Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal CD138⁻ and CD138⁺ plasma cells

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Design and Methods

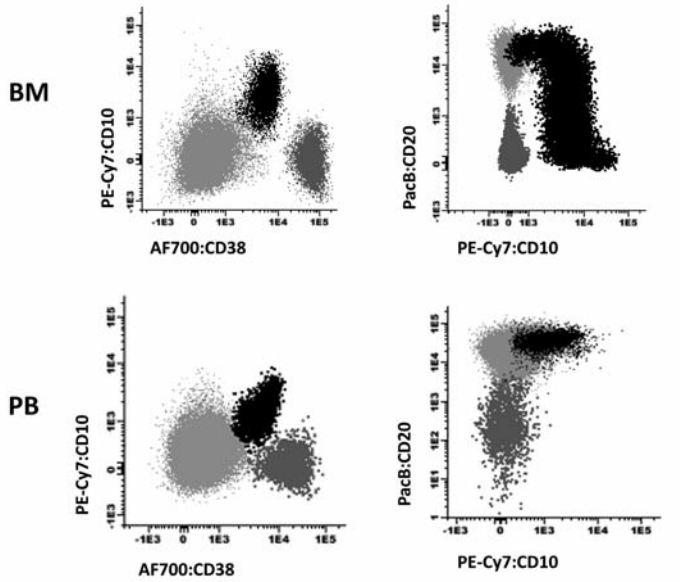
Cell samples

EDTA-anticoagulated peripheral blood from a total of 106 adult healthy donors (63 men and 43 women; age range: 20-82 years), was analyzed in this study, after informed consent was given by each subject according to the Local Ethical Committees. Leukocyte concentrates from healthy donors were obtained from the French Blood Center (Toulouse, France).

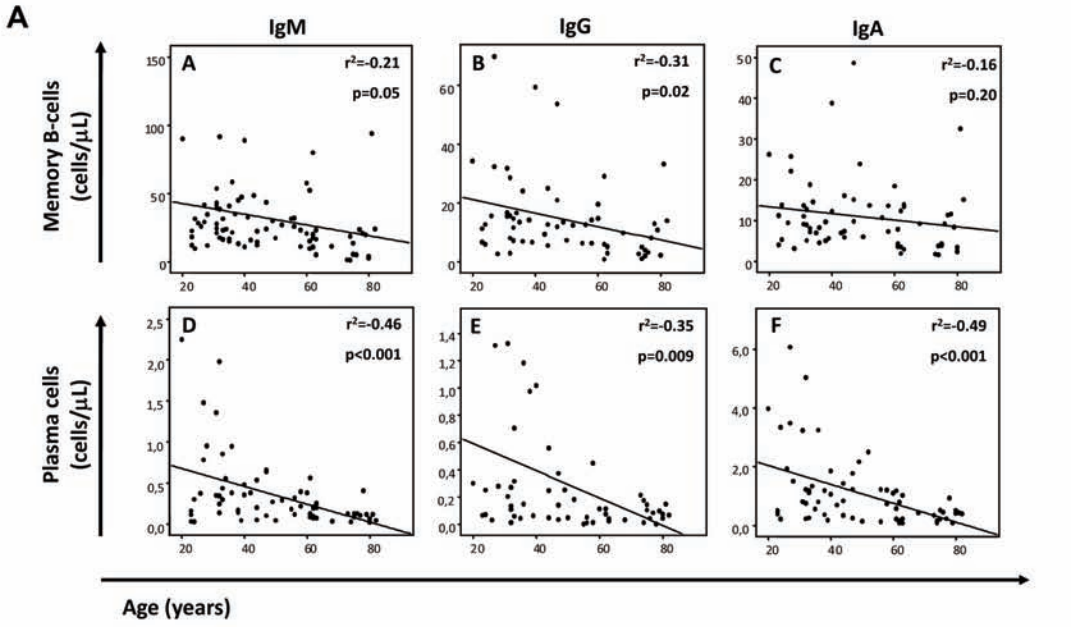
Antibodies (Abs) conjugated with pacific blue (PacB), anemonia majano cyan (AmCyan), fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein/cyanin 5 (PerCP/Cy5), PerCP/Cy5.5, PE/cyanin 7 (PE/Cy7), allophycocyanin (APC), alexafluor 700 (AF700), AF750, APC/Cy7, specific for human CD5 (clone L17F12), CD9 (clone M-L13), CD10 (clone HI10a), CD19 (clone SJ25C1), CD21 (clone B-ly4), CD22 (clone S-HCL-1), CD23 (clone EBVCS-5), CD24 (clone ML5), CD25 (clone 2A3), CD27 (clone L128), CD29 (β1 Integrin–ITGβ1, clone MAR4), CD38 (clone HIT2 or HB7), CD40 (clone 5C3), CD43 (clone 1G10), CD45 (clones 2D1 and HI30), CD49d (ITGα4, clone 9F10), CD49e (ITGα5, clone SAM1), CD53 (clone HI29), CD56 (N-CAM, clone B159), CD62L (clone DREG-56), CD70 (clone Ki-24), CD80 (clone L307.4), CD81 (clone JS-81), CD86 (clone IT2.2), CD95 (clone DX2), CD106 (VCAM-1, clone 51-10C9), CD138 (clone MI15), CD184 (CXCR4, clone 12G5), CCR6 (clone 11A9), HLA-DR (clone L243), HLA-DR, DP, DQ (clone Tu39), ITGβ7 (clone FIB504), Ig light chain lambda (Igλ, clone JDC-12), Ig light chain kappa (Igκ, clone TB 28-2), IgG (clone G18-145), IgM (clone G20-127), and KI-67 (clone B56) were purchased from Becton/Dickinson (BD) Biosciences (San Jose, CA); CD20 (clone B9E9), CD58 (LFA-3, clone AICD58) and CD138 (clone B-A38) from Beckman Coulter (Fullerton, CA); CCR10 (clone 314305) from R&D Systems (Minneapolis, MN); CD19 (clone HIB19), CD20 (clone 2H7), and CD200 (clone OX104) were from Biosciences (San Diego, CA); CD38 (clone HI72) from ExBio (Vestec, Czech Republic); CD43 (clone TP1/36) from Immunostep (Salamanca, Spain); IgM (polyclonal rabbit Ab), Igλ (polyclonal rabbit Ab), and Igκ (polyclonal rabbit Ab) from Dako (Glostrup, Denmark); IgA (polyclonal goat antibody), IgG (polyclonal goat Ab), and IgD (polyclonal goat Ab) from Southern Biotech (Birmingham, AL).

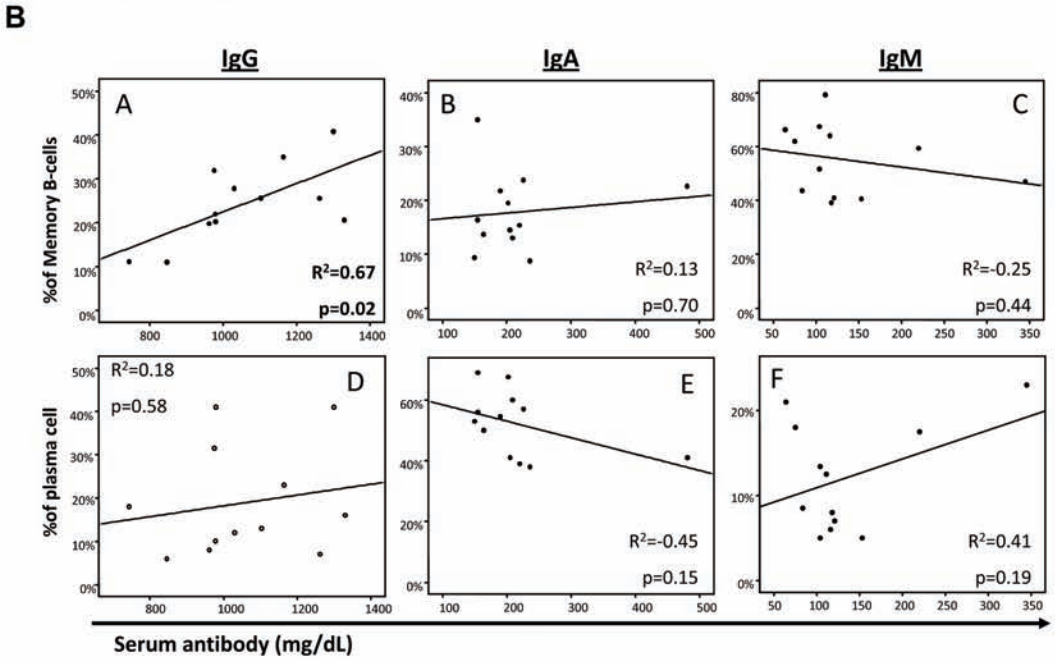
Immunophenotypic studies

Erythrocyte-lysed whole peripheral blood samples or mononuclear cells obtained by Ficoll-hypaque density gradient centrifugation were labeled with Abs conjugated with different fluorochromes (2.10^6 cells in 100μL/test) as described.[1] In some experiments, T and natural killer cells were removed with anti-CD2 magnetic beads (DYNAL, dynabeads M-450 CD2 Pan T) to enrich for B cells. For intracellular staining of Ig or KI-67, cells were fixed and permeabilized with the Cytofix/Cytoperm kit (BD Biosciences). B-cell subpopulations were identified using a combination of 7-8 fluorochrome-conjugated Abs. The fluorescence was analyzed with a FACSCanto II or a FACSAria flow cytometer, driven by the FACSDiva 6.1 software (BD Biosciences). Data acquisition was performed using a two-step procedure. Information from the total cellularity was first acquired using 5.10^4 cells/tube, and then full data was specifically acquired for ≥2.10^5 cells showing CD19 and/or CD38 expression and low-to-intermediate sideward light scatter (SSC) values (where B lymphocytes and plasma cells are located). In multiparameter phenotyping, we used a first gating on $CD38^{high}$ cells because we have checked that $CD38^{high}$ cells comprise all and only cytoplasmic Ig plasma cells in 10 peripheral blood

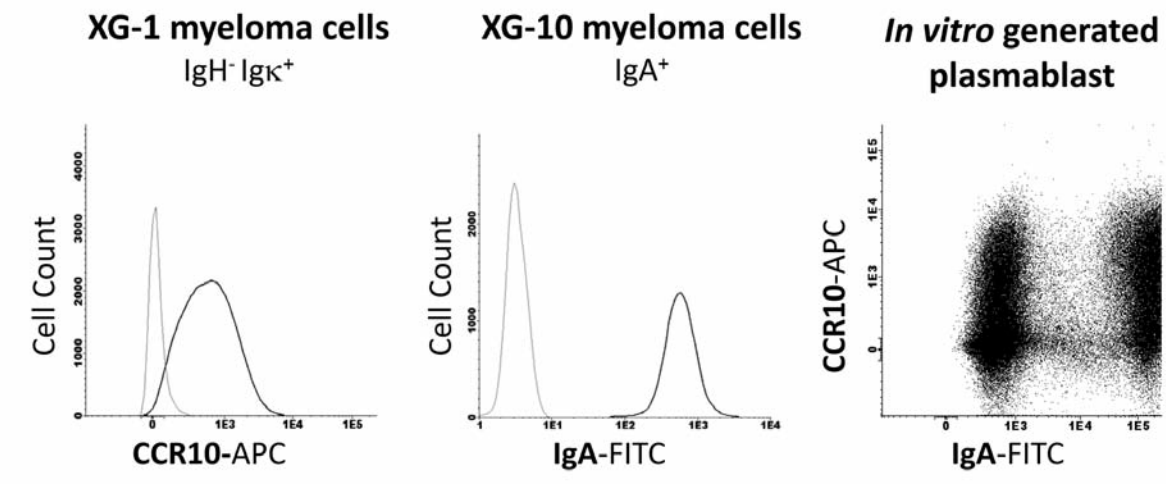


Online Supplementary Figure S1. Expression of CD10, CD20 and CD38 on immature, naïve B cells and plasma cells. Distribution of the different subsets: immature (■), naïve (■) B lymphocytes and plasmablasts/plasma cells (■) in bone marrow and in peripheral blood according to the expression of CD10, CD27 and CD38.





Online Supplementary Figure S2. Age-related changes in the immunoglobulin heavy chain isotype subsets of memory B lymphocytes or plasma cells and correlation between serum concentration of IgG, IgA and IgM antibodies and the percentage of the corresponding Ig heavy chain isotype-specific memory B cells. (A) Data plotted in each diagram represent correlation between the age of each healthy individual donor and the absolute count of memory B lymphocytes and plasma cells expressing IgG (panels A and D, respectively), IgM (panels B and E), IgA (panels C and F). (B) Correlation between the concentration of IgG, IgA and IgM antibodies in the serum and the percentage of the corresponding Ig heavy chain isotype-specific memory B cells (panels A, B and C) and plasma cells (D, E, and F). Serum IgM, IgG and IgA levels (mg/dL) were determined by nephelometry.



Online Supplementary Figure S3. Labeling of myeloma cells or *in vitro* generated plasmablasts with anti-human CCR10 or anti-human IgA mAbs. The anti-CCR10 mAb (clone 314305 from R&D systems) used was the same as that used by Mei *et al.* This mAb strongly stained the CCR10⁺ XG1 human myeloma cell line, or *in vitro* generated plasmablasts from healthy donors. The mAb to human IgA was also validated by labeling the IgA⁺ XG-10 myeloma cell line after cell permeabilization.

Table 1. Distribution and immunophenotypic profile of peripheral blood B-cell subsets.

PB Distribution*:	Immature	Naive	Memory	Plasma cells
% from all PB B cells	3.4%±2.4% (1.8%-4.5%)	65%±12% (57%-73%)	31%±12% (22%-39%)	1.3%±1.4% (0.5%-1.4%)
Absolute count (N. of cells/μL)	6±6 (2-9)	125±90 (63-156)	58±42 (28-68)	2.1± 2.1 (0.6-2.9)
Phenotypic profile:**				
CD5	++	-/+	-	-
CD10	+	-	-	-
CD19	+	+	+	+[d]
CD20	+	+	+	-
CD21	+	+	+	-
CD22	+	+	+	-
CD23	-	-/++	-	-
CD24	+	+[d]	+	-
CD25	-	-	++	-
CD27	-	-	+	++
CD34	-	-	-	-
CD38	+	-/+[d]	-/+[d]	++
CD40	++	++	++	+
CD43	-	-	-	+
CD45	++	++	++	+/++
CD53	+	+	++	+[d]
CD80	-	-	-	-
CD81	++	+	+	+
CD86	-	-	-	+
CD95	-	-	-/+	+
CD138	-	-	-	-/+[d]
CCR6	+	+	+	-
HLA-DR	++	++	++	+/++
sIgH	++ (M&D)	+ (M&D)	++ (M&D or G or A)	+[d] (M or D or G or A)
sIgL	++	+	+	+[d]

**Results expressed as mean ± one standard deviation (p25-p75). **Intensity of the staining expressed as -: negative, +d: dimly positive, +: positive, ++: brightly positive.*

samples. Multiparameter phenotyping of B lymphocytes was performed on gated $CD19^+CD20^+$ cells.

Data were analyzed with the Infinicyt 1.3 software (Cytognos SL, Salamanca, Spain). For some mAbs, a bimodal fluorescence distribution was observed and the MFI was that of the positive population. The fluorescence intensity of the cell populations was compared using the staining index (SI) provided by the formula: (mean fluorescence intensity -MFI- obtained from the given monoclonal Ab -mAb- minus MFI obtained with a control mAb)/(2 times the standard deviation -SD- of the MFI obtained with the same control mAb).[2]

Cell isolation and purification

Plasma cell subpopulations, $CD20^-CD38^{++}CD138^-$ cells and $CD20^-CD38^{++}CD138^+$ cells, were sorted with a FACSAria flow cytometer to perform cytospins. The purity of the sorted cells was ≥90%. Cells were stained with May-Grünwald-Giemsa and cytology pictures were acquired with a DM LB microscope (Leica, Wetzlar, Germany).

Statistical methods

Mean values and their SD, median and range were calculated for continuous variables with SPSS statistical software package (SPSS 10.1 Inc., Chicago, IL). Student's *t* test was used to evaluate the statistical significance of differences observed between groups for paired and unpaired variables. Correlation studies were performed using the Pearson test. *P* values less than 0.05 were considered to be associated with statistical significance.

References

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2. Maecker HT, Frey T, Nomura LE, Trotter J. Selecting fluorochrome conjugates for maximum sensitivity. Cytometry A. 2004;62(2):169-73.