Immunology 1994 **83** 171–179

Antigen-specific human immunoglobulin production in SCID mice transplanted with human peripheral lymphocytes is dependent on $CD4^+$ $CD45RO^+$ T cells

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SUMMARY

Severe combined immunodeficient (SCID) mice, lacking mature T and B cells and virtually devoid of endogenous serum immunoglobulins, spontaneously produce large amounts of human immunoglobulin after transplantation with human peripheral blood lymphocytes (PBL). Moreover, after immunization with antigen an active immune response resulting in a production of specific antibodies can be induced. Here we report that human T cells must be co-transplanted with B cells into the SCID mice for immunoglobulin production to occur. Resting human B cells could be activated to immunoglobulin production in the absence of human monocytes and a specific antibody response to tetanus toxoid (TT) could be induced, suggesting that the human B cells could present antigen to T cells in the SCID environment. Production of human immunoglobulins, as well as specific antibodies, was obtained only if $CD4^+$ T cells of the memory phenotype, i.e. expressing CD45RO, were present. No human immunoglobulin, either of IgM or of IgG isotype, was found in SCID sera if mice were co-transplanted with human B cells and CD45RA expressing $CD4^+$ T cells. However, FACS analysis revealed that the transplanted $CD45RA^+$ cells became activated and differentiated towards $CD45RO^+$ cells within 1–2 weeks. These cells also gained the lymphokine gene expression pattern associated with $CD45RO^+$ cells, as demonstrated by polymerase chain reaction (PCR) analysis, and could support immunoglobulin production in SCID mice transplanted with fresh B cells. In fact, after differentiation of $CD4^+$ $CD45RA^+$ T cells towards expression of CD45RO, either *in vivo* in the SCID mouse or *in vitro*, these cells could interact with and activate human B cells to immunoglobulin production. Furthermore, *in vitro* activated and differentiated $CD4^+$ $CD45RA^+$ T cells from vaccinated donors were also able to support production of TT-specific antibodies provided the antigen was administered.

INTRODUCTION

Mice suffering from severe combined immunodeficiency (SCID) due to a defect in recombination of the antigen receptor genes, are deficient in mature T and B cells.[1,2] Therefore these SCID mice tolerate allogeneic or xenogeneic transplants.[2] Thus, SCID mice have been used as recipients of human tumour cells in the study of tumour biology and for the testing of various anti-tumour compounds in a manner similar to how the nude mice were used.[3–5] It has also been demonstrated that SCID mice accept grafts containing human lymphoid cells of various origin.[6–10] We and others have been interested in this model since it offers possibilities to manipulate human antibody specificities outside the human body.[6,11] It is possible to obtain production of human immunoglobulins in peripheral blood lymphocyte (PBL)-transplanted SCID mice even in the absence of deliberate immunization,[11,12] despite the fact that the transferred human immune cells derived from PBL do not constitute an intact immune system. However, immunization with antigen, especially recall antigens, can result in production of specific antibodies detectable in the sera of immunized mice.[6,11] Antibody specificities developed in the SCID-hu-PBL system have, in fact, been immortalized either through cellular[11,13] or molecular biology[14] techniques, demonstrating the feasibility of the system for production of human monoclonal antibodies.

While it has been relatively easy to induce human antibody production in SCID-hu-PBL mice against recall antigens such as tetanus toxoid (TT), antibodies against primary antigens have been much more difficult to elicit. To reach an understanding of the reasons for these difficulties we have

Received 28 October 1993; revised 26 April 1994; accepted 26 May 1994.

Abbreviations: SCID, severe combined immunodeficiency; TT, tetanus toxoid.

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studied basic immune parameters of human mature lymphocytes in SCID mice. We have been especially interested in the relationship between human T and B cells known to be of importance for immunoglobulin production to occur.

Antigen-specific interactions between T helper and B cells are mediated through binding of the T-cell receptor (TCR) to peptide/class II major histocompatibility complexes (MHC) on the surface of B cells. In addition to these and other contact-mediated signals soluble lymphokines influence B-cell proliferation and differentiation. T-helper cell subsets differing in their capabilities to deliver lymphokine-mediated signals to B cells have been identified. Thus, $CD4^+$ T cells expressing CD45RO can act as helper cells for immunoglobulin production whereas the subset expressing high levels of the CD45RA isoform cannot.[15,16] The CD45RA expressing $CD4^+$ T cells acquire the phenotype and function of the CD45RO T-helper cells after activation,[16,17] and T cells specific for recall antigens are mainly, if not exclusively, found in the CD45RO population. Thus, this subset is considered to represent memory cells or preactivated cells, whereas the CD45RA-expressing cells are regarded as being naive T cells.[16–19]

In this paper we have investigated the ability of the different $CD4^+$ T-cell subsets to interact with and activate human B cells when transplanted into SCID mice. It was found that T-helper cells, in particular $CD4^+CD45RO^+$ T cells, were mandatory for activation and differentiation of human B cells. In addition, purified human CD45RA expressing $CD4^+$ T cells differentiated phenotypically and functionally in the SCID mice and became, after this process, able to activate human B cells to immunoglobulin production.

MATERIALS AND METHODS

Animals

C.B-17 *scid/scid* mice, referred to as SCID mice, kindly provided by Dr Donald Mosier (Scripps Research Institute, La Jolla, CA), were bred and maintained in our specific pathogen-free facility at the Wallenberg Laboratory, Lund University (Sweden). The mice were kept in sterilized cages with filter tops and given acidified, sterilized water (pH 3) and sterile pellets (Special Diets Services, Witham, UK). The mice were bled between 7 and 9 weeks of age to analyse the presence of murine immunoglobulin, and only those with $< 1\ \mu g$/ml serum were used in the study.

Cell separation and repopulation of SCID mice with human cells

PBL were obtained either from buffy coats from the University Hospital Blood Bank (Lund, Sweden), or from whole blood from healthy volounteers previously vaccinated against TT. Mononuclear cells were prepared by density centrifugation on Ficoll–Hypaque (Kabi-Pharmacia AB, Uppsala, Sweden). Platelets were removed by centrifugation through a Percoll gradient.[20] The resulting cells were depleted of monocytes by plastic adherence for 30 min at 37°. B cells were separated from the non-adherent cells using PanB magnetic beads (Dynal, Oslo, Norway).[16,21] The resulting B-cell population was routinely $> 99{\cdot}5\%$ $CD20^+$, as determined by flow cytometry. The non-B cells were depleted of natural killer (NK) cells and residual monocytes by treatment with L-leucyl-L-leucine methyl esther (LeuLeuOMe; Bachem, Budendorf, Switzerland).[22] For further enrichment of T cells the remaining cell fraction was rosetted with neuraminidase-treated sheep red blood cells (SRBC)[23] and then subjected to a negative panning procedure. To eliminate residual contaminating B cells, monocytes and NK cells the purified T cells were incubated in phosphate-buffered saline, pH 7·4, containing 1% bovine serum albumin (PBS–BSA) with anti-HLA-Dr (Dakopatts, Glostrup, Denmark) and anti-HNK-1 (Sera-Lab, Crawely Down, UK) monoclonal antibodies for 30 min at 4°. For selection of $CD4^+$ T cells a monoclonal antibody directed against CD8 (Dakopatts) was added as well. After incubation the lymphocytes were washed on a cushion of 40% Ficoll–Hypaque in PBS–BSA and were then plated on rabbit anti-mouse immunoglobulin-coated plastic Petri dishes (Dakopatts), 40×10^6 lymphocytes/Petri dish. After incubation for 1 hr at 37°, non-attached lymphocytes were gently removed, washed and resuspended in complete RPMI-1640 medium supplemented with 2 mM L-glutamine, 1% non-essential amino acids (Gibco, Grand Island, NY), 50 μg/ml gentamicin (Biological Industries, Haemek, Israel) and 10% fetal calf serum (Gibco).

Cells prepared this way were routinely $> 97\%$ $CD4^+$ T cells and contained less than $0{\cdot}1\%$ $CD56^+$ NK cells, $0{\cdot}5\%$ $CD20^+$ B cells, $0{\cdot}1\%$ $CD14^+$ monocytes and 1% $CD8^+$ T cells. To isolate the $CD45RA^+$ and $CD45RO^+$ populations from the $CD4^+$ T cells, these cells were stained with monoclonal antibodies directed against CD45RA (anti-Leu-18; Becton Dickinson, San José, CA) or CD45RO (UCHL-1; Dakopatts) and subjected to negative panning as described above. The staining and panning procedure was performed twice. The resulting negatively panned $CD45RA^+$ and $CD45RO^+$ T cells always exceeded 96% purity, as shown by flow cytometry. The contaminating cells were double-positive for CD45RA and CD45RO, expressing low levels of CD45RO or CD45RA, respectively. After several rounds of washing the human cells were injected intraperitoneally (i.p.) in 0·5 ml of phosphate-buffered saline (PBS) into SCID mice.

Cell culture conditions

$CD4^+$ T cells expressing CD45RA or CD45RO were cultured in complete RPMI-1640 medium. To differentiate CD45RA into CD45RO-expressing $CD4^+$ cells, the $CD45RA^+$ cells were seeded in T75 tissue culture bottles (Costar, Cambridge, MA) at a concentration of 1×10^6 T cells/ml together with 10% formalin-fixed cells from the B lymphoma Raji cell line and 1 ng/ml of staphylococcal enterotoxin A (SEA)[25] (Toxin Technology, Madison, WI) and cultured for 7 days. Raji cells were fixed in 1% formaldehyde in PBS for 1 hr at room temperature. After fixation the cells were washed three times in PBS and resuspended in complete RPMI-1640 medium. Cells obtained from the peritoneal cavity of SCID mice were restimulated in U-bottomed 96-well tissue culture plates (Sarstedt, Newton, IA) in the presence of 10% formalin-fixed Raji cells and 1 ng/ml SEA. Samples of 5×10^4 cells were taken for mRNA preparation at different time-points after initiation of the cultures.

In vivo *immunization*

SCID mice repopulated with human cells derived from vaccinated donors, were immunized i.p. with 0·25 ml of 40 μg/ml TT (National Bacteriological Laboratory, Stockholm, Sweden) diluted in PBS. The first injection was given 1 day

after the transplantation of human cells and the immunization was repeated with 2 week intervals.

Preparation of cells from SCID mice

The mice were killed by cervical dislocation and cells were obtained from the peritoneal cavity under aseptical conditions. The cells were harvested by flushing the peritoneum with complete medium containing 5% fetal calf serum. The cell preparations were washed twice in PBS containing 1% bovine serum albumin, before staining with monoclonal antibodies (mAb). Spleen and lymph nodes were not examined as previous studies have shown that human T and B cells are mainly found in the peritoneal cavity of repopulated SCID mice.[11]

Analytical procedures

Flow cytometry was performed on a FACStar Plus cytofluorograph (Becton Dickinson). The purity and identity of the purified human cells were analysed using the following antibodies specific for human cell-surface markers: anti-CD4 Tri-Color (Caltag Laboratories, San Francisco, CA), UCHL-1 phycoerythrin (PE) (anti-CD45RO; Dakopatts), anti-Leu-2a FITC (anti-CD8), anti-Leu-18 FITC (anti-CD45RA), anti-Leu-16 FITC (anti-CD20), anti-Leu-19 PE (anti-CD56) and anti-Leu-M3 FITC (anti-CD14) (all from Becton Dickinson). Staining of cells was performed by incubating the cells with proper amounts of the monoclonal antibodies diluted in PBS–BSA for 30 min on ice. After incubation the lymphocytes were washed using a cushion of 40% Ficoll–Hypaque in PBS–BSA, and then analysed on a flow cytometer. Analysis was performed on 3000–5000 viable cells, as determined by their forward and side light scatter characteristics. Cells from the peritoneal cavity were stained with murine mAb specific for human cell-surface markers for three-parameter analysis using anti-Leu-4 FITC (anti-CD3), anti-HLA-DR PE, anti-IL-2R PE (anti-CD25), anti-Leu-18 FITC and UCHL-1 PE. Murine cells in the cell suspensions were identified by staining with biotinylated anti-mouse H-2K^d (Pharmingen, San Diego, CA) followed by streptavidin Tri-Color. All peritoneal exudate cells from mice not transplanted with human lymphocytes stained with this antibody

For assessment of total and anti-TT specific human antibody production, SCID mice were bled every second week after repopulation with human cells and their sera tested by ELISA.[11] Briefly, 96-well microtitre plates (Costar) were coated with 100 μl (1 μg/ml) of goat anti-human IgG, IgM or total immunoglobulin (Zymed Laboratories, Inc., San Francisco, CA) or with TT diluted in 50 mM sodium bicarbonate buffer, pH 9·5. Samples to be analysed, as well as horseradish peroxidase-conjugated goat anti-human IgG, IgM or total immunoglobulin antibodies (Zymed Laboratories) used for detection of bound antibody, were all diluted in 10 mM sodium phosphate buffer, pH 8·0, containing 0·5 M sodium chloride and 0·05% Tween 20. The amount of bound antibody was determined with orthophenylenediamine (Sigma, St Louis, MO) (67 μg/well) and hydrogen peroxide as chromogen and substrate, respectively.

Analysis of lymphokine gene expression

Messenger RNA preparation, cDNA synthesis and polymerase chain reaction (PCR) were performed as previously described.[17] To control the quality of the mRNA preparations, all samples were examined for the presence of β-actin-specific mRNA. Only those experiments where all preparations were positive for β-actin were further investigated for interleukin-specific mRNA. All primers used for the PCR reaction have been described in detail elsewhere.[17]

The PCR products were identified by agarose gel electrophoresis of 10 μl amplified DNA on 2% agarose gels in Tris–acetate–EDTA (TAE) buffer, containing 0·25 μg/ml ethidium bromide. Molecular weight standard number VI (Boeringer Mannheim, Mannheim, Germany) was used at 0·5 μg DNA/lane.

RESULTS

Ability of CD45RA$^+$ and CD45RO$^+$ human T cells to provide help for immunoglobulin production in SCID mice

Previous studies have demonstrated production of human immunoglobulin in SCID mice transplanted with human PBL.[6,11,12] Initial studies performed by us demonstrated human immunoglobulin production, spontaneous as well as antigen induced, to be totally dependent on the presence of human T cells. Moreover, human monocytes were not needed for activation of the T cells and the B cells themselves could act as antigen-presenting cells (data not shown). It is well known from studies *in vitro* that CD4$^+$ T cells of the memory phenotype (expressing CD45RO) contain helper activity for immunoglobulin production, whereas the subpopulation expressing CD45RA is incapable of providing such help.[15,16] To investigate the effect of these T-cell subpopulations when transplanted into SCID mice, human CD4$^+$ T cells expressing either CD45RA or CD45RO were injected into SCID mice together with purified human B cells and these mice were subsequently immunized with TT. Sera were obtained from the animals at 2-week intervals for a period of 8 weeks after transplantation of the human cells. Levels of human IgM and IgG in these sera were detected only when CD4$^+$ T cells or CD4$^+$ T cells of the memory phenotype (CD45RO$^+$) had been co-transplanted with the B cells. No human immunoglobulin, either of IgM or of IgG isotype, could be detected in mice given purified CD4$^+$ CD45RA$^+$ T cells at any time during the whole 8-week period studied. Data obtained after analysis of sera from mice obtained 4 weeks after transfer of human cells is shown in Fig. 1. Furthermore, only mice given CD45RO$^+$ T-helper cells and immunized with TT developed a high titred specific IgG antibody response, whereas specific antibodies of the IgM isotype were much less prevalent (Fig. 1c, d). The positive sera did not bind to the control antigens KLH and BSA indicating the specificity of the antibodies.

Ability of CD45RA$^+$ T-helper cells to act as helper cells for immunoglobulin production in SCID mice after differentiation into CD45RO$^+$ T cells *in vitro* or *in vivo*

Studies *in vitro* have shown that CD4$^+$ CD45RA$^+$ human T cells achieve the phenotype as well as function of memory cells after activation and differentiation.[16,17] Cultivation for 7 days of highly purified CD4$^+$ CD45RA$^+$ T cells together with 1 ng/ml SEA and 10% formalin-fixed Raji cells, as antigen-presenting cells, resulted in blast-transformation and expression of CD45RO on about 80% of the cells (data not shown).

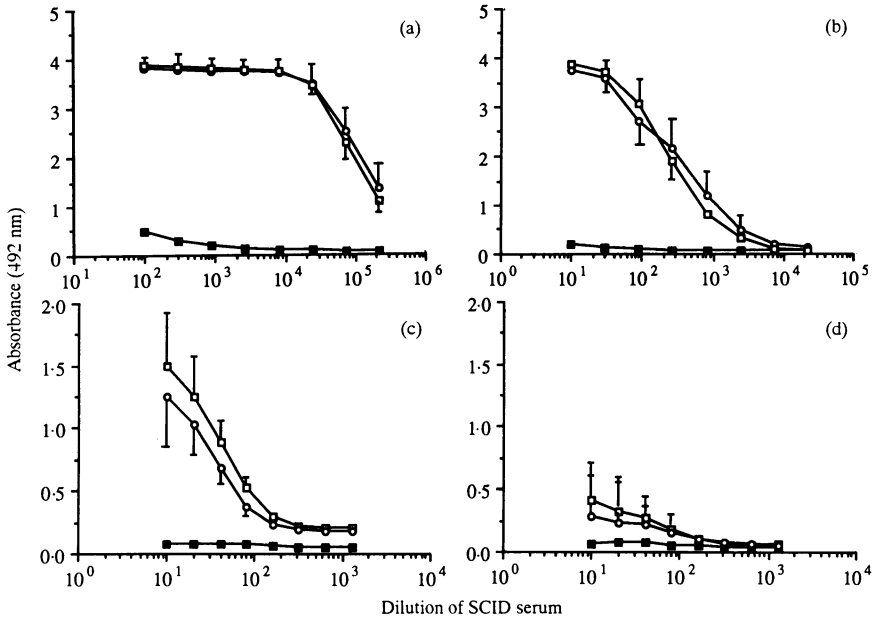

Figure 1. Effect of different subpopulations of human T cells on production of human IgG (a), human IgM (b), anti-TT IgG (c) and anti-TT IgM (d) antibodies in SCID mice 4 weeks after transplantation with lymphocytes. The mice were injected with $6{\cdot}5 \times 10^6$ B cells (99·2% $CD20^+$) and 15×10^6 $CD4^+$ $CD45RA^+$ T cells (■), $CD4^+$ $CD45RO^+$ T cells (○) or $CD4^+$ T cells (44% $CD45RO^+$, 56% $CD45RA^+$) (□). All mice were immunized with 10 μg of TT. Each data point represents the mean ± SEM from three individuals. Two identical experiments were performed and gave the same results.

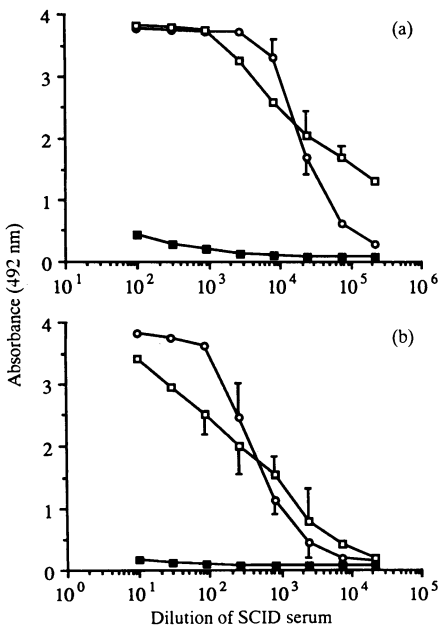

Figure 2. Ability of *in vitro* differentiated $CD45RA^+$ T-helper cells to support human IgG (a) and IgM (b) production in SCID mice 4 weeks after transplantation. The mice were transplanted with 3×10^6 B cells and 15×10^6 $CD4^+$ $CD45RO^+$ T cells (○), $CD4^+$ $CD45RA^+$ T cells (■) or *in vitro* differentiated $CD4^+$ $CD45RA^+$ T cells (□). $CD45RA^+$ T-helper cells were cultured *in vitro* with 1 ng/ml SEA and 10% Raji cells for 7 days to differentiate into CD45RO-expressing T-helper cells. The *in vitro* differentiated $CD4^+$ $CD45RA^+$ T cells were 80% positive for CD45RO. Each data point represents the mean ± SEM from three mice. The figure shows results from one experiment representative of four identical experiments.

When these T cells were injected together with purified B cells into SCID mice, substantial levels of immunoglobulin, both of IgM and of IgG isotype, were detected in the sera of the SCID mice by 2 weeks after transplantation of the cells. These serum levels increased further during the following weeks and became comparable to those found in sera of mice transplanted with fresh $CD4^+$ $CD45RO^+$ T cells together with B cells. In contrast, as was demonstrated earlier, fresh $CD4^+$ $CD45RA^+$ T cells did not support immunoglobulin production (Fig. 2a, b). As demonstrated by FACS analysis human cells isolated from the peritoneal cavity of mice 7–14 days after transplantation were activated and blast transformed. (Fig. 3a). A portion of the isolated human cells also expressed activation-associated antigens, such as interleukin-2 receptor (IL-2R) and HLA-DR (Fig. 3b, c). Moreover, some of the $CD45RA^+$ cells had differentiated towards expression of CD45RO and lost expression of the CD45RA isoform (Fig. 3d). Human B cells as defined by lack of $H2K^d$ and CD3 markers but positive for HLA-Dr (Fig. 3c), did not produce immunoglobulin after transplantion together with fresh $CD4^+$ $CD45RA^+$ T cells. However, they were alive and activated as they had light scatter characteristics typical for blast-transformed lymphocytes (Fig. 3a). Note that human B cells expressing CD45RA are also found in the lower right corner of the dot plot in Fig. 3d. The percentage of T cells expressing CD45RO is, thus, higher than the figure shows and can be estimated to be around 40–50%. These $CD45RO^+$ T cells were sorted out, stimulated *in vitro* with SEA, and were found to have a lymphokine mRNA profile characteristic of the memory phenotype since they produced mRNA encoding IL-2, IL-4, IL-5 and, occasionally, interferon-γ (IFN-γ) (Fig. 4). As controls, fresh $CD45RA^+$ and $CD45RO^+$ T cells were isolated from PBL, stimulated with

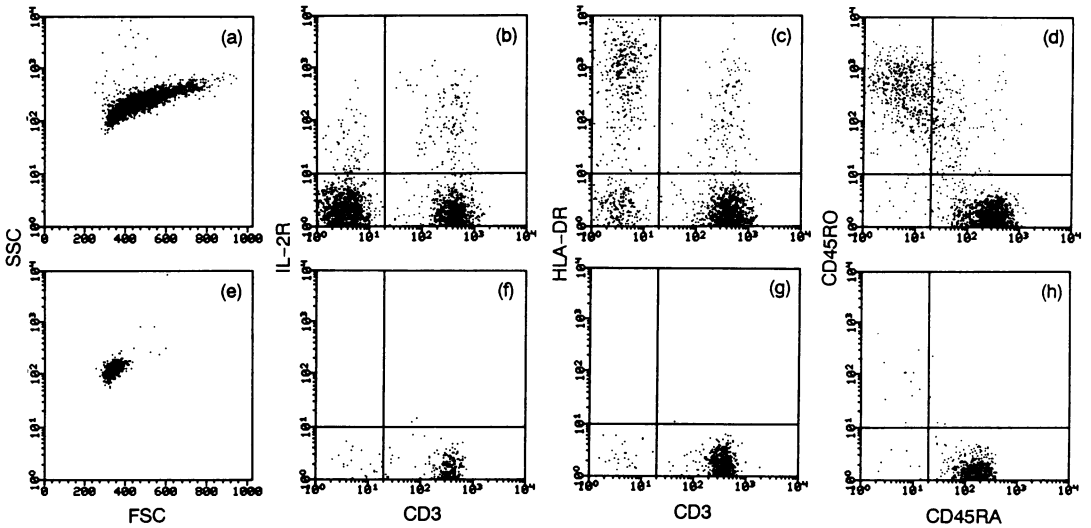

Figure 3. Phenotypic characterization using flow cytometry of peritoneal cavity cells obtained from SCID mice 2 weeks after transplantation with human lymphocytes. $CD4^+$ $CD45RA^+$ human T cells (65×10^6) were injected into SCID mice either in combination with 25×10^6 human B cells (a–d) or separately (e–h). Analysis was performed on cells which did not stain with the anti-mouse antibody, anti-$H\text{-}2K^d$.

SEA and investigated for lymphokine mRNA. In accordance with previous results[17] the $CD45RO^+$ T cells produced IL-2-, IL-4- and IL-5-specific mRNA, whereas $CD45RA^+$ T cells produced mRNA encoding IL-2 only. In contrast, $CD4^+$ $CD45RA^+$ human T cells transferred to SCID mice in the absence of human antigen-presenting cells, such as monocytes or B cells, showed no evidence of activation. The transplanted cells were not blast transformed (Fig. 3e), did not express either IL-2R or HLA-DR molecules (Fig. 3f, g) and retained their expression of CD45RA (Fig. 3h).

The experiments described above show that $CD45RA^+$ T cells can be activated and differentiated towards functional $CD45RO^+$ cells *in vivo* provided human antigen-presenting cells are present. Moreover, human B cells have the ability to interact with and activate the $CD45RA^+$ T cells in the SCID environment. Despite the fact that the co-transplanted B cells were activated, as indicated by blast transformation and expression of IL-2R, they failed to secrete immunoglobulin. Eight weeks after transfer of the human cells, still no human immunoglobulin was detected. However, when the mice which had differentiated $CD45RA^+$ T-helper cells *in vivo* were given a second graft of fresh autologous human B cells, production of immunoglobulins to levels comparable to that found in CD45RO grafted mice occurred (Fig. 5). These results demonstrated that *in vivo* differentiated $CD45RA^+$ $CD4^+$ T cells became functional helper cells for human immunoglobulin production after differentiation in the presence of human B cells.

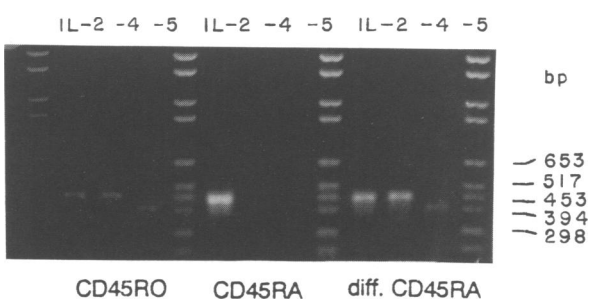

Figure 4. IL-2-, IL-4- and IL-5-specific mRNA production of *in vivo* differentiated $CD4^+$ $CD45RA^+$ T cells. SCID mice were transplanted with 65×10^6 isolated $CD45RA^+$ T-helper cells in combination with 25×10^6 B cells. After 13 days human cells in the peritoneal cavity expressing CD45RO were isolated by sorting using a FACStar Plus cell sorter. The cells were stimulated for 13 hr *in vitro* with 1 ng/ml SEA and 10% fixed Raji cells. Lymphokine mRNA expression was investigated after PCR amplification. For comparison, freshly isolated $CD45RA^+$ and $CD45RO^+$ T cells were studied for lymphokine production as well, after stimulation *in vitro* with SEA and 10% fixed Raji cells.

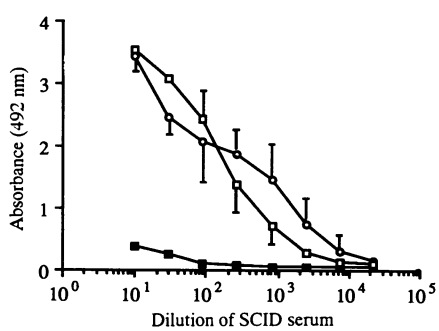

Figure 5. Ability of *in vivo* differentiated $CD4^+$ $CD45RA^+$ T cells to support immunoglobulin production in SCID mice. Human immunoglobulin production in SCID mice 4 weeks after transplantation with 7×10^6 B cells and 17×10^6 $CD4^+$ $CD45RA^+$ fresh T cells (■), 13×10^6 $CD4^+$ $CD45RO^+$ fresh T cells (○), and in mice receiving a second injection of 5×10^6 B cells (□). These mice had 1 week earlier obtained both B cells and $CD4^+$ $CD45RA^+$ T cells as above. The first two groups consisted of three mice each, whereas the third group contained six mice. Each data point represents the mean value ± SEM from the number of mice used in each group.

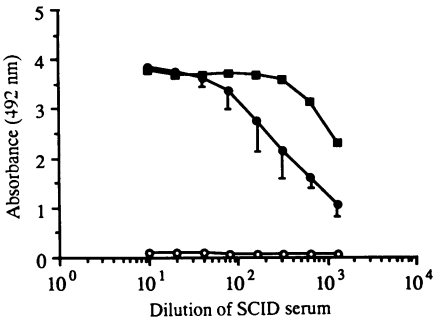

Figure 6. Specific antibody response to TT in SCID mice transplanted with B cells and either with *in vitro* differentiated autologous $CD4^+$ $CD45RA^+$ T cells (○) or freshly isolated $CD4^+$ $CD45RO^+$ T cells (■). Filled symbols represent mice immunized with 10 μg TT and empty symbols mice given PBS. Each mouse was transplanted with 3×10^6 B cells and 15×10^6 *in vitro* differentiated $CD45RA^+$ or fresh $CD45RO^+$ T-helper cells. Serum was analysed for content of TT-specific antibodies 4 weeks after the transplantation of cells by ELISA. Each data point represents the mean value ± SEM from three mice. The figure shows results from one experiment of three performed, all with similar results.

Ability of differentiated $CD4^+$ $CD45RA^+$ T cells to support specific antibody production *in vivo*

Memory T cells can be found within the $CD45RO^+$ subpopulation, whereas the frequency of antigen-specific T cells is much lower in the $CD45RA^+$ compartment.[19] We wanted to investigate whether differentiation of $CD45RA^+$ T cells which enabled these cells to provide help for unspecific immunoglobulin production, would also allow antigen-specific help. Cells were obtained from three donors which had all been vaccinated with TT during the past 1–2 years. Mice were transplanted with *in vitro* differentiated $CD4^+$ $CD45RA^+$ T cells, obtained from these donors, together with autologous human B cells, and immunized with TT or given PBS only. Mice which were transplanted with fresh $CD45RO^+$ or $CD45RA^+$ $CD4^+$ T cells from these donors responded as described above to immunization with TT. In Fig. 6 the results from one experiment of the three performed are shown. Mice transplanted with fresh CD45RO expressing $CD4^+$ T cells developed high titres of specific anti-TT antibodies. In addition, antigen-dependent production of specific antibodies was also demonstrated in mice transplanted with *in vitro* differentiated $CD45RA^+$ $CD4^+$ T cells and autologous B cells, since only mice immunized with TT and not those given PBS developed seroreactivity against TT. The levels of anti-TT antibodies in these responding mice were somewhat lower than in mice transplanted with fresh $CD45RO^+$ T cells (Fig. 6).

DISCUSSION

In this report we demonstrate that production of human antigen-specific as well as unspecific immunoglobulins in repopulated SCID mice depends on the presence of human T-helper cells of the memory phenotype. We also demonstrate that the presence of monocytes is not obligatory for T-cell activation to occur. The requirement of human T cells for immunoglobulin production to occur indicates that murine cells or factors in the murine environment are incapable of stimulating human B cells to immunoglobulin production. This has been reported earlier by us[26] and was recently confirmed by others.[27] To further investigate the role of human T cells in the SCID immune response, two subsets of CD4-positive cells were isolated according to their expression of different isoforms of the CD45R molecule. These subsets of T-helper cells differ in lymphokine production[28] and ability to act as helper cells for immunoglobulin production.[15,16] Similar to what has been found from studies *in vitro*,[15,16] human immunoglobulin was only produced when SCID mice were transplanted with human B cells in combination with T-helper cells expressing CD45RO. In addition, a specific immune response against TT was obtained only when $CD45RO^+$ T cells were co-transplanted with B cells, which suggested that antigen-specific human $CD4^+$ T cells of the memory phenotype can activate human B cells in an antigen-restricted manner in the SCID system. The lack of human immunoglobulin in mice co-transplanted with B cells and $CD45RA^+$ T cells could be explained by the death of B cells after tranplantation in combination with $CD45RA^+$ T cells. However, FACS analysis of human cells from the peritoneal cavity 2 weeks after transplantation showed the presence of activated and blast-transformed human B cells, indicating that the B cells were in a state of anergy possibly caused by inadequate signalling from the $CD45RA^+$ T cells. Results supporting this contention has been obtained from *in vitro* cell culture systems. Culture of $CD4^+$ $CD45RA^+$ T cells together with human B cells in the presence of SEA resulted in a similar state of B-cell anergy as, although the B cells proliferated, addition of $CD45RO^+$ T-helper cells to the cultures more than 24 hr after culture initiation did not restore immunoglobulin production (S. Ingvarsson, personal communication).

Recent studies have shown that human T cells found in SCID mice after injection of adult peripheral blood were all positive for CD45RO and negative for CD45RA.[29] Whether or not this reflected differentiation of the $CD45RA^+$ cells or merely a selection of $CD45RO^+$ T cells was unclear. To investigate if naive human T cells were able to differentiate into CD45RO-expressing cells in the SCID mouse, highly purified CD45RA-positive T-helper cells were co-transplanted with human B cells. The results demonstrated that the $CD45RA^+$ cells could differentiate into $CD45RO^+$ cells. However, not all of the $CD45RA^+$ T cells had done so and only few cells double-positive for CD45RA and CD45RO were found. The fact that human antibodies against murine components exist in serum of SCID-hu-PBL[30] could explain the pattern of the FACS analysis, since it is possible that only a limited portion of human T-helper cells are able to respond to murine antigens presented by human B cells. However, this differentiation required the presence of human antigen-presenting cells, since in the absence of B cells no activation or differentiation of the CD45RA-expressing T cells could be observed. This suggests that murine antigen-presenting cells could not present antigen to human $CD45RA^+$ T cells in a functional manner.

From *in vitro* studies, using FACS analysis and limiting dilution experiments, it was quite clear that $CD4^+$ $CD45RA^+$ human T cells may be activated using human B cells as antigen-presenting cells.[31] The results obtained in the present study also demonstrated that human naive $CD4^+$ T cells, as defined by expression of CD45RA, were able to interact with human B cells and become activated by such accessory cells. These results might be in variance with the results reported by other

groups,[32,33] demonstrating that murine naive T cells cannot be activated on B cells *in vivo*. Although much experimental data favour the view that CD45RA-expressing T cells are naive; (1) memory T cells to recall antigens are found in $CD45RO^+$ and not in $CD45RA^+$ populations;[19] (2) $CD45RA^+$ T cells gain phenotype and function of $CD45RO^+$ T cells after activation;[16–18] and (3) the majority of T cells found in fetuses and newborns are of the CD45RA phenotype,[34] it is not entirely clear that this is a correct subdivision of $CD4^+$ T cells. Recent investigations have casted some doubt on this nomenclature since it has become evident that the phenotypic change is not completely unidirectional.[35] In some experimental systems $CD4^+$ $CD45RO^+$ cells have been shown to revert to expression of CD45RA,[36] and recently Michie *et al.*[36] proposed a model based on *in vivo* studies suggesting that memory T cells of the CD45RO phenotype may loose expression of the RO marker and regain expression of the CD45RA isoform, perhaps as a consequence of lacking continous antigenic stimulation of the T cells.[37] The $CD45RA^+$ subset would thus contain not only naive cells but also some post-memory T cells with, for example, TT specificity. The data presented here indicate that $CD4^+$ $CD45RA^+$ T cells can support B-cell activation and production of immunoglobulin after differentiation *in vitro* or *in vivo*. Also, the presence of antigen-specific T-helper cells in the $CD45RA^+$ subpopulation was indicated, since $CD4^+$ $CD45RA^+$ T cells after differentiation into CD45RO-expressing cells were able to support a specific immune response against TT. This suggested that the differentiation procedure had made $CD45RA^+$ T cells, specific for TT, capable of interacting with B cells in an antigen-restricted and cognate manner. Such a restriction was recently indicated after immunization of repopulated SCID mice with primary antigens coupled to the recall antigen TT, as only mice immunized with the conjugate and not with a mixture of the antigens developed antibodies specific for the primary antigen.[39] It is unlikely that the presence of a low amount of contaminating $CD45RO^+$ T cells, among the $CD45RA^+$ cells, could have caused the specific immune response, since no immunoglobulin production let alone specific immunoglobulin production was observed in mice transplanted with fresh $CD45RA^+$ T cells from the same donors.

Alternatively, differentiated $CD4^+$ $CD45RA^+$ T cells might support immunoglobulin production in the SCID mice as a result of non-cognate interactions between T and B cells, similarly to what has been described from *in vitro* studies using activated T and B cells.[40] However, our data show not only production of unspecific immunoglobulin, which would be the expected result of such cellular interactions, but also production of specific antibodies provided the mice were immunized with antigen. Possibly stimulation via the surface immunoglobulin molecules with antigen might lower the activation threshold of the specific B cells, making it more susceptible for signals provided by activated T cells in a non-cognate manner. Support for this contention was recently reported as it was found that stimulation of B cells with a combination of anti-CD40 antibodies and antibodies directed towards surface IgM was more effective in activating the B cells than either one of the antibodies.[41] Obviously, more experiments are needed to fully clarify whether non-cognate or cognate interactions between T and B cells are responsible for the antigen-specific activation of human B cells observed in SCID-hu-PBL mice.

From previous studies it is clear that the level of total human immunoglobulin in the SCID sera does not depend on immunization, whereas the level of specific antibody does.[11] The specificity of the human immunoglobulin produced in the SCID system in the absence of immunization is largely unknown, but antibody specificities against human autoantigens and viral antigens reflecting the antigenic history of the donor,[13,42] as well as antibodies against murine components, have been demonstrated.[30] The fact that antibodies towards murine components can be elicited suggests that human naive B cells can become activated in SCID mice and that a primary immune response has occurred. It can be concluded from the present data that this immune response depends on human $CD45RO^+$ T-helper cells. Probably these T cells are not specific for the murine antigens to which the antibodies are directed as it seems unlikely that human $CD45RO^+$ population should comprise memory cells with specificities towards murine components. The reason why human B cells with specificities towards murine antigens become activated is presently not known. The specificities of these B cells are, however, not useful for diagnostic or therapeutic purposes. Deliberate primary immunizations of SCID mice transplanted with human mature lymphocytes have hitherto met with limited success.[43,44] In this paper we have demonstrated that human B cells have to interact with human $CD4^+$ $CD45RO^+$ T cells to become activated and produce immunoglobulins. This suggests that by coupling a primary antigen to a recall antigen to which a high frequency of memory T cells could be found, a primary immune response might be elicited. In fact, recent experiments in this direction have shown the validity of this concept as primary antibody responses against a variety of different antigens could be demonstrated in SCID mice immunized with such conjugates.[39]

ACKNOWLEDGMENTS

This work was supported by grants from the Biotechnology programme of the EC commission, NUTEK and the Swedish Cancer Society.

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