

Appearance of Human Plasma Cells Following Differentiation of Human B Cells in NOD/SCID Mouse Spleen

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Relatively little is known for the differentiation and maturation process of human B cells to plasma cells. This is particularly important in reconstitution work involving transfer of autoantibodies. To address this issue, we transplanted human peripheral blood mononuclear cells (PBMC) directly into the spleen of irradiated NOD/SCID mice depleted of natural killer cell activity. Within 6 weeks, naïve B cells differentiated into memory B cells and, importantly, the numbers of human CD138⁺ plasma cells in spleen increased by 100 fold after transplantation. Plasma cell numbers correlated with the detection of human IgM and IgG in serum, indicating that human B cells had differentiated into mature plasma cells in the murine spleen. In addition to CD19⁺ plasma cells, a distinct CD19⁻ plasma cell population was detected, suggesting that downregulation of CD19 associated with maturation of plasma cells occurred. When purified human B cells were transplanted, those findings were not observed. Our results indicate that differentiation and maturation of human B cells and plasma cells can be investigated by transplantation of human PBMC into the spleen of NOD/SCID mice. The model will be useful for studying the differentiation of human B cells and generation of plasma cells.

Keywords: Autoantibodies; B cell ontogeny; Reconstitution; NOD/SCID mice

INTRODUCTION

Plasma cells are terminally differentiated B cells that produce and secrete antibodies. Little is known about the development and differentiation of human B cells into plasma cells due to the low frequency of plasma cells in the peripheral blood. Murine models, especially immunodeficient Prkdc^{scid}/Prkdc^{scid} (SCID) mice engrafted with human cells (SCID-Hu), have been used to investigate the development, differentiation and *in vivo* functions of human lymphocyte subsets (McCune *et al.*, 1988; Mosier *et al.*, 1988). Differentiation of human B cells have been examined by transplanting umbilical cord blood cells into the scid/scid mice (Mosier *et al.*, 1988; Pflumio *et al.*, 1996; Hogan *et al.*, 1997; Novelli *et al.*, 1999; Rossi *et al.*, 2001). However, when peripheral blood mononuclear cells (PBMC) were transplanted intraperitoneally, the majority of the identifiable human cell population were T cells (Mosier *et al.*, 1988). This has been a particular problem in attempts to transfer autoimmune disease using human PBMCs to SCID mice.

Naïve B cells, following generation in the bone marrow, migrate to secondary lymphoid organs before they

differentiate into memory B cells and plasma cells under complex environmental stimuli (Calame, 2001). The spleen is known to be a major site of B cell differentiation. To explore the differentiation process of human B cells, we directly engrafted human PBMC into spleens of NOD/LtSz-scid/scid (NOD/SCID) mice.

MATERIALS AND METHODS

Engraftment of Human PBMC into NOD/SCID Mice

NOD/SCID mice, aged 4–5 weeks, were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and subsequently maintained by the Animal Resource Service of the University of California at Davis. Prior to engraftment, each mouse received 2 Gy of total body irradiation. Each mouse also received an intraperitoneal injection of an optimal dose of 20 µl of rabbit anti-mouse/rat asialo GM1 polyclonal antibody (Cedarlane Laboratories, Hornby, Ont., Canada), which recognizes murine natural killer (NK) cells and depletes NK activity *in vivo* (Kasai *et al.*, 1980; Shpitz *et al.*, 1994).

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A suspension of 2×10^7 healthy control human PBMC or $4\text{--}10 \times 10^6$ B cells purified by anti-human CD19 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) obtained following leukapheresis, in $50 \mu\text{l}$ of phosphate-buffered saline (PBS), was directly injected into the spleen under general anesthesia in groups of at least 30 mice. After the injection, physiological salt solution was frequently injected into the peritoneum to correct for dehydration. Twenty microliters of anti-asialo GM1 antibody was injected i.p. into each mouse every 5 days. To prevent infection, mice were maintained in microisolator cages and provided drinking water supplemented with antibiotics. Three mice were examined weekly. Single cell suspensions of splenocytes were prepared by slicing cells from spleen using the end of slideglass and washed with 0.5% bovine serum albumin (BSA) in PBS (washing buffer). The cells were counted in a hemocytometer and viability of cells determined using trypan blue exclusion.

Flow Cytometric Analysis

PBMC or splenocytes were resuspended in staining buffer (0.5% BSA, 0.05% sodium azide in PBS) and $25 \mu\text{l}$ aliquots dispensed into individual tubes. The cells were first pre-incubated with anti-human FcR blocking reagent (Miltenyi Biotec) and anti-mouse Fc receptor antibody (eBioscience, San Diego, CA, USA). Cells were stained with different combinations of the following antibodies for 15 min at 4°C : FITC conjugated anti-human CD45, PE conjugated anti-human CD138, PE-Cy5 conjugated anti-human CD38, APC conjugated anti-human CD19 or CD27 (eBioscience), and Cy-Chrome conjugated anti-human IgM (BD Biosciences, San Diego, CA, USA). The cells were then washed with washing buffer, resuspended with 0.05% EDTA in washing buffer and analyzed with a FACSCalibur (BD Biosciences) flow cytometer. The acquired data were analyzed with CELLQUEST software (BD Biosciences).

Determination of Human Ig Levels

Serum samples were collected from three mice every week and the concentration of human IgG and IgM determined utilizing an Immuno-Tek human IgM and IgG EIA Kit (ZeptoMetrix Corporation, Buffalo, NY, USA). Known positive and negative control samples were included throughout.

RESULTS

Transfer of Human PBMC into NOD/SCID Mouse Spleen

To examine the development and differentiation of human B cells into plasma cells in mouse spleen, we injected 2×10^7 human PBMC or 4 to 10×10^6 B cells directly

into the spleens of NOD/SCID mice that had been γ -irradiated and depleted of NK cell activity. After the injection, spleens were removed from groups of recipient mice at weekly intervals and examined by flow cytometric analysis. As shown in Fig. 1, the number of viable human mononuclear cells (human CD45⁺ cells, Fig. 1A) from each spleen remained relatively stable for up to 6 weeks after transplantation (Fig. 1C). By week 7, human cells were no longer detected in the spleen. Human B cells and plasma cells, identified as CD45⁺CD19⁺CD138⁻ and CD45⁺CD138⁺ cells respectively (Fig. 1B) (Sanderson *et al.*, 1989), were also enumerated weekly. The number of CD19⁺ B cells in each spleen remained essentially unchanged for the first 2 weeks after transplantation and declined gradually thereafter. In contrast, CD138⁺ plasma cells while barely detectable in the PBMC before transplantation (week 0), increased by more than 100-fold by week 3 and remained readily detectable up to week 5 (Fig. 1C). The frequency of plasma cells

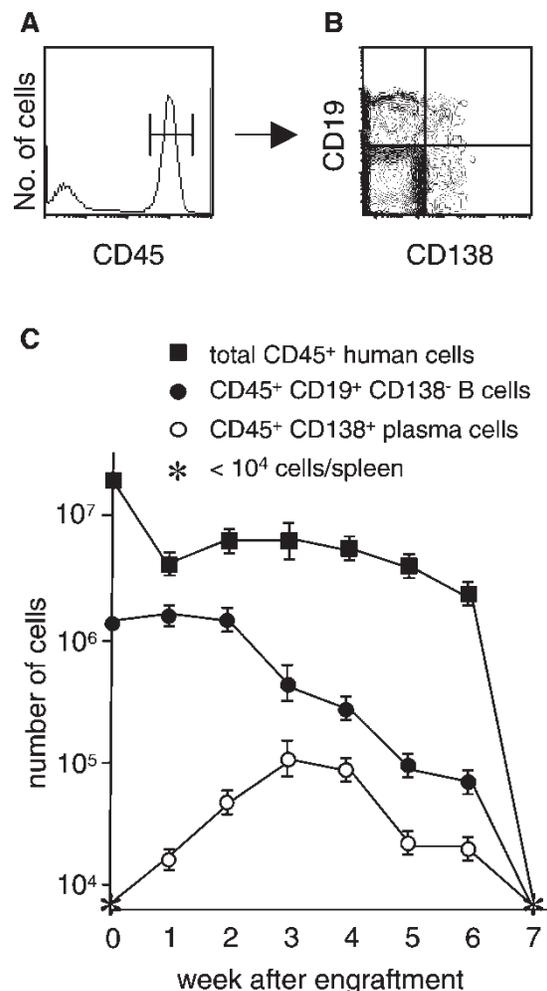


FIGURE 1 Kinetics of human PBMC subsets grafted into NOD/SCID mouse spleen. (A) Human cells are defined as CD45⁺. (B) Human B cells and plasma cells are defined as CD45⁺CD19⁺CD138⁻ and CD45⁺CD138⁺, respectively. (C) The number of total human cells, human B cells and human plasma cells in each spleen at different time points following transplantation.

increased from 0.06% in PBMC before transplantation to 2.1% of the human cells recovered from mouse spleen at week 3. These data indicate that human B cells had differentiated into plasma cells. When purified human B cells were transplanted, no cells were detected even 1 week after transplantation (data not shown).

Phenotypic Analysis of Human B Cells

Expression of CD27 has been used as a marker to distinguish memory B cells from naïve B cells

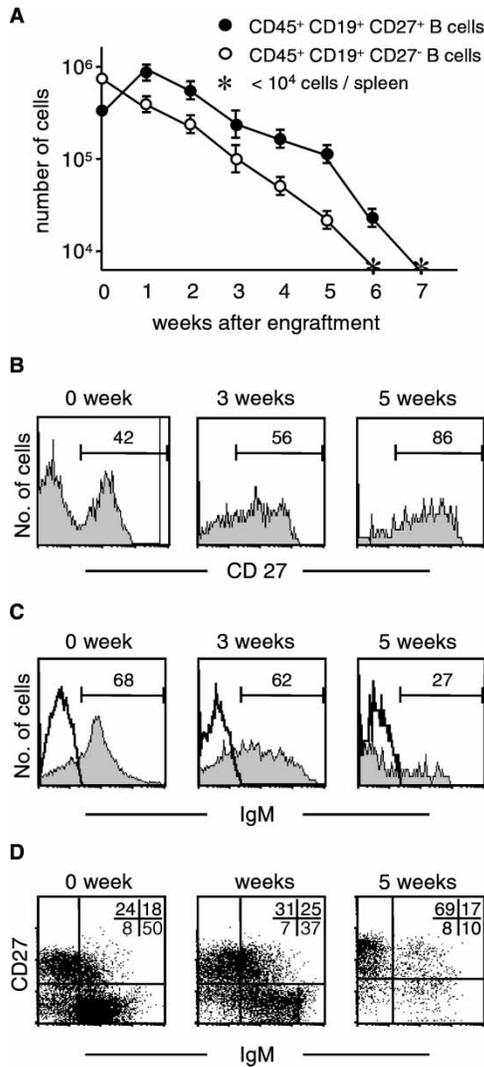


FIGURE 2 Kinetics and phenotype of engrafted human B cells. Human naïve and memory B cells are defined as CD45⁺CD19⁺CD27⁻ and CD45⁺CD19⁺CD27⁺, respectively. (A) The number of human naïve and memory B cells in each spleen at different time points following transplantation. (B) Kinetics of memory B cell frequency; the number in the histogram is the percentage of CD27⁺ cells in the CD45⁺CD19⁺ B cell population. (C) Kinetics of surface IgM⁺ B cell frequency; the number in the histogram is the percentage of surface IgM⁺ cells in the CD45⁺CD19⁺ B cell population. The unfilled graph indicates the histogram of cells with isotype control antibody. (D) Transition of surface IgM⁺ cell frequency in naïve and memory B cells. Cells are gated on the CD45⁺CD19⁺ B cell populations. The numbers in the dot plots are percentage of cells in each quadrant.

(Klein *et al.*, 1998; Tangye *et al.*, 1998). We quantified CD19⁺ B cells that expressed CD27 before transplantation (week 0) and at different time points after transplantation. At week 1 post-transplantation, the number of CD27⁺ memory B cells per spleen increased while that of CD27⁻ naïve B cells decreased when compared to those in the transplanted PBMC (week 0) (Fig. 2A), suggesting that differentiation of naïve B cells into memory B cells occurred during the first week after transplantation. The frequency of CD27⁺ memory B cells in the total CD19⁺ B cell population increased steadily while the number of both memory and naïve B cells declined after week 1 (Fig. 2B), suggesting that memory B cells have a longer life span than naïve B cells in the mouse spleen. Alternatively, this may indicate that some naïve B cells continued to differentiate into memory B cells during this time period.

We also examined the expression of sIgM by B cells. At all time points examined, both IgM⁺ and IgM⁻ memory and naïve B cells were detected. The overall frequency of IgM⁺ B cells declined from 68% at week 0 to 27% at week 5 after transplantation (Fig. 2C). Of note, most of this change is attributed to the decrease of naïve B cells (CD27⁻) that expressed IgM. In PBMC before transplantation, 50% of B cells are CD27⁻IgM⁺; while 5 weeks after transplantation, 69% of B cells are CD27⁺IgM⁻ (Fig. 2D), again suggesting that IgM⁻ memory B cells have a longer life span.

Phenotypic Analysis of Human Plasma Cells

We analyzed the expression of the cell surface markers CD19, CD38 and IgM on CD138⁺ plasma cells recovered from mouse spleen. As shown in Fig. 3A, plasma cells were found in both CD19⁺ and CD19⁻ compartments at all time points examined (Fig. 3A). The majority of plasma cells expressed CD38, however, 13% of plasma cells in the CD19 negative population were CD38⁻ at week 5 (Fig. 3B). Changes in the frequency of IgM⁺ plasma cells were within the CD19⁺ and CD19⁻ populations. Between week 3 to week 5, it declined from 39 to 10% in CD19⁺ population, but remained unchanged in the CD19⁻ population (Fig. 3C).

Detection of Human Immunoglobulin in Mouse Serum

As shown in Fig. 4, the concentration of both human IgG and IgM in mouse serum increased significantly after transplantation. The peak concentration correlated with the peak of plasma cells, indicating that the human plasma cells generated in the mouse spleen are functional. The concentration of IgM started to decline at week 5 and became undetectable at week 7, while that of IgG remained little changed until week 6. By week 7 a significant level of IgG can still be detected, suggesting that the IgG-producing plasma cells have a longer life-span than IgM-producing cells.

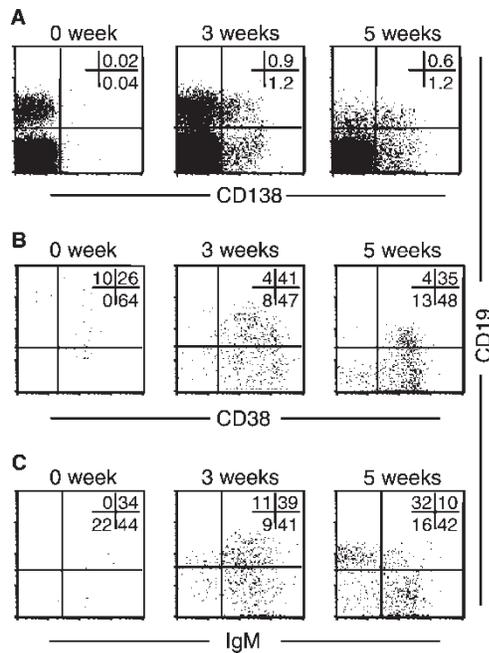


FIGURE 3 Phenotype of human plasma cells recovered from mouse spleen. Human plasma cells are defined as CD45⁺CD138⁺. (A) Frequency of CD19⁺ and CD19⁻ plasma cells; the numbers in the dot plots are the percentage of CD19⁺ and CD19⁻ plasma cells in the recovered CD45⁺ human cells. (B) Expression of CD38 and CD19 by CD138⁺ plasma cells. Cells are gated on CD45⁺CD138⁺ plasma cell population; the numbers in the dot plots are percentage of cells in each quadrant. (C) Expression of cell surface IgM and CD19 on CD138⁺ plasma cells; the numbers in the dot plots are the percentage of cells in each quadrant.

DISCUSSION

We studied herein the differentiation of human B cells using a SCID-Hu mouse model. Human PBMC were transplanted into spleens of NOD/SCID mice following irradiation and depletion of NK cell activity. Human cells were found to survive in the mouse spleen for up to 6 weeks. The number of plasma cells greatly increased, with high circulating levels of both human IgG and IgM. Our results indicate that human B cells can differentiate into mature plasma cells in the murine spleen. To our knowledge this is the first observation of newly differentiated plasma cells in SCID-Hu mice using the direct intrasplenic injection method.

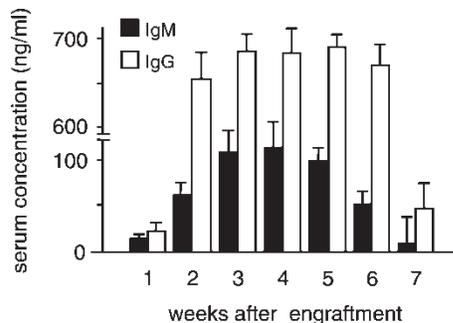


FIGURE 4 Serum concentration of human immunoglobulins in mice receiving human PBMC.

Spleen is known to be a major site for B cell differentiation and plasma cell maturation, a process that has not been fully characterized. A recent study found that when human PBMC were engrafted into the spleen of irradiated NOD/SCID mice, B cells became activated and differentiated into plasmacytoid cells (Depraetere *et al.*, 2001). However, the engrafted B cells were followed for a relatively short time period, because almost all mice died of graft-vs-host disease (GVHD) within 3–4 weeks after transplantation. For this reason, terminally differentiated plasma cells have not been characterized in detail (Depraetere *et al.*, 2001). Successful transplantation of human cells into mice requires both irradiation and depletion of NK cell activity in recipient mice (Shultz *et al.*, 1995). Irradiation of the mice may augment the capacity of murine spleen for the differentiation of transplanted human cells. However, high dose irradiation may also cause severe GVHD, resulting in early death of mice that limits the time span for following transplanted human cells. In this study, we irradiated the mice with a lower dose than that previously reported (2 Gy vs. 3 Gy) (Depraetere *et al.*, 2001). Our procedure resulted in a significantly improved animal survival of 9 weeks after transplantation (data not shown), without compromising the capability of irradiated mouse spleen to support the differentiation of engrafted B cells.

Antigen stimulation followed by T cell help initiates naïve B cells differentiation into memory B cells and plasma cells. For B cell differentiation to occur, activated T cell help including CD40/CD40L interactions are required. This requirement has also been shown in SCID-Hu mice. In addition, interaction of tumor-necrosis factor (TNF) family ligands and their receptors play an important role in the splenic environment for B cell differentiation (Fu and Chaplin, 1999). Recently, a member of TNF family, BLyS (B lymphocyte stimulation, also named BAFF or TALL-1) has been found to express on T cells, dendritic cells, monocytes and macrophages. We postulate that injection of human PBMC into the spleen of NOD/SCID mouse leads to dysregulation of BLyS and/or its receptor, resulting in the differentiation of B cells and generation of plasma cells. Since transplantation of purified human B cells into the mouse spleen did not lead to similar results, we believe that other non-B cell factors are necessary in the differentiation and maturation of B cells.

Naïve B cells are generated in the bone marrow. From there they migrate into spleen, some stay at the marginal zone while others localize to the follicle. In the marginal zone, naïve B cells are activated by antigen. The activated B cells form foci in the extrafollicular region and differentiate into plasma cells that produce low-affinity IgM as a primary antibody response (Calame, 2001). The IgM-producing plasma cells die by apoptosis after a short life span (Smith *et al.*, 1996; Dal Porto *et al.*, 1998). On the other hand, if the follicle occurs within the germinal center, they become memory B cells and switch isotype into high affinity IgG-producing plasma cells facilitated by CD40/CD40L interactions (Arpin *et al.*, 1995). These cells home to the bone marrow and their life span is long

(Slifka *et al.*, 1998). For germinal center B cells, the expression of CD38 inhibits apoptosis (Zupo *et al.*, 1994), suggesting that the CD38⁺ population is long-lived. It has been reported that plasma cells which differentiate in the spleen germinal center were CD19⁺CD138⁺ (Angelin-Duclos *et al.*, 2000), a phenotype called early plasma cells (Jego *et al.*, 1999).

Our finding is in agreement with the thesis that naïve B cells are activated and differentiate into memory B cells within the spleen. During the first week after engraftment, the number of CD27⁻ naïve B cells declined while that of CD27⁺ memory B cells increased (Fig. 2A). The greatly increased number of plasma cells after transplantation as well as the concurrent detection of high levels of human IgG and IgM in the mouse serum indicates that human B cells have differentiated into functional plasma cells. At week 3 after transplantation, most plasma cells express IgM (Fig. 3C), suggesting these are the short-life IgM-producing plasma cells which differentiate in the extrafollicular region (Smith *et al.*, 1996; Dal Porto *et al.*, 1998). By week 5, most of the plasma cells were CD38⁺IgM⁻, suggesting that they are IgG-producing long-life plasma cells (Arpin *et al.*, 1995; Slifka *et al.*, 1998).

In this work we used CD138 as the marker to define plasma cells because it has been reported to be a more specific marker for plasma cells than CD38, as the latter is also expressed by activated B cells or plasmablasts (Sanderson *et al.*, 1989; Wijdenes *et al.*, 1996; Jego *et al.*, 1999; Deaglio *et al.*, 2001). With the use of this marker, we found that in addition to CD19⁺CD138⁺ plasma cells as previously described (Jego *et al.*, 1999), CD19⁻CD138⁺ plasma cells were also detected (Fig. 3A). It is known that when CD19⁺ plasma cells migrate from the peripheral blood to bone marrow, CD19 expression is down-regulated during the process of maturation (Medina *et al.*, 2002). Our observation suggests that the downregulation of CD19 expression may also occur in the spleen. However, the origin and function of the CD19⁻ plasma cells we identified need to be further characterized.

Thus we have established a SCID-Hu mouse model that appears to be physiologically capable to allow the differentiation process of human B cells into mature and functional plasma cells. A traceable population of CD138⁺ plasma cells can be followed for up to 6 weeks after transplantation of human PBMC into the mouse spleen. Differentiation of B cells and generation of plasma cells plays important roles in the regulation of normal and abnormal B cell immunity. This system will be useful for studying the generation of protective antibodies against infection, as well as the generation of antibodies involved in autoimmune diseases.

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