ABC$^+$ | Spleen CD19$^+$ | Spleen CD3$^+$ |
|---|---|---|---|---|---|---|
| D1 | 57 | 3/6 | – | – | – | – |
| D1 | 52 | 10/11[2] | 8/13 | – | – | – |
| D1 | 50 | 10/10[2] | – | 8/10[2] | 2/10 | – |
| D1[1] | 47 | – | – | 5/8[3] | 1/8 | 2/8 |
| D2 | 55 | – | – | 6/10[2] | 1/10 | 5/10 |
| D3 | 55 | – | – | 5/10[3] | 2/10 | 0/10 |

[1]PBL were freeze/thawed in liquid nitrogen prior to reconstitution. Significant positive correlation's were found between the percentage of HLA-ABC$^+$ human cells and the total serum levels of both human IgG and IgM [2]($P < 0{\cdot}05$), or human IgM alone [3]($P < 0{\cdot}05$).

from donor 3 (7/10), had total human IgM levels of greater than 200 μg/ml. These high concentrations were only found after day 30 p.r., and were comparable to the reported total human IgM serum concentrations of hu-PBL-SCID mice developing Epstein-Barr virus (EBV) associated B cell lymphomas (23–25). The high IgM concentrations did not show any relation to the antigen specific responses reported below, and occurred after the antigen specific antibody responses were initially detected. We did not test for the presence of EBV associated B cell lymphomas in our experiments.

To follow the migration of the human PBL, we used the pan human cell marker HLA-ABC. HLA-ABC$^+$ cells could be detected in the peritoneal cavity, bone marrow, and spleen of the hu-PBL-SCID mice from days 47–57 p.r. (Table 3). The percentages of human cells recovered were variable and, in hu-PBL-SCID mice with detectable numbers of HLA-ABC$^+$, human cells ranged from 0·1 to 71% in the peritoneal lavage fluid, 0·1–75·2% in the spleen and 0·01–0·78% in the bone marrow. Human HLA-ABC$^+$ cells were also detected in the pooled inguinal and popliteal lymph nodes from vaccinated hu-PBL-SCID mice reconstituted with PBL from donor 1 (1·3% of total lymph node cells), but not in the mock-vaccinated group nor in the hu-PBL-SCID mice engrafted with PBL from donors 2 or 3.

Despite the high incidence of human IgM and IgG production, human CD19$^+$ B cells could not be detected in the peritoneal lavage fluid or bone marrow. They were detected in the spleen of a fraction of the hu-PBL-SCID mice with percentages ranging from 0·1 to 1·3% (Table 3). The percentages of HLA-ABC$^+$ cells in the peritoneal lavage fluid and spleen, however, did show significant positive correlations with the serum levels of total human IgM and/or IgG. It has been reported that CD19 expression on human B cells is down-regulated after prolonged exposure to the SCID environment, possibly explaining this discrepancy (23,26). Human CD3$^+$ cells could be detected in the spleens of some hu-PBL-SCID mice (Table 3), with the percentages ranging from 0·1% to 56%. Freeze thawing the human PBL in liquid nitrogen prior to reconstitution did not adversely affect their ability to reconstitute the SCID mice (Tables 1 to 3).

### Vaccination of hu-PBL-SCID mice with *T. muris* E/S Ag results in the production of *T. muris* specific human IgG2

To determine whether hu-PBL-SCID mice were capable of responding to antigenic challenge the hu-PBL-SCID mice were vaccinated s.c. with 0·5 mg of *T. muris* E/S Ag on days 1 and 15 p.r. The serum levels of *T. muris* specific human IgG1, IgG2, IgG3, and IgG4 were then followed every 7–10 days throughout the course of the experiment. Due to the limited serum volumes obtained at the intermediate time points, the results shown below concentrate on the antibody responses at the time of autopsy when the full range of assays were performed. Compared with the mock-vaccinated hu-PBL-SCID mice, vaccination with *T. muris* E/S Ag using either IFA or alum as an adjuvant resulted in higher serum levels of *T. muris* E/S specific human IgG2 at days 57 and 52 p.r., respectively (Figures 1a,b). *T. muris* specific human IgG2 could be detected by day 20 post reconstitution, and the kinetics of its production differed between individual hu-PBL-SCID mice with maximal levels occurring at any time point from day 20 onwards (Figures 2a,b). There was no relationship between the *T. muris* specific human IgG2 response within each hu-PBL-SCID mouse and its overall level of human cell engraftment. Negligible levels of *T. muris* E/S specific human IgG1, IgG3 or IgG4 were detected in the sera.

*T. muris* E/S specific human IgG2 could be detected in the sera of donor 1 at greater or equal levels to the vaccinated hu-PBL-SCID mice (Figures 1a,b); however, it should be noted that the donor's total IgG serum concentration was at least twice those of the hu-PBL-SCID mice (Table 1). The levels of *T. muris* specific human IgG in the donor's

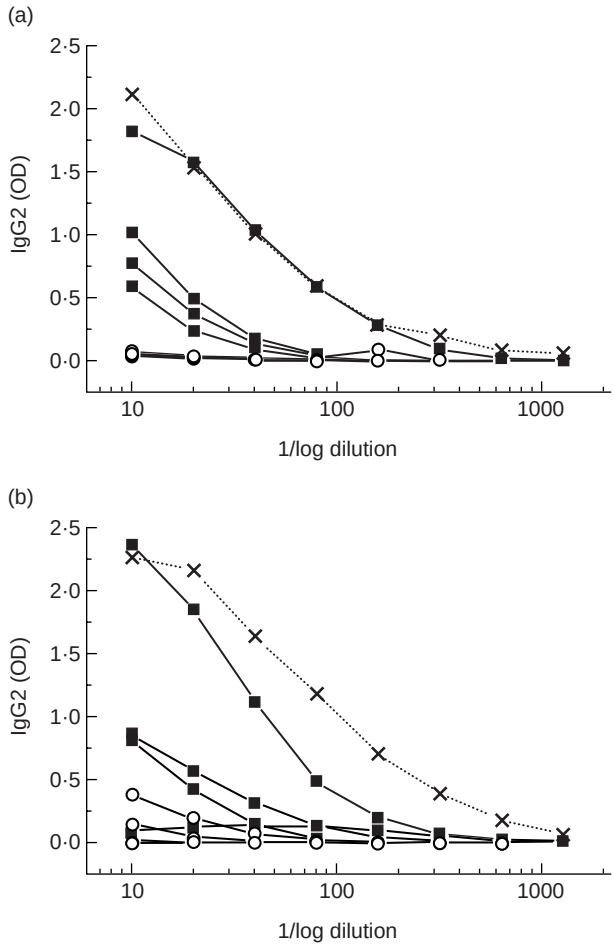


**Figure 1** Serum levels of *T. muris* E/S specific human IgG2 of donor 1 (crosses), E/S vaccinated hu-PBL-SCID mice (closed squares) and mock-vaccinated hu-PBL-SCID mice (open circles). The donor's sera was sampled at the time of reconstitution. (a) Hu-PBL-SCID mice were reconstituted with PBL from donor 1 and vaccinated with *T. muris* E/S Ag in IFA. Autopsy was performed at day 57 p.r. ($n = 5$ for vaccinated group, $n = 6$ for mock-vaccinated group). The three non-reconstituted SCID controls showed negligible levels of *T. muris* E/S specific human IgG2 (OD at a 1 : 10 dilution = 0·05 ± 0·03). (b) Hu-PBL-SCID mice were reconstituted with PBL from donor 1 and vaccinated with *T. muris* E/S Ag in alum. Autopsy was performed at day 52 p.r. ($n = 7$ for vaccinated group, $n = 6$ for mock-vaccinated group, $n = 3$ for non-reconstituted SCID controls). The non-reconstituted SCID controls were negative for *T. muris* E/S specific human IgG2.

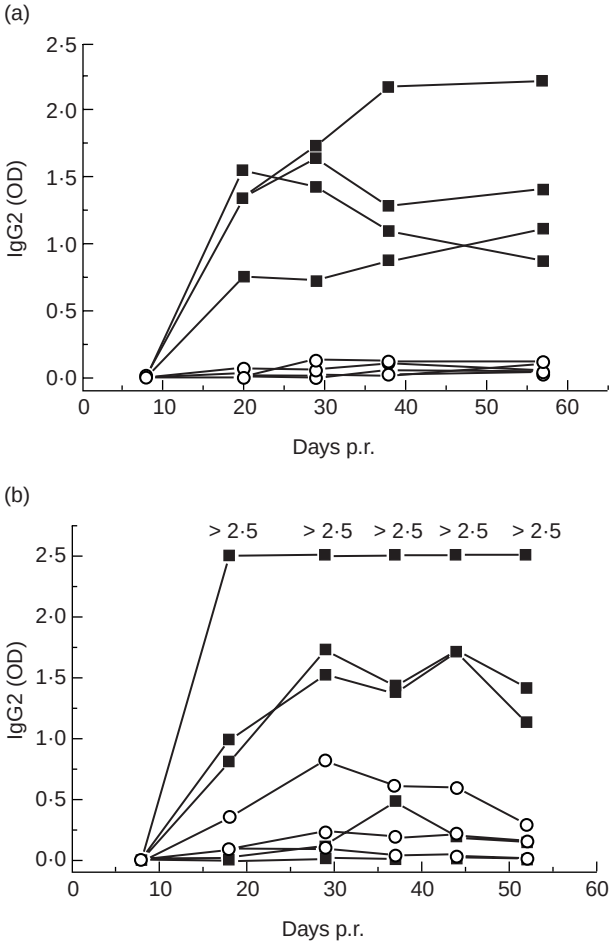


**Figure 2** Time course of *T. muris* specific human IgG2 serum levels in vaccinated (closed squares) and mock-vaccinated (open circles) hu-PBL-SCID mice reconstituted with PBL from donor 1. The serum level of *T. muris* specific human IgG2 represents the OD when a serum dilution of 1 : 10 was used. (a) Hu-PBL-SCID mice were vaccinated with *T. muris* E/S antigens in IFA on days 1 and 15 p.r. The three non-reconstituted SCID mice showed an OD of 0·015 ± 0·012 across all the time points. The results are from the same experiment as shown in Figure 1(a). (b) Hu-PBL-SCID mice were vaccinated with *T. muris* E/S antigens in alum on days 1 and 15 p.r. The results are from the same experiment as shown in Figure 1(b), but only show five of the seven vaccinated hu-PBL-SCID mice and three of the six mock-vaccinated hu-PBL-SCID mice. The remaining mice were all negative for *T. muris* specific IgG2 at the time of autopsy. One non-reconstituted SCID mouse was also assayed and was negative for *T. muris* specific human IgG2 at all time points.

sera were much lower than those reported in *T. trichiura* infected people, and showed a more restricted antigen recognition profile (9). Their presence is probably due to a cross-reactive B cell response or low levels of natural antibodies (27,28), and their transfer presumably explains the detection of low levels of *T. muris* specific human IgG2 in the sera of some mock-vaccinated hu-PBL-SCID mice. There was no relationship between the serum concentration of total human IgG and the serum levels of *T. muris* specific human IgG2 (Figure 3). Thus, the *T. muris* E/S specific human IgG2 responses seen in the sera of the vaccinated mice cannot simply be due to passive transfer of cross-reactive cells.

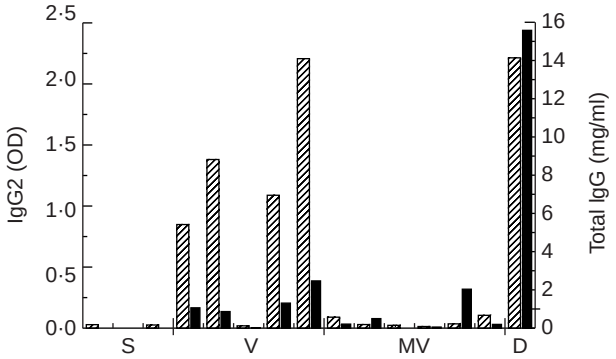


**Figure 3** Comparison of serum levels of *T. muris* specific human IgG2 (hatched bars, left axis) and serum concentrations of total human IgG (solid bars, right axis) in individual non-reconstituted SCID mouse controls (S), vaccinated hu-PBL-SCID mice (V), mock-vaccinated hu-PBL-SCID mice (MV) and the donor (D). SCID mice were reconstituted with PBL from donor 1 and vaccinated with *T. muris* E/S Ag in IFA. The donors serum was sampled at the time of reconstitution. Autopsy was performed at day 57 p.r. The serum level of *T. muris* specific human IgG2 represents the OD when a serum dilution of 1 : 10 was used.

### Vaccination of hu-PBL-SCID mice with *T. muris* homogenate in IFA results in the production of *T. muris* specific human IgG1 and IgG2

To study the effects of using different antigenic mixtures in our system, we vaccinated hu-PBL-SCID mice with 0·5 mg *T. muris* homogenate using the protocol described above. Vaccination of hu-PBL-SCID mice with *T. muris* homogenate in IFA resulted in the detection of both *T. muris* homogenate specific human IgG1 and IgG2 in the sera of vaccinated hu-PBL-SCID mice reconstituted with PBL from either donor 1 or donor 2 at days 50 and 55 p.r., respectively (Figure 4). The levels of *T. muris* specific human IgG1 and IgG2 reached peak levels by days 26/35 for donor 1 and days 19/30 for donor 2 (results not shown). No *T. muris* homogenate specific human IgG3 or IgG4 was detected in the vaccinated or mock-vaccinated hu-PBL-SCID mice, and none of the five SCID mice reconstituted with PBL from donor 3 responded to vaccination.

One of the mock-vaccinated hu-PBL-SCID mice reconstituted with PBL from donor 2 had *T. muris* homogenate specific IgG2 serum levels equal to or greater than those of the hu-PBL-SCID mice vaccinated with antigen (Figure 4d). This shows that the transfer of *T. muris* cross-reactive B cells from the donor without exposure to antigen was sufficient to produce an equivalent antibody response to hu-PBL-SCID mice vaccinated with antigen. Across all the experiments, however, vaccination with antigen resulted in consistently higher *T. muris* specific human IgG2 responses. Also, despite the presence of *T. muris* specific IgG1 antibodies in the sera of both donors 1 and 2, *T. muris* specific human IgG1 was not detected in the sera of mock-vaccinated hu-PBL-SCID mice (Figures 4a,c). Exposure to antigen within the SCID environment was therefore required to induce the production of *T. muris* specific human IgG1.

### SCID mice reconstituted with freeze/thawed human PBL can still respond to vaccination

Applying this model to a field situation will likely require human PBL to be frozen in liquid nitrogen for storage. To determine whether human PBL can still respond within SCID mice after such treatment, we freeze/thawed PBL from donor 1 in liquid nitrogen prior to reconstitution. The hu-PBL-SCID mice were then vaccinated with *T. muris* homogenate in alum using the protocol described above. *T. muris* homogenate specific human IgG2 was detected in the sera of one of four vaccinated hu-PBL-SCID mice at day 47 p.r. (Figure 5). Unlike the results obtained when IFA was used as the vaccinating adjuvant, no homogenate specific human IgG1 could be detected in any of the vaccinated hu-PBL-SCID mice. No *T. muris* homogenate specific human IgG3 or IgG4 could be detected in the sera.

### Vaccination with *T. muris* homogenate promotes an antibody response against a 66-kDa component of *T. muris* homogenate

We wanted to ascertain whether the IgG responses seen after vaccination with *T. muris* antigens were based on the existing *T. muris* cross-reactive B cell responses of the donor, or whether vaccination induced novel antibody responses. To this end, we used Western immunoblotting to compare the antigen recognition profiles of IgG from the donor and the vaccinated and mock-vaccinated hu-PBL-SCID mice against *T. muris* homogenate. At days 47 and 52 p.r., the antigen recognition profiles of the hu-PBL-SCID mice reconstituted with PBL from donor 1 and vaccinated with *T. muris* homogenate were almost identical to the antigen recognition profile of the donor. This was true whether IFA or alum was used as the adjuvant, and also for the mock-vaccinated hu-PBL-SCID mice that had detectable levels of *T. muris* specific human IgG2 (representative profiles shown in Figure 6a). A different picture was obtained when hu-PBL-SCID mice reconstituted with PBL from donor 2 were studied. In this experiment, hu-PBL-SCID mice vaccinated with *T. muris* homogenate showed strong recognition of a component of *T. muris* homogenate of approximately 66 kDa at day 55 p.r. (Figure 6b). This response was not novel as the serum IgG of the donor and the mock-vaccinated hu-PBL-SCID mice saw recognition of this component, but their

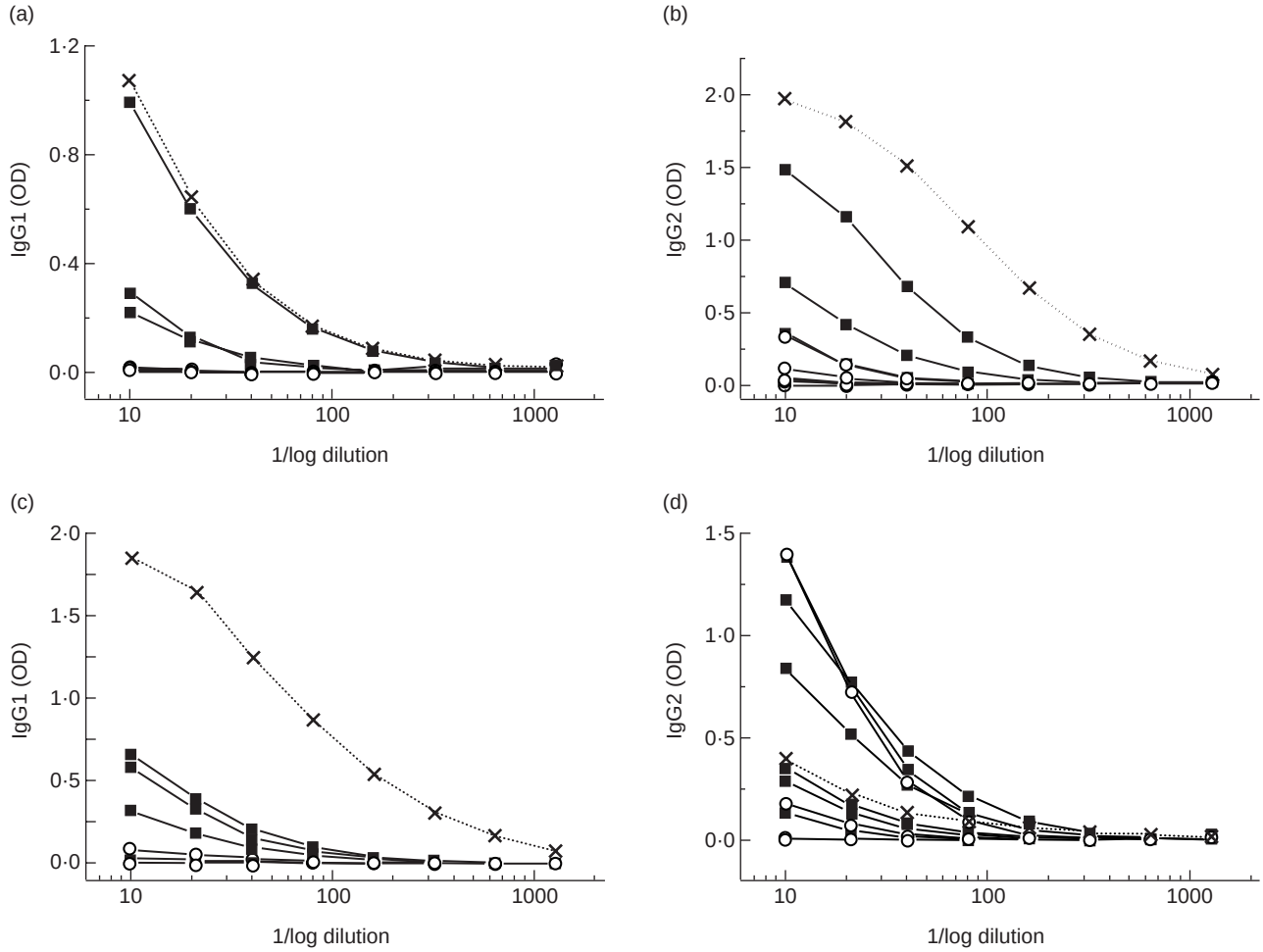


**Figure 4** Serum levels of *T. muris* specific human IgG1 (a,c) and IgG2 (b,d) of homogenate vaccinated hu-PBL-SCID mice (closed squares), mock-vaccinated hu-PBL-SCID mice (open circles) and the respective donors (crosses). Sera from the donors was sampled at the time of reconstitution. (a,b) SCID mice were reconstituted with PBL from donor 1 and vaccinated with *T. muris* homogenate in IFA. Autopsy was performed at day 50 p.r. ($n$ = 5 for vaccinated group, $n$ = 5 for mock-vaccinated group, $n$ = 3 for non-reconstituted SCID controls). Non-reconstituted controls were negative for *T. muris* E/S specific human IgG1 and IgG2. (c,d) SCID mice were reconstituted with PBL from donor 2 and vaccinated with *T. muris* homogenate in IFA. Autopsy was performed at day 55 p.r. ($n$ = 6 for vaccinated group, $n$ = 5 for mock-vaccinated group, $n$ = 3 for non-reconstituted controls). Non-reconstituted controls were negative for *T. muris* E/S specific human IgG2.

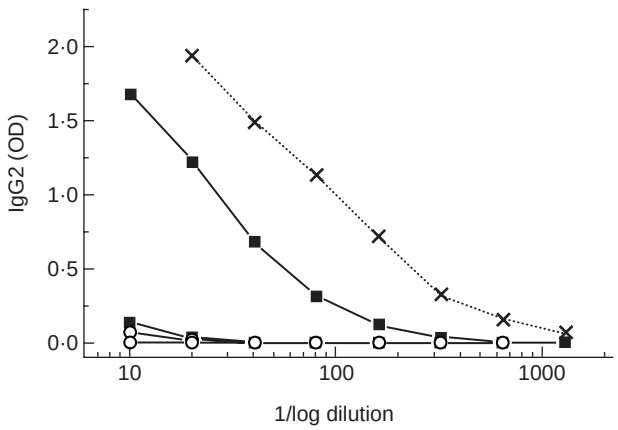


**Figure 5** Serum levels of *T. muris* specific human IgG2 of donor 1 (crosses), homogenate vaccinated hu-PBL-SCID mice (closed squares) and mock-vaccinated hu-PBL-SCID mice (open circles). The donors sera was taken at the time sampling the PBL for reconstitution. SCID mice were reconstituted with freeze/thawed PBL sampled from donor 1, followed by vaccination with *T. muris* homogenate in alum. Mice were autopsied at day 47 p.r. ($n$ = 4 for vaccinated group, $n$ = 4 for mock-vaccinated group, $n$ = 2 for non-reconstituted controls). Non-reconstituted controls were negative for *T. muris* E/S specific human IgG2.

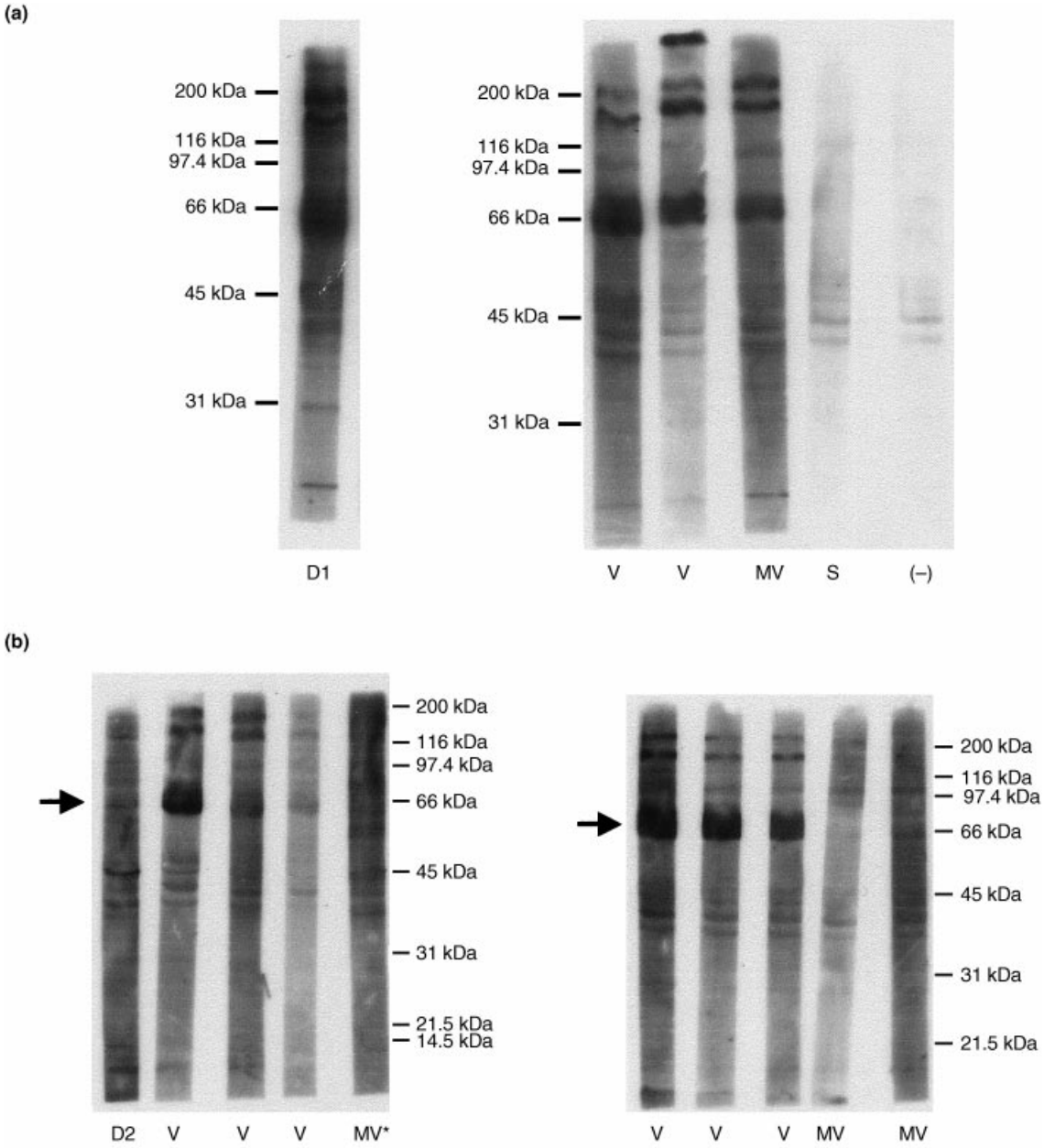


**Figure 6** Antigen recognition profiles of serum IgG of *T. muris* vaccinated (V) and mock-vaccinated (MV) hu-PBL-SCID mice, non-reconstituted SCID control (S), detection antibody without sera (–) and the respective donor (D1 or D2). (a) SCID mice were reconstituted with PBL from donor 1 followed by vaccination with *T. muris* homogenate in alum. Autopsy was performed at day 47 p.r. (b) SCID mice were reconstituted with PBL from donor 2 and vaccinated with *T. muris* homogenate in IFA. Non-reconstituted SCID mouse sera did not show any recognition of *T. muris* homogenate, neither did the detection antibody alone. MV* denotes the mock-vaccinated hu-PBL-SCID mouse from Figure 4(d) that showed a strong *T. muris* specific human IgG2 response.

responses to the 66 kDa component were less prominent. Interestingly, the mock-vaccinated hu-PBL-SCID mouse that showed serum levels of homogenate specific human IgG2 equal to or greater than the vaccinated hu-PBL-SCID mice only weakly recognized the 66 kDa component, and had a similar antigen recognition profile to that of the donor (Figures 4d and 6b). Therefore, vaccination with *T. muris* antigen did specifically promote an IgG response against a 66-kDa component of homogenate that could not be reproduced through the transfer of cross-reactive B cells alone.

## DISCUSSION

The ability to reconstitute SCID mice with human peripheral blood lymphocytes (hu-PBL-SCID) taken from individuals infected with parasitic infections, and then to be able to study the human immune responses in the murine host would provide many advantages for immunological field studies. In this study, we show the potential for using the hu-PBL-SCID model to dissect out human antibody responses against different antigens of the intestinal helminth *Trichuris trichiura*. Intra-peritoneal injection of human PBL resulted in successful engraftment in greater than 80% of the SCID mice receiving human cells, with human cells and immunoglobulins still detectable 47–57 days post reconstitution. Vaccination of hu-PBL-SCID mice with *T. muris* E/S antigens resulted in the elevated production of parasite specific human IgG2 in comparison to the hu-PBL-SCID mice mock-vaccinated with PBS. This was despite the SCID mice being reconstituted with PBL taken from naïve donors, and was independent of the adjuvant types used. The class of antibody response that could be invoked was not solely limited to IgG2 and was dependent upon the antigen mixture; when the hu-PBL-SCID mice were vaccinated with *T. muris* homogenate in IFA, an elevated production of both *T. muris* specific human IgG1 and IgG2 was seen. Negligible levels of *T. muris* specific human IgG3 and IgG4 were seen in the hu-PBL-SCID mice. These results relate with published field studies on human antibody responses to *T. trichiura* in which the main serum responses were also of the IgG1 and IgG2 subclasses (14). In these field studies, the patients' serum levels of *T. trichiura* specific IgG3 were negligible, and their levels of specific IgG4 were low.

The application of this model to field studies will likely require human PBL to be frozen in liquid nitrogen prior to reconstitution, and in other studies freeze/thawed human PBL have been less efficient at reconstituting SCID mice (29). In our hands, however, we found no difference in the total levels of reconstitution between 'fresh' and freeze/thawed PBL. The SCID mice reconstituted with freeze/thawed PBL were also capable of producing a *T. muris* specific human IgG2 response after vaccination with *T. muris* homogenate mixed with alum. Interestingly, these mice did not produce a *T. muris* specific human IgG1 response in contrast to those mice vaccinated with homogenate emulsified in IFA. As a *T. muris* specific antibody response was seen, we do not believe that this was due to the freeze/thawing of the PBL, and it may be related to the use of alum as an adjuvant rather than IFA. The variability seen in this model combined with the small group size makes it difficult to draw a firm conclusion; however, it does provide an interesting area to test in the future.

The levels of parasite specific human antibodies seen after vaccination of the hu-PBL-SCID mice were much lower than those reported in *T. trichiura* infected humans (9). In fact, in the majority of cases, they were equal to or lower than the levels of *T. muris* specific human antibodies in the donor's sera, despite the donors being naïve in respect to *T. muris* and *T. trichiura*. What should be remembered in interpreting these results is that the total human IgG concentrations of the hu-PBL-SCID were at least half, and often one-tenth, of the donor's serum IgG concentration. Thus, vaccination did increase the serum levels of *T. muris* specific human IgG in relation to the total human IgG serum concentrations. In general, it has been very difficult to generate naïve human antibody responses in hu-PBL-SCID mice (30–33), although several groups have succeeded (8,34,35). The low levels of the antibody responses seen in our vaccination protocol are likely to be due to the naïve immunological status of the donors. The difficulties probably lie in a limited 'humanization' of the SCID mice through the transfer of human PBL, which contains a restricted repertoire of human cells and in particular has a low percentage of dendritic cells. The lack of human dendritic cells within the hu-PBL-SCID mice could greatly hinder the correct priming of a naïve T cell response. In support of this, the most convincing generation of a primary human immune response was shown when a variety of tissues were transplanted to the SCID host, namely the mesenteric lymph nodes, thymus, skin and fetal bone (26). One way of improving the immune responses in hu-PBL-SCID mice could be to culture dendritic cells from the donor's blood and reconstitute SCID mice with both human PBL and dendritic cells, although this obviously adds extra logistical problems. Strong recall responses have been seen in hu-PBL-SCID mouse models, however, and generally the induction of recall as opposed to naïve immune responses have been much easier (30,32,36). We therefore expect that reconstitution of SCID mice with PBL from *T. trichiura* infected donors will result in a much stronger response to vaccination than seen with PBL from naïve donors. A stronger antibody response to vaccination should also result in a greater separation of the responses between the vaccinated and mock-vaccinated hu-PBL-SCID mice, helping to alleviate the problem of the low levels of *T. muris* specific IgG2 seen in the mock-vaccinated hu-PBL-SCID mice.

One common problem in all the studies using hu-PBL-SCID mice is the variation seen, even between SCID mice reconstituted with PBL from the same donor (7,21,22). Similar variation was seen in our study both in the levels of total engraftment, and in the parasite specific antibody responses detected. In a group of SCID mice reconstituted at the same time with PBL from the same donor, not all would respond to the vaccination and those that did

responded to different extents. It is reported in the literature that PBL from different donors show different compatibilities with the SCID environment (7,29,37) and, similarly, in our experiments, SCID mice reconstituted with PBL from one of our three donors failed to respond to vaccination. The variability in the specific antibody responses was not related to the variability in the total levels of engraftment (except in those mice which failed to show any human cell engraftment), and there was no obvious factor we noted that could account for the differences seen. This variability does warn against using this model when group sizes are limiting, and the results from each hu-PBL-SCID mouse should be considered individually in their own right. The variability does not discount the use of this model, just that careful planning and interpretation of experiments will be required to avoid artefacts. In a practical sense, this will either entail reconstituting a group of SCID mice with PBL from one donor requiring relatively large blood volumes, or using one SCID mouse per donor and attempting to overcome the variation through sampling a large population of donors.

Low levels of *T. muris* reactive IgG1 and IgG2 were detected in the naïve donors which were presumably either due to cross-reactive or 'natural antibodies' (27,28). A resultant complicating factor of this study was the production of *T. muris* specific human IgG2 by hu-PBL-SCID mice never exposed to *T. muris* antigens. It therefore appeared that transfer of the cross-reactive B cells from the donor was sufficient to generate *T. muris* specific IgG2 responses in hu-PBL-SCID mice in the absence of vaccination. A similar phenomenon has been noted for recall antigens in other hu-PBL-SCID mouse vaccination models (30,38). Overall, the hu-PBL-SCID mice vaccinated with *T. muris* antigens produced more consistent *T. muris* specific human IgG2 responses at higher levels than the mock-vaccinated hu-PBL-SCID mice (with the exception of one mock-vaccinated hu-PBL-SCID mouse discussed below) leading us to believe that vaccination was at least enhancing the donor's cross-reactive response. Also, the production of *T. muris* specific human IgG1 required the hu-PBL-SCID mice to be vaccinated with *T. muris* homogenate, and was never found in the mock-vaccinated hu-PBL-SCID mice. It is possible that the production of IgG2 in the absence of *T. muris* antigens may be related to the 'T-cell independent' nature of the antibody response reported for this isotype, and would be an interesting area for future study (39,40).

This ability to transfer cross-reactive B cells from the naïve donor suggests that the responses generated by the vaccination protocol were based on the donor's cross-reactive responses. This idea was supported by the antigen recognition profiles of *T. muris* homogenate by the vaccinated and mock-vaccinated hu-PBL-SCID mice reconstituted with PBL from donor 1. Their profiles were almost identical to those of the donor, and thus vaccination with antigen had not generated a novel antibody response even though it had boosted the serum levels of *T. muris* specific antibodies. When SCID mice were reconstituted with PBL from donor 2, the antibody responses were also based on the donors cross-reactive response, but the vaccination appeared to bias the hu-PBL-SCID mouse response towards a component of *T. muris* homogenate of approximately 66KDa. Importantly, such a bias was not seen in the *T. muris* cross-reactive IgG responses of any of the mock-vaccinated hu-PBL-SCID mice, including the one that produced equivalent or higher levels of *T. muris* reactive antibodies than the hu-PBL-SCID mice in the vaccinated group. Thus, although cross-reactive B cells can survive and produce *T. muris* specific antibodies in the SCID environment without exposure to *T. muris* antigens, vaccination with *T. muris* antigens does boost the antibody responses seen and can also direct the response towards a particular antigen. A strong IgG response against the 66 kDa component was already present in the sera of donor 1, possible explaining why a similar enhancement was not seen in the hu-PBL-SCID mice engrafted with PBL from this donor. Although a novel antibody response was not detected, the reflection of the donor's 'cross-reactive' responses in the hu-PBL-SCID mice does suggest that the responses of the hu-PBL-SCID mice reconstituted with PBL from *T. trichiura* exposed individuals will mirror the infection history and status of the donor.

The successful vaccination of hu-PBL-SCID mice reconstituted with PBL from naïve human donors demonstrates the potential for studying human antibody responses against *Trichuris* antigens using this model. This is especially so considering that the use of SCID mice reconstituted with PBL from *T. trichiura* exposed individuals should be much stronger than those in 'naïve' hu-PBL-SCID mice. Donor and antigen-dependent differences were seen in the human antibody responses of the hu-PBL-SCID mice, and thus it should be possible to see similar differences in the antibody responses of *T. trichiura* infected humans. The infection of hu-PBL-SCID mice with *T. muris* may also provide a method for comparing the cellular and cytokine responses seen in the murine model to those produced by the human immune system. Human cells have been retrieved from the mesenteric lymph nodes of one hu-PBL-SCID mouse noted to have intestinal inflammation, but a key question will be to determine whether human cells are capable of migrating sufficiently within SCID mice to show a normal response to a live infection. In the future, the model could also be used to study how to manipulate a vaccination protocol to produce an antibody response of a desired phenotype, or to redirect it towards a particular antigen.

## ACKNOWLEDGEMENTS

We would like to thank the human donors for giving up their blood, and the staff of the BSU at the University of Manchester for care of the mice. We would like to thank Richard Grencis, Eric Bell and Peter Wood for helpful discussions. This work was funded by the Wellcome Trust (grant N°: 44494/Z/95/Z & 047366/Z/96/Z).

## REFERENCES

1 Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science* 1988; **242**: 1706–1709.
2 McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 1988; **241**: 1632–1639.
3 Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* 1988; **335**: 256–259.
4 Goldstein H, Pettoello-Mantovani M, Katopodis NF, Kim A, Yurasov S, Kollmann TR. SCID-hu mice: a model for studying disseminated HIV infection. *Semin Immunol* 1996; **8**: 223–231.
5 Tighe H, Silverman GJ, Kozin F, *et al.* Autoantibody production by severe combined immunodeficient mice reconstituted with synovial cells from rheumatoid arthritis patients. *Eur J Immunol* 1990; **20**: 1843–1848.
6 Ifversen P, Borrebaeck C. A SCID-hu-PBL: a model for making human antibodies? *Semin Immunol* 1996; **8**: 243–248.
7 Murphy WJ, Taub DD, Longo DL. The huPBL-SCID mouse as a means to examine human immune function *in vivo*. *Semin Immunol* 1996; **8**: 233–241.
8 Mazingue C, Cottrez F, Auriault C, Cesbron JY, Capron A. 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. *Eur J Immunol* 1991; **21**: 1763–1766.
9 Roach TI, Wakelin D, Else KJ, Bundy DAP. Antigenic cross-reactivity between the human whipworm, *Trichuris trichiura*, and the mouse trichuroids *Trichuris muris* and *Trichinella spiralis*. *Parasite Immunol* 1988; **10**: 279–291.
10 Artis D, Grencis RK, Kennedy MW, Harnett W, eds. In *Parasitic Nematodes. Molecular Biology, Biochemistry and Immunology*. Oxon: CABI Publishing; 2001, 331–372.
11 Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 1994; **179**: 347–351.
12 Bancroft A, McKenzie AN, Grencis RK. A critical role for IL-13 in resistance to intestinal nematode infection. *J Immunol* 1998; **160**: 3453–3461.
13 Blackwell NM, Else KJ. B cells and antibodies are required for resistance to the parasitic gastrointestinal nematode *Trichuris muris*. *Infect Immun* 2001; **69**: 3860–3868.
14 Needham CS, Lillywhite JE. Immunoepidemiology of intestinal helminthic infections. 2. Immunological correlates with patterns of *Trichuris* infection. *Trans Roy Soc Trop Med Hyg* 1994; **88**: 262–264.
15 Needham CS, Bundy DAP, Lillywhite JE, Didier JM, Simmons I, Bianco AE. The relationship between *Trichuris trichiura* transmission intensity and the age-profiles of parasite-specific antibody isotypes in two endemic communities. *Parasitology* 1992; **105**: 273–283.
16 Cooper ES, Whyte-Alleng CA, Finzi-Smith JS, MacDonald TT. Intestinal nematode infections in children: the pathophysiological price paid. *Parasitology* 1992; **104**: S91–S103.
17 MacDonald TT, Choy MY, Spencer J, *et al.* Histopathology and immunohistochemistry of the caecum in children with the *Trichuris* dysentery syndrome. *J Clin Pathol* 1991; **44**: 194–199.
18 MacDonald TT, Spencer J, Murch SH, Choy MY, Venugopal S, Bundy DAP, Cooper ES. Immunoepidemiology of intestinal helminthic infections. 3. Mucosal macrophages and cytokine production in the colon of children with *Trichuris trichiura* dysentery. *Trans Roy Soc Trop Med Hyg* 1994; **88**: 265–268.
19 Else KJ, Betts CJ. Antibody isotype responses to *Trichuris* infection in humanized SCID mice. *Parasite Immunol* 1997; **19**: 485–491.
20 Artis D, Potten CS, Else KJ, Finkelman FD, Grencis RK. *Trichuris muris*: host intestinal epithelial cell hyperproliferation during chronic infection is regulated by interferon-γ. *Exp Parasitol* 1999; **92**: 144–153.
21 Murphy WJ, Bennett M, Anver MR, Baseler M, Longo DL. Human-mouse lymphoid chimeras: host-vs.-graft and graft-vs.-host reactions. *Eur J Immunol* 1992; **22**: 1421–1427.
22 Williams SS, Unemoto T, Kida H, Repasky EA, Bankert RB. Engraftment of human peripheral blood leukocytes into severe combined immunodeficient mice results in the long term and dynamic production of human xenoreactive antibodies. *J Immunol* 1992; **149**: 2830–2836.
23 Donze HH, Cummins JE, Schwiebert RS, Fultz PN, Jackson S, Mestecky J. Human and nonhuman primate lymphocytes engrafted into SCID mice reside in unique mesenteric lymphoid structures. *J Immunol* 1998; **161**: 1306–1312.
24 Duchosal MA, Eming SA, McConahey PJ, Dixon FJ. Characterization of hu-PBL-SCID mice with high human immunoglobulin serum levels and graft-versus-host disease. *Am J Pathol* 1992; **141**: 1097–1113.
25 Picchio GR, Kobayashi R, Kirven M, Baird SM, Kipps TJ, Mosier DE. Heterogeneity among Epstein-Barr virus-seropositive donors in the generation of immunoblastic B-cell lymphomas in SCID mice receiving human peripheral blood leukocyte grafts. *Cancer Res* 1992; **52**: 2468–2477.
26 Carballido JM, Namikawa R, Carballido-Perrig N, Antonenko S, Roncarolo MG, de Vries JE. Generation of primary antigen-specific human T- and B-cell responses in immunocompetent SCID-hu mice. *Nature Med* 2000; **6**: 103–106.
27 Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F, Hengartner H, Zinkernagel R. Control of early viral and bacterial distribution and disease by natural antibodies. *Science* 1999; **286**: 2156–2159.
28 Borrebaeck CA. Human mAbs produced by primary *in-vitro* immunization. *Immunol Today* 1988; **9**: 355–359.
29 Hesselton RM, Koup RA, Cromwell MA, Graham BS, Johns M, Sullivan CA. Human peripheral blood xenografts in the SCID mouse: characterization of immunologic reconstitution. *J Infectious Dis* 1993; **168**: 630–640.
30 Markham RB, Donnenberg AD. Effect of donor and recipient immunization protocols on primary and secondary human antibody responses in SCID mice reconstituted with human peripheral blood mononuclear cells. *Infect Immun* 1992; **60**: 2305–2308.

31 Kim HM, Han SB, Hong DH, Yoo BS, Oh GT. Limitation of *Hu*-PBL-*scid* mouse model in direct application to immunotoxicity assessment. *J Pharmacol Toxicol Meth* 1997; **37**: 83–89.
32 Duchosal MA, Eming SA, Fischer P, *et al.* Immunisation of hu-PBL-SCID mice and the rescue of human monoclonal Fab fragments through combinatorial libraries. *Nature* 1992, 355: 258–262.
33 Abedi MR, Christensson B, Islam KB, Hammarström L, Smith CIE. Immunoglobulin production in severe combined immunodeficient (SCID) mice reconstituted with human peripheral blood mononuclear cells. *Eur J Immunol* 1992; **22**: 823–828.
34 Walker W, Roberts CW, Brewer JM, Alexander J. Antibody responses to *Toxoplasma gondii* antigen in human peripheral blood lymphocyte-reconstituted severe-combined immunodeficient mice reproduce the immunological status of the lymphocyte donor. *Eur J Immunol* 1995; **25**: 1426–1430.
35 Sandhu J, Shpitz B, Gallinger S, Hozumi N. Human primary immune response in SCID mice engrafted with human peripheral blood lymphocytes. *J Immunol* 1994; **152**: 3806–3813.
36 Nonoyama S, Smith FO, Ochs HD. Specific antibody production to a recall or a neoantigen by SCID mice reconstituted with human peripheral lymphocytes. *J Immunol* 1993; **151**: 3894–3901.
37 Uchibayashi N, Sasada R, Shino A, *et al.* A human monoclonal antibody to a human self-antigen, CD2 derived from human peripheral blood lymphocytes engrafted in SCID mice. *Hybridoma* 1995; **14**: 313–321.
38 Lucas AH, Siff TE, Trujillo KH, Kitamua MY. Vaccine-induced human antibody responses to the *Haemophilius influenzae b* polysaccharide in severe combined immunodeficient mice engrafted with human leukocytes. *Pediatr Res* 1992; **32**: 132–135.
39 Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. *Annu Rev Immunol* 1995; **15**: 655–692.
40 Ravindran B, Satapathy AK, Das MK, Pattnaik NM, Subramanyam VR. Antibodies to microfilarial sheath in Bancroftian filariasis – prevalence and characterization. *Ann Trop Med Parasitol* 1990; **84**: 607–613.