Blood-borne human plasma cells in steady state are derived from mucosal immune responses

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Introduction

Protective humoral immunity is provided by plasma cells through the production of antibodies. Because the half-life of the secreted immunoglobulin (Ig) is limited to a maximum of 2 to 3 weeks,1 persisting humoral immunity must be regulated through mechanisms controlling generation, survival, and homeostasis of plasma cells, that is, terminally differentiated B cells that are generated from activated B cells.2 In a secondary systemic immune response to a protein antigen such as tetanus toxoid, antigen-specific IgG-secreting plasmablasts with somatically mutated VH gene rearrangements are generated from memory B cells.6,7 After leaving the secondary lymphoid tissues, they are detectable in human blood between days 6 and 8 after vaccination. At this time, they are migratory and attracted by CXCL12, that is, could migrate to tissues, such as bone marrow.6 In the bone marrow, plasmablasts, expressing high human leukocyte antigen–DR (HLA-DR), proliferating and being migratory, can differentiate into long-lived plasma cells, which are no longer migratory, express low HLA-DR, and do not proliferate and continue to secrete their antibodies, thus maintaining humoral memory.8,9 Independent of circulating CD20+ B cells, as shown in patients treated with anti-CD20 (rituximab),10,11 Humoral immunity can also be driven by persisting antigen and the continuous differentiation of B cells into short-lived plasma cells.3,12

Survival of plasma cells in vivo depends on specific signals from their environment, the plasma cells’ niche.13 The number of niches most probably limits the number of plasma cells in the body.14 Entry into and egress from survival niches thus probably determine the homeostasis of plasma cells specific for a certain antigen. Recently, in accordance with this hypothesis, the mobilization of plasma cells of diverse specificities by tetanus toxoid-specific plasmablasts after tetanus vaccination has been reported.6 Trafficking and localization of plasmablasts and plasma cells in the body/tissue are mediated by homing receptors and chemokine receptors.15 The adhesion molecules CD62L (L-selectin) and α7β7 integrin initiate transmigration from blood into tissue by transient interactions with carbohydrates (eg, peripheral lymph node addressin [PNAd]) expressed by endothelial cells in the peripheral lymph nodes, as for CD62L,16,17 and with the mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) expressed on intestinal endothelial cells, as for α4β7 integrin.18,21 Plasma cells isolated from human lamina propria express high levels of α4β7 integrin.19 Further, rotavirus-specific IgD+/CD38high/CD27high plasma cells induced in the gut-associated lymphoid tissue express α4β7 integrin and C-C motif receptor 10 (CCR10),21 a receptor for chemokinesis toward CCL28 of mucosal tissues and the skin.24 Interaction between α4β7 integrin and MAdCAM-1 initiates transmigration25,26 and is indispensable for maintenance of secretory IgA levels.20,27 It has also been demonstrated that CCR9 and its ligand CCL25 mediate homing of plasmablasts expressing this chemokine receptor into intestinal tissue.28,29 Plasmablasts generated in systemic immune responses do not express CCR9 or CCR10 but do express CXCR4.30,31 CXCL12, the ligand of CXCR4, is expressed abundantly in human tissues, including spleen, bone marrow, lymph nodes,32 and mucosal tissues,33 and mediates recruitment of...
CXCR4+ plasmablasts into bone marrow.\textsuperscript{30,31} In short, subsets of plasmablasts have been described expressing distinct chemokine receptors or combinations thereof, enabling them to migrate to mucosal tissue, bone marrow, or inflamed tissue, settle there, and differentiate into plasma cells with different function and fate.

Based on phenotype, human antibody-secreting cells of different anatomic localization, that is, spleen, blood, and bone marrow, have been viewed as distinct successive developmental stages of plasma cell differentiation.\textsuperscript{34-36} Here, we provide evidence for an additional layer of complexity, namely, the mucosal versus systemic origin of plasmablasts and plasma cells in blood, indicating the independent chronic generation of mucosal plasmablasts versus the induced generation of parenteral plasmablasts. In steady state, the few antibody-secreting cells detectable express IgA, β7 integrin and CCR10, and most of them, but not all, are HLA-DR\textsuperscript{high}, qualifying them as plasmablasts of mucosal immune reactions. Between days 6 and 8 after parenteral (systemic) vaccination with tetanus/diphtheria toxoid, additional prominent populations of IgG, HLA-DR\textsuperscript{high}, CCR10\textsuperscript{−}, CXCR4\textsuperscript{+}, vaccine-specific plasmablasts and HLA-DR\textsuperscript{low} plasma cells with different specificity appear in the blood. Plasmablasts migrate toward gradients of the chemokines they have receptors for, in contrast to plasma cells, confirming our earlier notion that these plasma cells might be no longer migratory and have been dislocated from the plasma cells niche by systemic immune reaction.\textsuperscript{6}

The ethics committee of the medical faculty of the Humboldt University (Charité) approved the study, and patients' informed consent was obtained before enrollment in accordance with the Declaration of Helsinki.

**Cytometry**

Surface antigens were stained by coinoculation of mononuclear cells with monoclonal antibodies (mAbs) for 10 minutes at 4°C in PBS/BSA. Cells were washed once. Dead cells were electronically excluded by adding 4.6 diamidino-2-phenylindole (DAPI; Invitrogen, Carlsbad, CA) directly before acquisition and subsequent electronic gating on DAPI\textsuperscript{−} cells.

Intracellular (ic) antigens were stained after surface staining. Cells were washed twice in PBS, resuspended in 2% formaldehyde solution (Merck), and fixed for 20 minutes at room temperature. Cells were then washed twice in PBS. Saponin (Sigma Chemie, Deisenhofen, Germany) was used as permeabilizing agent at 0.5% solution (saponin buffer) in PBS/BSA containing 0.02% sodium azide (PBS/BSA/azide) for the intracellular staining and at 0.1% solution in PBS/BSA/azide for the washing steps. Cells were labeled with biotinylated or digoxigenated mAb in 0.5% saponin buffer for 15 minutes at room temperature. Cells were then washed with 0.1% saponin buffer. For secondary detection, the procedure was repeated using streptavidin–peridinin chlorophyll protein (PerCP; BD Pharmingen, San Diego, CA), streptavidin–allophycocyanin (APC)–Cy7 (Invitrogen), and antidualoxigenein (Roche Diagnostics, Mannheim, Germany) coupled to Alexa 350 (Invitrogen). Finally, cells were analyzed cytometrically on an LSRII cytometer (BD Biosciences) equipped with an additional UV laser and a DivaSoft operation system (BD Biosciences). Data were analyzed using FlowJo software (TreeStar, Ashland, OR). Cell aggregates were excluded according to peak vs area of the forward scatter signal. Cytometric fluorescence data are displayed as 2-color plots in log10 scale, light scatter in a linear scale.

Antibodies used included the following: CD19–phycocerythrin (PE; clone HD37; DakoCytomation, Glostrup, Denmark), CD19-PerCP or -PE-Cy7 (SJ25C1; BD Biosciences), CD27-Cy5 or –fluorescein isothiocyanate (FITC; 2E4; kind gift from René van Lier, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands), biotinylated and FITC-labeled and unlabeled κ (G20-193; BD Biosciences) and λ Ig light chain (JDC-12; BD Biosciences), CD38-FITC, -PE or -APC (HIT2; BD Biosciences), CD20-FITC (2H7; BD Biosciences), CD20-PerCP (L27; BD Biosciences), HLA-DR, coupled to FITC or Cy5 (L243; Deutsches Rheumafororschungszentrum [DRFZ], Berlin, Germany), β7 integrin–PE (FIB504; BD Biosciences), CD62L-FITC (145; Miltenyi Biotec, Auburn, CA), CD62L-PE-Cy5 (Dreg-56; BD Biosciences), Ki-67–FITC (MB-1; Dako North America, Carpinteria, CA), CD138-PE (B-B4; Chemicon International, Temecula, CA), IgG1-biotin or –FITC (G18-145; BD Biosciences), IgG2a-biotin (G20-359; BD Biosciences), IgG3-biotin (G20-127; BD Biosciences), CD45-PerCP (2D1; BD Biosciences), CD3-FITC (UCHT-1; DRFZ), and CD14-Cy5 (TM1; DRFZ), CD3-PacificBlue (UCHT1; BD Biosciences), CD14-PacificBlue (M5E2; BD Biosciences), IgM-PE (G20-127; BD Biosciences), CCR10-PE or -APC (314305; R&D Systems Europe, Abingdon, United Kingdom), CCR9-PE (112509; R&D Systems Europe), CCR10-PE or -APC (314305; R&D Systems Europe, Abingdon, United Kingdom), CCR9-PE (112509; R&D Systems Europe), CCR10-PE or -APC (314305; R&D Systems Europe, Abingdon, United Kingdom), CCR9-PE (112509; R&D Systems Europe), CCR10-PE or -APC (314305; R&D Systems Europe, Abingdon, United Kingdom), CCR9-PE (112509; R&D

**Methods**

**Preparation of blood and bone marrow samples**

Citrate or heparinized whole blood from healthy donors (18-57 years of age; average, 35 years) was collected, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation as described before,\textsuperscript{9} using lymphocyte separation medium (PAA Laboratories, Coelbe, Germany).

Some donors were immunized with tetanus/diphtheria vaccine (Sanofi-Aventis, Bridgewater, NJ) after informed consent had been obtained from each donor.

Leukocyte filters were obtained from the blood bank of the Charité University Hospital, Institute of Transfusion Medicine, immediately after preparation. They were flushed reversely with 20 mL cold phosphate-buffered saline (PBS), and PBMCs were isolated by density gradient centrifugation as described before.\textsuperscript{6} All donors were healthy and fulfilled criteria for blood donation. Initial studies of a cohort of blood donors (shown in Figure 1, and Figures S1C,S3, available on the Blood website; see the Supplemental Materials link at the top of the online article) were further expanded by detailed phenotypic and functional analyses of additional healthy volunteers before and after vaccination.

Bone marrow cells were obtained from patients (55-80 years of age; average, 68 years) undergoing hip joint endoprosthesis surgery at the Department for Orthopedics at the Charité Berlin. As previously reported, different age of bone and bone marrow donors does not cause a significant change of total IgG- or IgA-secreting cells in the body.\textsuperscript{38} The material obtained was flushed with cold PBS supplemented with 0.5% bovine serum albumin (BSA) and 5 mM ethylenediaminetetra-acetic acid (PBS/BSA/ethylenediaminetetra-acetic acid; Merck, Darmstadt, Germany) and was filtered using cell strainers (70 μm; BD Biosciences Discovery Labware, Bedford, MA). Bone marrow mononuclear cells were isolated by subsequent density gradient centrifugation as described before for PBMCs.\textsuperscript{6} No enzymatic digestion was used.

Blood serum was collected from healthy donors using the Vacutainer system according to the manufacturer’s instructions (BD Biosciences, San Jose, CA).
(Invitrogen) at a concentration of 10 μg/mL in distilled water and incubated for 1 hour at 37°C and 5% CO2. The solution was removed and the inserts were dried for 2 hours at 37°C.

PBMCs or bone marrow mononuclear cells were isolated as described in “Preparation of blood and bone marrow samples,” using prewarmed RPMI 1640 instead of buffers. For some experiments, B cells were enriched using RosetteSep technology (StemCell Technologies, Vancouver, BC). Cells were counted in a Neubauer chamber. The lower transwell chamber was filled with 600 μL assay medium with or without the human recombinant chemokines CXCL12 (stromal cell–derived factor 1α, 10 nM; R&D Systems, Minneapolis, MN), CCL25 (thymus-expressed chemokine or Ckβ-15, 300 nM; PeproTech, Rocky Hill, NJ), CCL28 (mucosae-associated epithelial chemokine, 300 nM, PeproTech) at optimal concentrations: 0.5 or 2 × 10^5 PBMCs or bone marrow mononuclear cells were added to the upper chamber. Cells were then allowed to migrate for 90 minutes at 37°C in a humid atmosphere (5% CO2). Finally, cells were added to the upper chamber. Cells were then allowed to migrate for 90 minutes at 37°C in a humid atmosphere (5% CO2). Finally, cells were collected from upper and lower wells and plasmablasts/plasma cells were enumerated cytometrically. Frequencies of migrated cells (migrated cells counted in the lower chamber divided by cells counted in the upper and lower chambers) are indicated.

**Statistical analysis**

Data were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA). Frequencies of various cell populations were calculated with FlowJo software. P values were calculated by Mann-Whitney test for unpaired observations and Wilcoxon test for paired data (both 95% confidence interval, 2-tailed).

**Identification and enumeration of blood plasmablasts and plasma cells**

As shown in Figure S1, blood plasmablasts/plasma cells were identified cytometrically as intracellular Ig^high^ (Ig^high^) cells, CD19^+^/CD27^high^/CD20^low^/CD38^high^ cells. Cells identified as Ig^high^ cells were stained brightly and exclusively by κ- or λ-light chain antibodies, and counterstaining for appropriate surface antigens confirmed the accuracy of this staining approach (Figure S1A). Less than 0.001% κ light chain/λ light chain double-positive cells were detected. Antigen specificity of plasmablasts/plasma cells was analyzed as described using recombinant tetanus toxoid C fragment (rTTC) coupled to digoxigenin. The specificity of staining for icIg and rTTC was confirmed by staining in the absence of saponin and by inhibition of the staining with 10-fold excess of unlabeled primary antibody and antigen, respectively, resulting in a 10-fold reduction of staining intensity (data not shown). Cytometric quantification of CD45^+^/SSClow/CD19^+^/CD20^+/−^ B cells, including plasmablasts/plasma cells, was performed using the TruCount system (BD Biosciences) according to the manufacturer’s instructions and antibodies detecting CD45, CD19, and CD20. Numbers of subfractions of plasmablasts and plasma cells were calculated based on previous findings that these cells are CD19^+^/CD27^high^/CD20^low^/Ig^high^ cells.

**Results**

Numbers of blood-borne plasmablasts and plasma cells in steady state and after vaccination

In steady state, plasmablasts and plasma cells were detected readily in the blood of healthy persons in the absence of apparent or intentional activation of the immune system. The median frequency of plasmablasts/plasma cells (either CD19^+^/CD27^high^ lymphocytes or icIg^high^ cells, Figure S1A,B) was 0.14% (± 39%; SD) of PBMCs (range, 0.03%-2.39%; 49 donors tested; Figure 1). Twenty-three persons were tested 6 to 7 days after secondary systemic vaccination with tetanus/diphtheria toxoid. In these donors, a significantly increased frequency of plasmablasts and plasma cells was observed (P < .001, Mann-Whitney test, 95% confidence interval) with a median of 0.46% (± 0.5%) of PBMCs (range, 0.08%-2.06%). The absolute count of blood-borne plasmablasts/plasma cells in steady state was 2307/mL (± 657/mL; 19 donors). In all donors, when absolute cell numbers were assessed directly before and 7 days after vaccination with tetanus/diphtheria toxoid, increased absolute counts of plasmablasts/plasma cells were observed after immunization (Figure S1C).

**Steady-state blood-borne plasmablasts/plasma cells express IgA, β7 integrin, and CCR10**

Plasmablasts/plasma cells were detected according to expression of icIg light chains (Ig^high^) and analyzed for expression of intracellular IgG (IgG), IgA (IgA), and IgM (IgM) (Figure 2A). In steady state, an average of 84% of plasmablasts/plasma cells expressed icIgA (range, 60%-92%; 8 donors). In the same representative persons, IgG^+^ and IgM^+^ cells were detected at median frequencies of 12% (range, 1%-38%) and 5% (range, 1%-10%) of total plasmablasts/plasma cells, respectively. Systemic tetanus/diphtheria vaccination resulted in a substantial relative increase of IgG^+^ cells on day 7 after vaccination (median, 81%; range, 73%-90%, 4 donors), whereas the frequencies of IgA^+^ and IgM^+^ cells were 15% (range, 8%-24%) and 4% (range, 1%-8%), respectively (Figure 2B). Before vaccination, the median frequency of IgG^+^ cells among PBMCs was 0.04% (0.00%-0.11%), of IgA^+^ cells 0.16% (0.07%-0.38%) and of IgM^+^ cells 0.01% (0.00%-0.02%), respectively. Seven days after vaccination, the frequencies of IgG^+^ and IgM^+^ cells among PBMCs remained constant (IgA^+^ 0.10%; range, 0.04%-0.31%, P = .283; IgM^+^ 0.03%; range, 0.01%-0.04%, P = .049), whereas frequencies of IgG^+^ cells were 10-fold increased to 0.67% (range, 0.37%-1.06%, P = .004) (Figure 2C).

Peripheral steady-state plasmablasts/plasma cells expressed β7 integrin (31.7% ± 23.1%; range, 7%-95%, 29 donors), indicating
Peripheral blood mononuclear cells

A

icIgA
icIgG
icIgM

B

IgG
IgM
IgA
day 0
day 7

C

% of PBMCs

icIgA
icIgG
icIgM

the potential of these plasmablasts/plasma cells to bind to MAdCAM-1 on high endothelial venules of gut-associated lymphoid tissues. After parenteral tetanus/diphtheria vaccination, the numbers of β7-integrin+ plasmablasts/plasma cells remained stable (median, 1.259/mL before and 1.702/mL after vaccination), whereas the numbers of β7-integrin− cells increased significantly on day 7 after vaccination (median, 1.393/mL before and 8.814/mL after vaccination). Of note, rTT.C-binding plasmablasts, representative of vaccine-specific plasmablasts, did not express β7 integrin (Figure 3A). In the same assay, CD62L was stained to discriminate antibody-secreting cells of systemic, not mucosal, origin. As expected, most of the vaccination-induced icIgM high plasmablasts/plasma cells expressed the lymph node homing marker CD62L (69%; range, 54%-88%), in particular nearly all plasmablasts. In steady state, all circulating IgA+ plasmablasts/plasma cells expressed CCR10 and thus qualified as antibody-secreting cells from mucosal immune reactions: 55% (± 15%; range, 31%-69%) of those coexpressed β7 integrin (Figure 3G), but not CCR9.

Steady-state blood-borne plasmablasts migrate toward CCL28 and CXCL12

Consistent with their expression of CCR10, steady-state plasmablasts spontaneously migrated toward a gradient of CCL28 (300 nM; Figure 4A), in an in vitro transwell migration assay. Individual frequencies of migratory CD19+CD27high plasmablasts were 16%, 21%, 37%, and 34% (4 donors). A total of 18%, 25%, 12%, and 30% of plasmablasts migrated toward a gradient of CXCL12 (10 nM), and less than 2% migrated toward the intestinal chemokine CCL25, the ligand of CCR9. Steady-state plasmablasts migrating toward CCL28 all expressed CCR10 (2 donors tested). Of the plasmablasts migrating toward CXCL12, most IgA+ cells also expressed CCR10, but only few of the IgA− cells (Figure 4D).

After vaccination with tetanus/diphtheria toxoid, the frequencies of plasmablasts migrating toward CXCL12 were 15% (± 13%; 10 donors, median ± SD), and barely any cells could be detected migrating toward CCL25 (< 1%), compared with assay medium (< 1%), in 2 donors tested (Figure 4B). At this time, antigen-specific cells and most of all plasmablasts/plasma cells did not express CCR10, implicating a significantly lower migration as response toward CCL28 compared with steady state.

As expected, less than 0.6% of plasma cells from bone marrow migrated to any of the chemokines tested, that is, more than 99% of bone marrow plasma cells were nonmigratory (Figure 4C), despite
the finding that bone marrow plasma cells do express CXCR4 and CCR10, but not CCR9 (Figure 6B).

Coexistence of plasmablasts and plasma cells in steady-state blood

The mature phenotype of bone marrow plasma cells, as shown in Figure 5, was consistent with previous results obtained in mice.30 Bone marrow plasma cells expressed high levels of CD38, CD138, and iIg (Figure S2). Few, if any, expressed Ki-67 (5% ± 3%), high levels of HLA-DR (12% ± 6%), or CD62L (2% ± 5%) (Figure 5B). Expression of β7 integrin was detectable on 49% (± 9%) of total bone marrow plasma cells. In peripheral blood, the CD62L≥β7 integrin+, HLA-DRlow, Ki-67− plasma cells had a phenotype similar to bone marrow plasma cells (Figure 5B). In contrast, blood plasmablasts expressing CD62L or β7 integrin were Ki-67+ and expressed high levels of HLA-DR (Figure 5A). Only HLA-DRhigh plasmablasts were capable of spontaneous migration toward 10 nM of CXCL12 in transwell-migration assays (Figure 5C).

Plasma cells of mucosal origin in bone marrow

The overall contribution of mucosal plasmablasts to the population of bone marrow plasma cells can be estimated according to expression of IgA and CCR10 by bone marrow plasma cells (Figures 6, S4). A total of 39.5% (± 9.8%; range, 27.6%-56.1%) of bone marrow plasma cells expressed iIgA, 55.1% (± 9.1%; range,
36.4%-63.2%) icIgG, and 6.7% (± 4.7%; range, 3.6%-16.3%) icIgM (Figure S2D). A total of 37% (± 10%; range, 28%-52%, 5 donors) of all CD38high bone marrow plasma cells, including IgA+ plasma cells, expressed CCR10 (Figure 6B). Of the IgA-secreting cells in the bone marrow, 43% (range, 25%-59%) expressed CCR10 and 33% (range, 25%-45%) β7 integrin (Figure S4). Thus, approximately 40% of IgA+ bone marrow plasma cells have a phenotype consistent with their mucosal origin. Migratory, mucosal steady-state plasmablasts are thus apparently not able to extinguish systemic humoral memory. Figure S3 demonstrates that most, if not all, occurrences of increased peripheral numbers of plasmablasts/plasma cells in apparently healthy donors (Figure 1) are the result of mucosal immune reactions.

**Discussion**

The homeostasis of antibody-secreting cells providing humoral immunity is still poorly understood. Plasma cells are residing mainly in bone marrow, but also in secondary lymphoid tissue and mucosal tissue. Their survival apparently depends on signals provided by their environment, the plasma cell niche. It remains controversial whether these plasma cells are continuously replaced or only as a consequence of subsequent immune reactions. Evidence for the mobilization of memory plasma cells into the blood in the course of an immune reaction has been provided by a previous study. It remained unclear whether such a mobilization does also occur continuously in steady state, implying a corresponding constant (chronic) generation of new plasmablasts to maintain the observed stability of humoral memory.

Antibody-secreting cells of blood in steady state have been analyzed phenotypically before. Arce et al identified IgG-secreting cells of blood by the cytometric secretion assay and showed that these cells are heterogeneous with respect to expression of HLA-DR and CD38, and speculated that the CD38low expressing IgG-secreting cells in blood might be plasmablasts. Moreover, Johansen et al have detected circulating CD19dim/IgAdim cells in steady state and speculated on their mucosal origin.

Here we have analyzed the phenotype and migratory potential of distinct antibody-secreting cells of peripheral blood to determine their possible origin and destination by applying different degrees of immune activation. In accordance with previous data, we found that, in steady state, 11.5 × 10⁹ antibody-secreting cells are circulating in 5 L of blood, compared with 5.5 × 10⁹ residing in bone marrow and 6.5 × 10⁹ in the gut-associated lymphoid tissue. More than 80% of the circulating antibody-secreting cells in steady state express IgA, αβ integrin, or CCR10. In contrast, 7 days after systemic immunization with tetanus toxoid, most of the antibody-secreting cells in blood express IgG and neither αβ integrin nor CCR10. This indicates that in steady state most, if not...
steady state, as we did in the context of systemic immune reactions, CD62L, and CD38 high plasma cells were counterstained for CD62L or β7 integrin (black gate represents histograms and numbers). (B) CD38 high bone marrow plasma cells were counterstained for CD62L, β7 integrin, Ki-67, HLA-DR (open histograms), and isotype controls (shaded histograms), revealing absence of CD62L and Ki-67 expression, a small subset of HLA-DR low cells, and expression of β7 integrin. The insert shows bone marrow mononuclear cells (Ki-67, open; control, shaded) positively stained for Ki-67. (C) PBMCs isolated 7 days after tetanus/diphtheria vaccination were migrated toward 10 nM of CXCL12. Migrated icIgG+ plasma blasts (black) and nonmigrated icIgG+ plasma blasts/plasma cells (gray) and their HLA-DR expression were detected cytometrically. Three different donors are shown.

Figure 6. Human bone marrow contains significant numbers of IgA+ plasma cells. (A) Proportions of bone marrow plasma cells expressing icIgG, icIgA, or icIgM. Bone marrow plasma cells and their isotype were assessed cytometrically as depicted in Figure S2. (B) Expression of CXCR4, CCR10, and CCR9 by CD38 high bone marrow plasma cells was analyzed (open histograms and black MFI values) and compared with control stainings (gray histograms and MFI values).

Figure 5. Coexistence of HLA-DR high plasmablasts and HLA-DR low plasma cells in steady state. (A) Intracellular IgG high plasmablasts/plasma cells circulating in steady state were stained for CD62L and β7 integrin and counterstained for HLA-DR or Ki-67. Plasma cells lacking Ki-67 expression and stained weakly for HLA-DR were also β7 integrin low/CD62L+. (Gray gate represents histograms and MFI ± SD values), whereas high expression of HLA-DR and Ki-67 on plasmablasts was associated with expression of CD62L or β7 integrin. (B) CD38 high bone marrow plasma cells were counterstained for CD62L, β7 integrin, Ki-67, HLA-DR (open histograms), and isotype controls (shaded histograms), revealing absence of CD62L and Ki-67 expression, a small subset of HLA-DR low cells, and expression of β7 integrin. The insert shows bone marrow mononuclear cells (Ki-67, open; control, shaded) positively stained for Ki-67. (C) PBMCs isolated 7 days after tetanus/diphtheria vaccination were migrated toward 10 nM of CXCL12. Migrated icIgG+ plasma blasts (black) and nonmigrated icIgG+ plasma blasts/plasma cells (gray) and their HLA-DR expression were detected cytometrically. Three different donors are shown.

Chemotactic responsiveness of peripheral plasmablasts and plasma cells towards 10nM CXCL12

HLA-DR

Migrated icIg+ cells  Non migrated icIg+ cells

Bone marrow plasma cells

Cell count

Figure 6. Human bone marrow contains significant numbers of IgA+ plasma cells. (A) Proportions of bone marrow plasma cells expressing icIgG, icIgA, or icIgM. Bone marrow plasma cells and their isotype were assessed cytometrically as depicted in Figure S2. (B) Expression of CXCR4, CCR10, and CCR9 by CD38 high bone marrow plasma cells was analyzed (open histograms and black MFI values) and compared with control stainings (gray histograms and MFI values).
Plasmablasts as well as nondividing, Ki-67−, HLA-DRlow plasma cells. A total of 25% of steady-state antibody-secreting cells are migrating toward gradients of CCL28 in transwell-migration assays. In vivo, this would allow them to home to the gut. Whether the presence of mature plasma cells in steady-state blood reflects the mobilization of resident mucosal plasma cells, in analogy to the mechanism postulated for long-lived IgG-secreting plasma cells of the bone marrow,6 could not be analyzed here because the specificity of the plasmablasts and plasma cells could not be compared.

Steady-state IgA+/CCR10+ antibody-secreting cells also express CXCR4 and are attracted by CXCL12 gradients. Interaction of CXCR4 with its chemokine ligand CXCL12 is involved in localization of plasma cells to the bone marrow33 but probably is also involved in recruitment and maintenance of mucosal IgA-secreting cells.56 For murine antibody-secreting cells, it has been shown that plasmablasts are attracted by CXCL12 and migrate in response to it. Plasma cells do not but instead use CXCL12 as a survival signal.13,30 We show here, for the first time, that human HLA-DRhigh plasmablasts migrate in response to CXCL12 gradients, whereas HLA-DRlow plasma cells of blood and bone marrow plasma cells do not. This confirms our original notion8 that plasma cells from blood are destined to death by neglect, whereas blood-borne plasmablasts have the potential to home to a niche providing survival signals.

For plasmablasts generated in systemic immune responses and expressing CXCR4 but not CCR10, it has been shown that their preferred homing organ is the bone marrow.57 Plasmablasts of steady state express both CXCR4 and CCR10 and would have a choice to home to either bone marrow or mucosa. Although 40% of bone marrow plasma cells express IgA or CCR10, it is doubtful that steady-state plasmablasts contribute to this population. Why plasmablasts of steady state express functional CXCR4 but do not home to bone marrow (as discussed above), remains unclear. It has been shown that bone marrow resident IgA-secreting cells secrete monomeric IgA, whereas steady-state antibody-secreting cells secrete dimeric, secretory IgA.50 IgA+ , CCR10+ bone marrow plasma cells are probably derived from distinct mucosal immune reactions. In 2 of 50 healthy blood donors analyzed, we detected significantly enhanced numbers of blood-borne antibody-secreting cells. These cells were probably not generated in an unintentional systemic immune response, but rather in an unintentional mucosal immune response, because they expressed αβ T integrin (Figure S3). The recruitment of IgA-secreting cells from distinct mucosal immune responses has been described for mice infected with rotavirus.78 The difference between steady-state plasmablasts and plasmablasts generated during infection with regard to their competence to join the pool of bone marrow memory plasma cells appears to be crucial for the development of mucosal vaccines and our understanding of immunity to mucosal virus challenge.

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Authorship
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Blood-borne human plasma cells in steady state are derived from mucosal immune responses

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