HLA-specific memory B cells : the missing link?

Karahan, G.E.

Chapter 7

Bone marrow plasma cells and memory B cells display a divergent immunoglobulin isotype distribution

Gonca E. Karahan[1], Juliette Krop[1], Helene Roelofs[1],
Cees van Kooten[2], Frans H. J. Claas[1], Sebastiaan Heidt[1]

[1]Department of Immunohematology and Blood Transfusion,
Leiden University Medical Center, Leiden, the Netherlands
[2]Department of Nephrology,
Leiden University Medical Center, Leiden, the Netherlands

ABSTRACT

Bone marrow transplantation is a curative treatment for various hematological disorders. In some instances, the transfer of humoral immunity from donor to recipient is observed, either in the form of protective immunity, or as detrimental immune responses such as auto- or allo-immunity. This could be due to the transfer of plasma cells, memory B cells, or both. By using highly sensitive ELISPOT methods, we characterized the bone marrow-residing plasma cells, as well as memory B cells present in the bone marrow and in peripheral blood. By using tetanus toxoid as a model antigen, we detected both IgG-producing plasma cells and memory B cells specific for the same antigen in bone marrow samples. IgG subclass distribution was similar for memory B cells in bone marrow and peripheral blood. However, immunoglobulin isotype distributions were distinctively different between plasma cells and B cells derived from the same bone marrow.

These data indicate that both cell types can contribute to humoral immune reconstitution after bone marrow transplantation with partially overlapping, but also different properties.

INTRODUCTION

Allogeneic and autologous hematopoietic stem cell transplantation (HSCT) are curative therapies for a wide range of disorders and malignancies affecting the immune system (1). Conditioning regimens administered before HSCT to eradicate malignant host cells lead to pancytopenia in almost all HSCT recipients. Therefore, red blood cell and platelet transfusions may be administered to patients in the pre- and post-transplantation period (2). Moreover, T and B lymphocyte reconstitution may be prolonged compared to the recovery of the innate immune cells leading to immuno-incompetence in these patients (3).

In contrast to the dominant repopulation of effector memory T cells expanding from mature T cells present in the donor bone marrow, immune restoration of B cells after HSCT has been reported to resemble normal B cell ontogeny (3). Indeed, the majority of repopulating B cells are transitional and naïve B cells with a minority of cells gradually differentiating into memory B cells upon immune challenge (4). A limited B cell repertoire resulting from the lack of somatically hyper-mutated B cells as well as the loss of protective humoral immunity acquired through childhood vaccinations render stem cell transplant recipients at risk for developing severe infections (5). Functional restoration of the B cell compartment may take up to 2 years (6) and during this recovery process most patients require immunoglobulin (Ig) replacement therapies and vaccinations (7, 8). However, B cell memory may be transferred by bone marrow transplantation (BMT), as anecdotally reported for a patient with leucocyte adhesion deficiency in whom transfer of donor memory B cells upon pre-BMT vaccination was found (9). With regard to antibodies, early studies have indicated that antigen-specific protective humoral immunity could be adoptively transferred from immune donors to recipients by allogeneic BMT (10-12). In addition to the transfer of humoral immunity to microbial antigens, there is also evidence that autoimmunity and allergy can be transferred via donor marrow cells (13, 14). Recently, several case reports have suggested that donor-derived HLA antibody production could be transferred to recipients of HSCT from HLA-immunized donors (15-17). From these reports, it is not clear whether plasma cells, memory B cells, or both, are responsible for the transfer of this pre-existing humoral immunity from donor to recipient.

In the present study, we aimed to characterize the bone marrow (BM) residing plasma cell and memory B cell compartments. Our data indicate that both memory B cells and plasma cells specific for the same antigen are present in bone marrow aspirates. While bone marrow residing plasma cells predominantly produce IgA and IgG antibodies, bone marrow B cells produce mainly IgM and IgG antibodies upon polyclonal activation resembling the isotype distribution of peripheral blood B cells.

Upon BMT, bone marrow residing plasma cells and memory B cells are potentially complementary in their contribution to humoral immune reconstitution. While overlapping specificities exist, the immunoglobulin isotype distributions of these cell types are distinctively different.

MATERIALS AND METHODS

Bone marrow and peripheral blood samples

Human bone marrow was drawn from punctures of the posterior iliac crests of ten individuals (female/male: 4/6). Of these samples, four were taken from individuals undergoing autologous BMT due to vascular disorders (median age: 79 y, range: 73-83 y), one was derived from a surplus clinical sample of a BMT donor (age: 28 y) and five were commercially obtained (Lonza, Maryland, USA) (median age: 28 y, range: 25-37 y). For 3 out of these 5 commercially obtained bone marrow samples we also had access to parallel blood samples. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Hypaque (Pharmacy LUMC, Leiden, the Netherlands) density gradient centrifugation. All bone marrow samples (and paired peripheral blood samples) were used fresh. Surplus clinical samples (n=5) were obtained from individuals with informed consent under guidelines issued by the Medical Ethics Committee of the Leiden University Medical Center (Leiden, the Netherlands).

Isolation of plasma cells from bone marrow aspirates

Bone marrow aspirates were either lysed with ammonium-chloride-potassium (ACK) lysing buffer (Life Technologies-Gibco, USA) for 5 min and centrifuged (n=4) or mononuclear cells were isolated by Ficoll Hypaque (pharmacy LUMC, Leiden, the Netherlands) density gradient centrifugation (n=6). Lysed bone marrow aspirates or isolated mononuclear cells of the bone marrow samples were suspended in Iscove's modified Dulbecco's medium (IMDM) (Gibco Invitrogen, Paisley, UK) containing 10% fetal bovine serum (FBS) (Gibco Invitrogen), supplemented with 50 µM 2-mercaptoethanol (Sigma-Aldrich, Zwijndrecht, the Netherlands), 2 mM L-glutamine (Gibco Invitrogen) and 100 U/ml penicillin with 100 µg/ml streptomycin (Gibco Invitrogen) and filtered through a 70 µm cell strainer (BD Falcon, Belgium). Hereafter, plasma cells were enriched using the EasySep CD138$^+$ plasma cell positive selection kit (Stem Cell Technologies, Grenoble, France), according to manufacturer's instructions. Positive selection of plasma cells enabled us to analyse the plasma cell-depleted B cell containing cell fraction from the same bone marrow sample.

Cell cultures

Polyclonal activation of bone marrow B cells and peripheral blood B cells was carried out in 10% FBS/IMDM with an activation cocktail consisting of 2.5 µg/ml Toll-like receptor (TLR) 7/8 agonist (Resiquimod-R848, Sigma) and 1000 IU/ml IL-2 (Proleukin, Novartis, Amsterdam, the Netherlands). The plasma cell-depleted cell fraction and PBMCs were cultured for 6 days by seeding 2x10^6 cells/well in 24-well flat-bottomed plates (Corning Inc., Corning, NY, USA) at 37^{0}C in a 5% CO_2 humidified incubator. Supernatants from the activated bone marrow and peripheral blood B cells at day 6 of culture were collected and stored at-20^{0}C for further use.

The sample workflow is schematically represented in Figure 1.

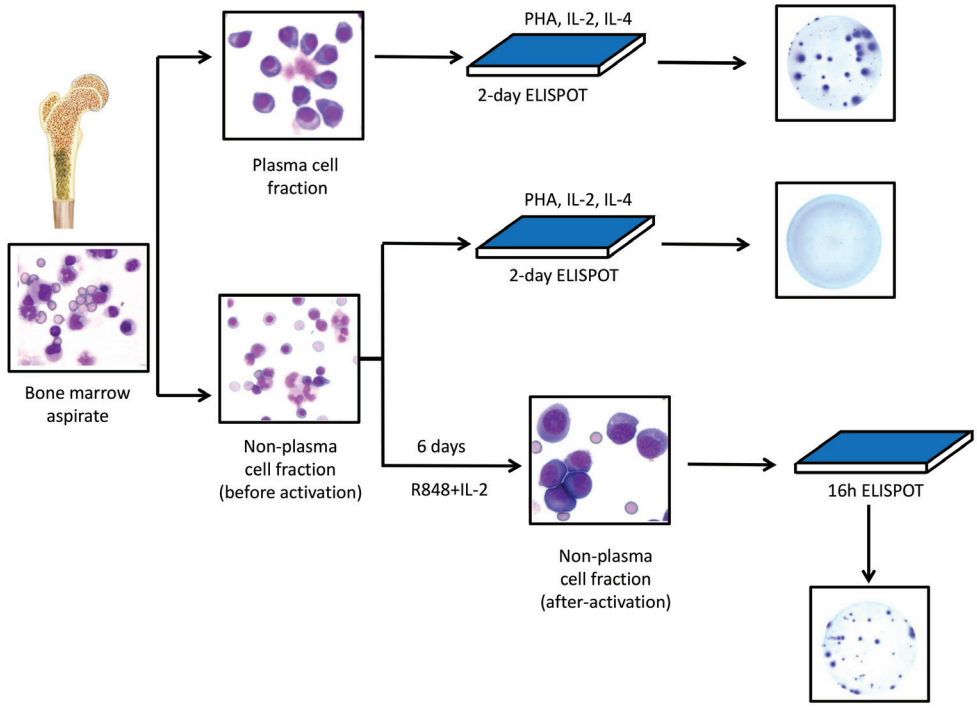

Figure 1. Workflow of processing bone marrow aspirates. *Plasma cells were enriched by CD138 positive selection upon lysing or mononuclear cell isolation of bone marrow aspirates. Plasma cell morphology was confirmed by the large cell size and eccentric localization of the nucleus in cytospin preparations. Positively selected plasma cells, as well as depleted non-plasma cell populations were incubated in ELISPOT plates for 2 days after which spontaneous antibody secreting cell (ASC) counts were assessed (n=10). Additionally, the depleted non-plasma cell fraction was polyclonally activated for 6 days and transferred to ELISPOT plates for ASC assessment (n=7).*

Morphologic assessment of bone marrow cells

Unseparated bone marrow cells, enriched plasma cells, and non-plasma cells (before and after polyclonal activation) were centrifuged onto slides (Thermo-Scientific, Braunschweig, Germany) and fixed with methanol (Merck, Darmstadt, Germany) at 4^0C for 3 min. Following fixation, slides were stained for 2 min with May-Grunwald (Merck) and 20 min with Giemsa (Merck), washed with tap water and air-dried. Visualization was performed using an Axioskop 40 microscope (Zeiss, Oberkochen, Germany).

Flow cytometry

Flow cytometry was performed according to standard protocols using the following antibodies (clone): IgD (IA6-2), CD3 (UCHT1), CD19 (J3119), CD27 (1A4CD27) and CD45 (J33) (all from Beckman Coulter, Woerden, the Netherlands), CD38 (HIT2) (eBioscience, San Diego, CA) and 7-AAD cell viability solution (BD Biosciences, Breda, the Netherlands).

B cell ELISPOT assays

Upon polyclonal activation, the frequencies of tetanus toxoid (TT), IgG subclass, as well as total IgM, IgG, and IgA antibody-secreting cells (ASC) were quantified by enzyme-linked immunosorbent spot (ELISPOT) assays. ELISPOT plates (Millipore, Billerica, MA, USA) were coated with 5 Lf/ml TT antigen (Dutch Vaccine Institute, Bilthoven, the Netherlands) to detect TT-specific ASC. For total immunoglobulin isotype analysis goat anti-human IgM (1.8 μg/ml), IgG (5 μg/ml) or IgA (2.5 μg/ml) (Jackson-Immunoresearch Laboratories, Inc., Baltimore, PA, USA) were used for coating. All coating antibodies were diluted in phosphate-buffered saline (PBS) and incubated overnight. Plates were blocked with 5% FBS/IMDM for at least 1h at 37^0C after which cells of interest were plated at a concentration of $2.5x10^5$ cells/well for TT-specific ELISPOT and 500 cells/well for total IgM, IgG, IgA ASC detection. After incubation for 16h at 37^0C in a 5% CO2 humidified incubator, either biotinylated (Novex Life Technologies, USA) or horse radish peroxidase (HRP)-conjugated (Southern Biotech, Birmingham, AL, USA) goat α-IgM, α-IgG or α-IgA antibodies were added to the appropriate wells for 2h at RT. Following washing, plates were incubated with streptavidin-conjugated alkaline phosphatase (Sigma-Aldrich) if biotinylated detection antibodies were used. Spots were visualized by addition of 5-Bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT), or 3, 3', 5, 5' tetramethylbenzidine (TMB) substrate (Mabtech, Nacka Strand, Sweden), where appropriate. The reaction was stopped by the addition of cold tap water and, after drying, the plates were analysed by an automated ELISPOT reader (Bio-Sys GmbH, Karben, Germany).

Plasma cell ELISPOT assays

To quantify the spontaneous TT-specific IgG and total IgM, IgG, IgA producing plasma cells, ELISPOT plates were coated as described above. Cells were plated at a concentration of $2.5x10^5$ cells/well for TT antigen-specific ELISPOT and 500 cells/well for the detection of immunoglobulin isotypes. Cells were incubated for 48h in the presence of 10% FBS/IMDM supplemented with ITS (5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite (Sigma-Aldrich), 200 IU/ml IL-2 (Proleukin,Novartis, Arnhem, the Netherlands), 50 IU/ml IL-4 (Gibco) and 2 μg/ml phytohaemagglutinin (PHA) (Remel, Dartford Kent, UK), as described elsewhere (18). Detection and visualization steps were performed as mentioned above.

IgG subclass ELISA

Supernatants from polyclonally activated bone marrow and peripheral blood B cells were tested for IgG1, IgG2, IgG3 and IgG4 levels by enzyme-linked immunoassay (ELISA) (Novex, Life Technologies, USA) following the manufacturer's description. The lower level of detection for IgG subclasses was: IgG1: 0.43 μg/ml; IgG2: 0.17 μg/ml; IgG3: 0.042 μg/ml; IgG4: 0.024 μg/ml.

Statistics

Spot forming unit (SFU) counts are expressed as the median of results from different experiments. The non-parametric Wilcoxon matched pairs signed rank test was used for all comparisons. Statistical level of significance was defined as $p<0.05$.

RESULTS

Bone marrow contains both plasma cells and memory B cells

Bone marrow, in addition to being a primary lymphoid organ, provides a microenvironment where billions of lymphocytes recirculate every day (19). In order to gain insight into antibody secreting cell composition of the bone marrow aspirates, we first enriched these samples for plasma cells. Upon enrichment, the median percentage of plasma cells ($CD27^+CD38^{high}$ in $CD19^+$ lymphocytes) in bone marrow aspirates increased from 6.1% to 87.2% (Figure 2A). Cells in these enriched fractions exhibited typical plasma cell morphology with a relatively large cell size and eccentric localization of the nucleus as demonstrated by cytospins (Figure 1). Flow cytometric analysis on non-plasma cell fractions revealed that the bone marrow (n=5) contained both $CD3^+$ T cells (median: 64.2%, range: 55.1-80.2%) and $CD19^+$ B cells (median: 19.7%, range: 6.3-30.2%). $CD19^+$ B cells in non-plasma cell fractions were composed of IgD^+CD27^- naïve B cells (median: 42%, range: 29-54%) and IgD^-CD27^+ memory B cells (median: 13%, range: 10-25%) in addition to IgD^+CD27^+ unswitched memory B cells (median: 7%, range: 4-12%) and IgD^-CD27^- exhausted memory B cells (median: 37%, range: 21-55%) (Figure 2B).

We next investigated whether we could activate these bone marrow B cells *in vitro*. To this aim, we polyclonally activated the B cells from the non-plasma cell fractions (devoid of plasma cells) for 6 days and assessed their phenotype and morphology. The median percentage of $CD19^+CD27^+CD38^{high}$ plasmablasts increased from 0.2% before activation to 38.2% at day 6 of polyclonal activation (Figure 2C). In addition, the B cells gained a plasma cell-like morphology as shown in cytospins (Figure 1).

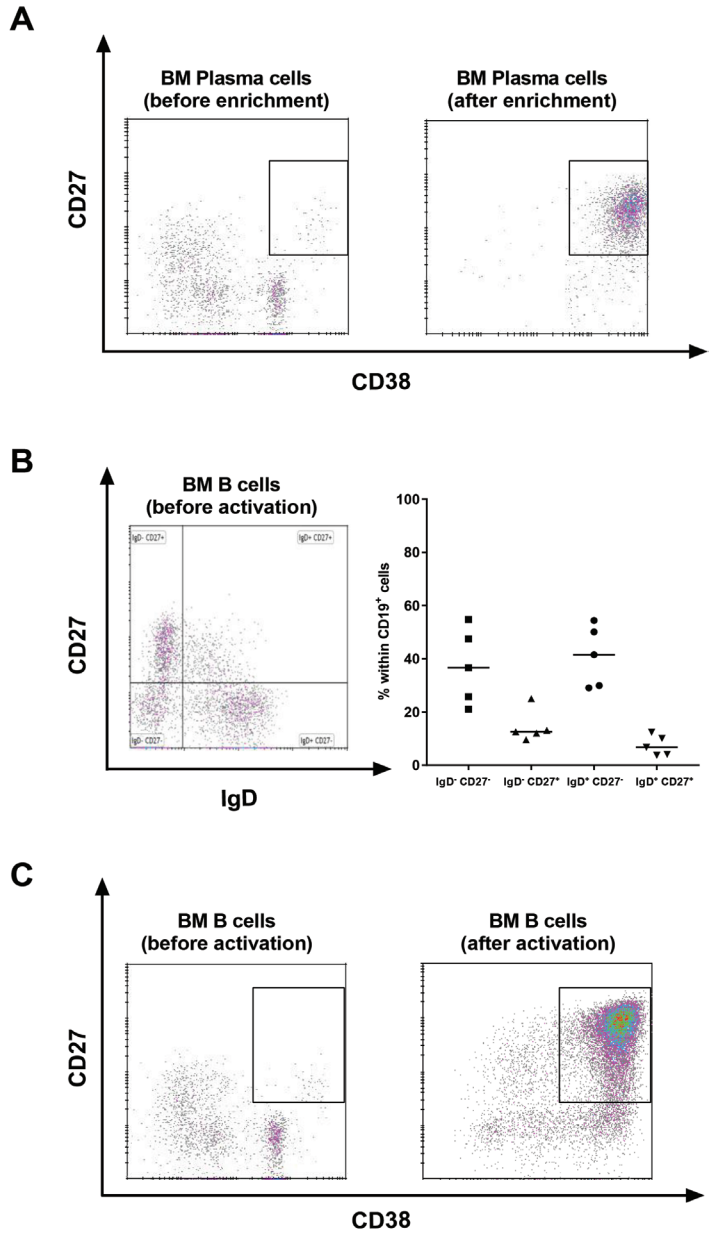

Figure 2. Plasma and B cell composition of bone marrow aspirates. *(A) Enrichment of $CD27^+$ $CD38^{high}$ plasma cells within $CD19^+$ lymphocytes from bone marrow samples. (B) The composition of $CD19^+$ B cells in the nonplasma cell fractions (BM B cells) are as follows: IgD^-CD27^- (median 37%, range: 21-55%); IgD^-CD27^+ (median 13%, range: 10-25%); IgD^+CD27^- (median 42%, range: 29-54%); IgD^+CD27^+ (median 7%, range: 4-12%). (C) Polyclonal activation of B cells in non-plasma cell fraction of bone marrow samples resulted in an increment in the percentage of $CD27^+CD38^{high}$ cells within $CD19^+$ lymphocytes. Horizontal lines represent median values. BM: bone marrow.*

Bone marrow harbours both TT-specific plasma cells and memory B cells

In order to gain insight into the presence of antigen-specific plasma cells and memory B cells in the bone marrow, we directly assessed plasma cells *ex vivo* as well as polyclonally activated bone marrow B cells in TT-specific ELISPOT assays (n=3, median age: 28 y, range: 25-35 y). We found TT-specific bone marrow plasma cells in 2 of the 3 individuals tested at a frequency of 106 and 382 TT-specific cells per 10^5 IgG producing cells (Figure 3A). Furthermore, we found TT-specific bone marrow memory B cell frequencies varying from 2 to 85 per 10^5 IgG producing cells (Figure 3B). No spot formation was found in non-stimulated cells from the same non-plasma cell fractions (data not shown). Polyclonally activated paired peripheral blood B cells showed TT-specific memory B cell frequencies at a range of 10 to 133 per 10^5 IgG producing cells. We found no differences in the bone marrow and peripheral blood TT-specific memory B cell frequencies (p=NS) (Figure 3B).

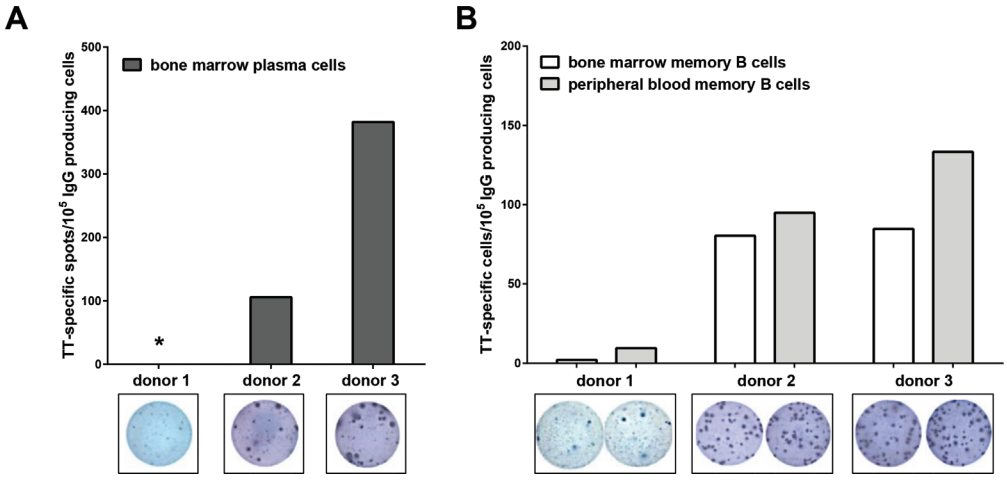

Figure 3. Frequencies of TT-specific memory and plasma cells in paired bone marrow and peripheral blood. *(A) TT-specific plasma cell frequencies in bone marrow samples. (B) TT-specific memory B cell frequencies in bone marrow and peripheral blood samples. Representative pictures are shown from a total of 3 experiments performed with different samples. TT: tetanus-toxoid.*

Bone marrow memory B cells and peripheral blood memory B cells display similar IgG subclass distribution

We next analyzed the B cell culture supernatants from paired bone marrow and peripheral blood samples for IgG subclass distribution. As shown in Figure 4, IgG1 was most dominant in the supernatants of both bone marrow memory B cells (5.7 µg/ml) and peripheral blood memory B cells (16 µg/ml), followed by IgG3 (0.7 µg/ml vs 1.1 µg/ml), IgG2 (0.4 µg/ml vs 0.8

μg/ml) and IgG4 (0.2 vs 0.3 μg/ml). No significant differences were found for any of the IgG subclass antibody concentrations between bone marrow and peripheral blood memory B cells (p=NS).

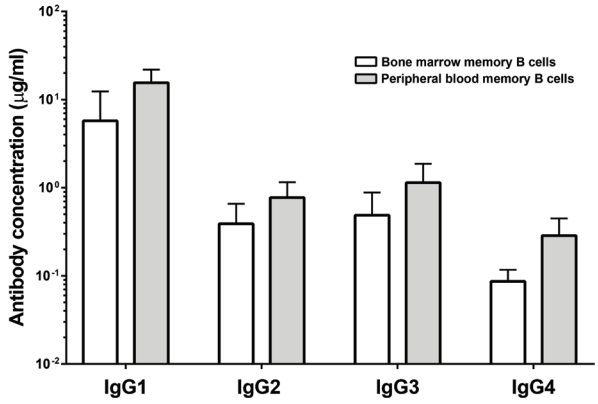

Figure 4. Distribution of IgG subclass antibody production in paired bone marrow and peripheral blood samples. *Supernatants from polyclonally activated bone marrow B cells and peripheral blood B cells showed same IgG subclass distribution with the following order: IgG1>IgG3>IgG2>IgG4. Results are expressed as the mean±SD of experiments performed with 3 different samples.*

Bone marrow plasma cell derived immunoglobulins display a different isotype distribution compared to bone marrow and peripheral blood B cells

In order to determine the isotypes of antibodies produced by plasma cells, we directly analyzed enriched bone marrow plasma cell antibody production *ex vivo*. Plasma cells spontaneously produced IgM, IgG and IgA when incubated for 2 days in ELISPOT plates (Figure 5A-left panel). Enriched bone marrow-derived plasma cells predominantly produced IgA (median SFU/500 cells: 23, range: 7-47) and IgG (median SFU/500 cells: 19, range: 11-51), and to a lesser extent IgM (median SFU/500 cells: 5, range: 1-22) (Figure 5B, n=8). Bone marrow B cells did not produce any IgM, IgG or IgA spots prior to activation (Figure 5A-middle panel), whereas upon polyclonal activation, these cells produced predominantly IgG (median SFU/500 cells: 82; range: 14-98) and IgM (median SFU/500 cells: 43, range: 8-82), and to a lesser extent IgA spots (median SFU/500 cells: 9; range: 0-24) (Figure 5A-right panel, 5C, n=7).

For the three paired bone marrow and PBMC samples we also had the opportunity to analyze bone marrow and peripheral blood B cell isotype distribution in a paired fashion. When analyzed separately we observed that the polyclonally activated bone marrow B cells (Figure 5D) and peripheral blood B cells (Figure 5E) from the same individuals showed predominance of IgM (BM: 38% vs PBMC: 30%) and IgG antibody producing cells (BM: 52% vs PBMC: 57%) and to a lesser extent IgA (BM: 10% vs PBMC: 13%).

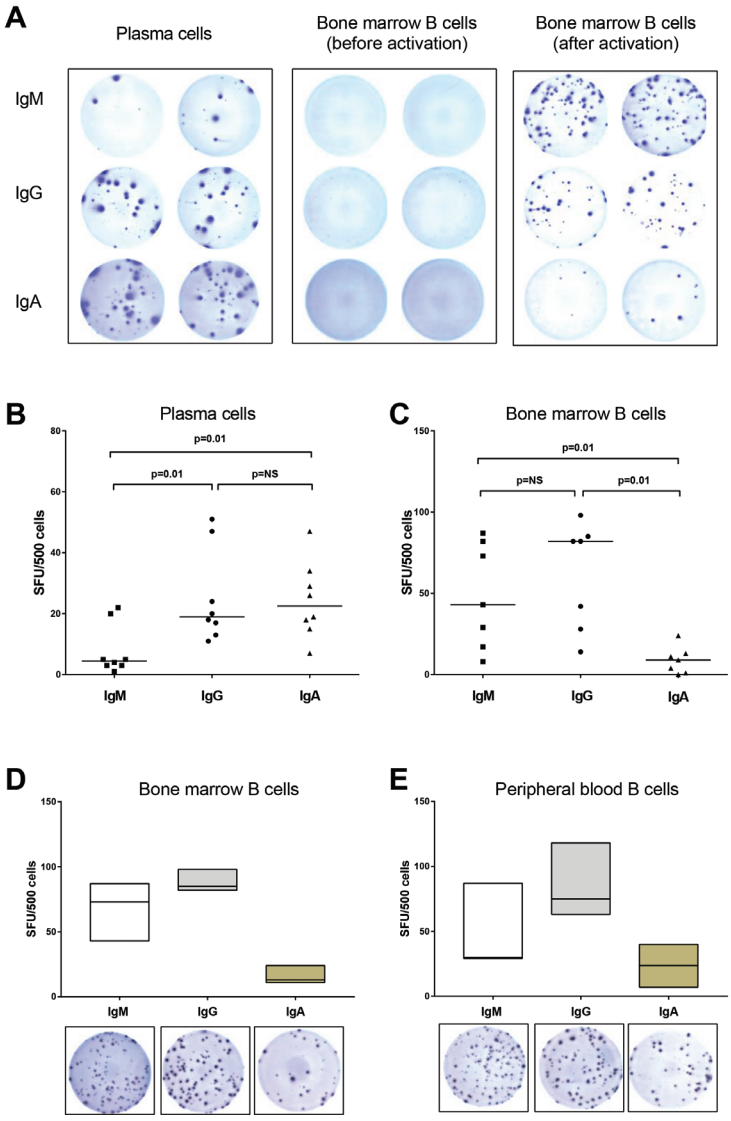

Figure 5. Characterization of the enriched plasma cell fractions, bone marrow and peripheral blood B cells for immunoglobulin production. *(A) Plasma cells were positively selected from bone marrow aspirates and immediately seeded onto ELISPOT plates for 2-day incubation. IgG and IgA producing cells constituted the majority of the antibody producing cells in the plasma cell fractions (left panel) whereas no spot formation was observed in bone marrow B cell fractions before activation (middle panel). When B cells were transferred to ELISPOT plates following 6-day polyclonal activation, comparable number of IgM and IgG spots were observed whereas IgA spot formation was scarce (right panel). (B) Significantly lower number of IgM spots (median SFU: 5) were found compared to IgG (median SFU: 19) (p=0.01) and IgA spots (median SFU: 23) (p=0.01) in enriched plasma cell fractions (n=8). (C) The number of IgM (median SFU: 43) and IgG (median SFU: 82) spots in polyclonally activated non-plasma cell fractions were significantly higher than the number of IgA spots (median SFU: 9) (p=0.01) (n=7). (D) Polyclonally activated B cells from paired bone marrow and (E) peripheral blood samples mainly produced IgM and IgG, and lower numbers of IgA spots. SFU: spot forming unit. Horizontal lines represent median values.*

DISCUSSION

Successful humoral memory depends on both pre-existing protective antibodies produced by plasma cells, as well as pathogen-experienced memory B cells in case circulating antibody levels are not sufficient (20). We here show that both levels of humoral immunity are present in bone marrow aspirates and that, while similarities exist, these compartments are not mirror images. Transfer of antigen-specific memory B cells via BMT may help immuno-compromised recipients to mount an effective, protective humoral immune response early after BMT and may also contribute to the pool of repopulating cells throughout the immune reconstitution period. Several studies have shown that the immune status of a bone marrow donor may affect the recipient's immune response to certain antigenic challenges after transplantation. In accordance, enhanced recipient antibody responses to infectious agents can be achieved if donors are vaccinated before the bone marrow is harvested (21, 22). Indeed, Lausen *et al*., described the transfer of donor Haemophilus influenza type b capsular polysaccharide-specific memory B cells that could be re-activated after antigen recall in a BMT recipient (9).

We tested the presence of TT-specific B cell memory in bone marrow from donors of whom we did not know the immunization history. TT was used as a model antigen because it is universally included in childhood vaccination schedules and evokes T cell-dependent B cell responses giving rise to high affinity antibodies and immunological memory. The fact that we found TT-specific memory B cells in all bone marrow samples analyzed suggests that indeed passive transfer of protective B cell memory may occur upon BMT. Whether these bone marrow derived B cells survive after infusion into the patient and become circulating ASC upon re-encounter with their cognate antigen remains to be studied.

We have recently shown that the current activation protocol did not induce any class switching in the naïve B cell population during the 6-day culture period which was evident by IgG spot production exclusively in memory B cell fractions. In the current study while plasma cells from the bone marrow produced predominantly IgG and IgA, the proportion of the IgA producing cells was remarkably low in B cells from both the bone marrow and the periphery. This relatively lower number of IgA memory B cells in bone marrow compared to IgG might be due to the differences in the homing and trafficking properties of these cells (24, 25). Furthermore, both bone marrow and peripheral blood B cell fractions contained IgM producing cells that were lacking in the plasma cell compartment. These data suggest that bone marrow plasma cells and memory B cells may differentially contribute to humoral immunity after BMT.

The assays described here may aid in estimating the magnitude of the memory B cell response of the donor before transplantation either in bone marrow harvest or peripheral blood samples to help clinicians understand the level of donor-derived antibody responses that may emerge in recipients after BMT. This information may be useful in scheduling the vaccination of the donor or recipient for certain antigens. Furthermore, screening of donors

with a history of immunization but lacking serum HLA antibodies for the presence of HLA-specific memory B cells may be informative to avoid platelet transfusion refractoriness in recipients by allowing for selection of acceptable platelet donors.

ACKNOWLEDGEMENTS

Authors thank Godelieve Swings for performing the cytospin preparations and Esther Steeneveld for her assistance in obtaining the samples.

REFERENCES

1. Ljungman P, Bregni M, Brune M, Cornelissen J, de Witte T, Dini G, et al. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. Bone Marrow Transplant. 2010;45(2):219-34.
2. Radia R, Pamphilon D. Transfusion strategies in patients undergoing stem-cell transplantation. Expert review of hematology. 2011;4(2):213-20.
3. Storek J. Immunological reconstitution after hematopoietic cell transplantation- its relation to the contents of the graft. Expert Opin Biol Ther. 2008;8(5):583-97.
4. Mackall C, Fry T, Gress R, Peggs K, Storek J, Toubert A, et al. Background to hematopoietic cell transplantation, including post transplant immune recovery. Bone Marrow Transplant. 2009;44(8):457-62.
5. Glas AM, van Montfort EH, Storek J, Green EG, Drissen RP, Bechtold VJ, et al. B-cell-autonomous somatic mutation deficit following bone marrow transplant. Blood. 2000;96(3):1064-9.
6. Storek J, Wells D, Dawson MA, Storer B, Maloney DG. Factors influencing B lymphopoiesis after allogeneic hematopoietic cell transplantation. Blood. 2001;98(2):489-91.
7. Storek J, Dawson MA, Lim LC, Burman BE, Stevens-Ayers T, Viganego F, et al. Efficacy of donor vaccination before hematopoietic cell transplantation and recipient vaccination both before and early after transplantation. Bone Marrow Transplant. 2004;33(3):337-46.
8. Sullivan KM, Storek J, Kopecky KJ, Jocom J, Longton G, Flowers M, et al. A controlled trial of long-term administration of intravenous immunoglobulin to prevent late infection and chronic graft-vs.-host disease after marrow transplantation: clinical outcome and effect on subsequent immune recovery. Biol Blood Marrow Transplant. 1996;2(1):44-53.
9. Lausen BF, Hougs L, Schejbel L, Heilmann C, Barington T. Human memory B cells transferred by allogenic bone marrow transplantation contribute significantly to the antibody repertoire of the recipient. J Immunol. 2004;172(5):3305-18.
10. Witherspoon RP, Schanfield MS, Storb R, Thomas ED, Giblett ER. Immunoglobulin production of donor origin after marrow transplantation for acute leukemia or aplastic anemia. Transplantation. 1978;26(6):407-8.
11. Wimperis JZ, Brenner MK, Prentice HG, Reittie JE, Karayiannis P, Griffiths PD, et al. Transfer of a functioning humoral immune system in transplantation of T-lymphocyte-depleted bone marrow. Lancet. 1986;1(8477):339-43.
12. Lum LG, Noges JE, Beatty P, Martin PJ, Deeg J, Doney KC, et al. Transfer of specific immunity in marrow recipients given HLA-mismatched, T cell-depleted, or HLA-identical marrow grafts. Bone Marrow Transplant. 1988;3(5):399-406.
13. Waters AH, Metcalfe P, Minchinton RM, Barrett AJ, James DC. Autoimmune thrombocytopenia acquired from allogeneic bone-marrow graft: compensated thrombocytopenia in bone marrow donor and recipient. Lancet. 1983;2(8364):1430.
14. Hallstrand TS, Sprenger JD, Agosti JM, Longton GM, Witherspoon RP, Henderson WR, Jr. Long-term acquisition of allergen-specific IgE and asthma following allogeneic bone marrow transplantation from allergic donors. Blood. 2004;104(10):3086-90.

15. Hatakeyama N, Hori T, Yamamoto M, Inazawa N, Iesato K, Miyazaki T, et al. Platelet transfusion refractoriness attributable to HLA antibodies produced by donor-derived cells after allogeneic bone marrow transplantation from one HLA-antigen-mismatched mother. Pediatric transplantation. 2011;15(8):E177-82.
16. Taniguchi K, Yoshihara S, Maruya E, Ikegame K, Kaida K, Hayashi K, et al. Donor-derived HLA antibody production in patients undergoing SCT from HLA antibody-positive donors. Bone Marrow Transplant. 2012;47(10):1338-42.
17. Fasano RM, Mamcarz E, Adams S, Donohue Jerussi T, Sugimoto K, Tian X, et al. Persistence of recipient human leucocyte antigen (HLA) antibodies and production of donor HLA antibodies following reduced intensity allogeneic haematopoietic stem cell transplantation. British journal of haematology. 2014;166(3):425-34.
18. Perry DK, Pollinger HS, Burns JM, Rea D, Ramos E, Platt JL, et al. Two novel assays of alloantibody-secreting cells demonstrating resistance to desensitization with IVIG and rATG. Am J Transplant. 2008;8(1):133-43.
19. Zhao E, Xu H, Wang L, Kryczek I, Wu K, Hu Y, et al. Bone marrow and the control of immunity. Cell Mol Immunol. 2012;9(1):11-9.
20. Kurosaki T, Kometani K, Ise W. Memory B cells. Nat Rev Immunol. 2015;15(3):149-59.
21. Saxon A, Mitsuyasu R, Stevens R, Champlin RE, Kimata H, Gale RP. Designed transfer of specific immune responses with bone marrow transplantation. J Clin Invest. 1986;78(4):959-67.
22. Parkkali T, Kayhty H, Hovi T, Olander RM, Roivainen M, Volin L, et al. A randomized study on donor immunization with tetanus-diphtheria, Haemophilus influenzae type b and inactivated poliovirus vaccines to improve the recipient responses to the same vaccines after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2007;39(3):179-88.
23. Karahan GE, de Vaal YJ, Krop J, Wehmeier C, Roelen DL, Claas FH, et al. A memory B cell crossmatch assay for quantification of donor-specific memory B cells in the peripheral blood of HLA-immunized individuals. Am J Transplant. 2017.
24. Kunkel EJ, Butcher EC. Plasma-cell homing. Nat Rev Immunol. 2003;3(10):822-9.
25. Williams MB, Rose JR, Rott LS, Franco MA, Greenberg HB, Butcher EC. The memory B cell subset responsible for the secretory IgA response and protective humoral immunity to rotavirus expresses the intestinal homing receptor, alpha4beta7. J Immunol. 1998;161(8):4227-35.