

# 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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### **SUPPLEMENTARY APPENDIX**

## **Design and methods**

#### Cell samples.

EDTA-anticoagulated PB from a total of 106 adult healthy donors (63 men and 43 women; age range: 20-82 years), were 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. 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 (\beta1 Integrin- ITG\beta1, clone MAR4), CD38 (clone HIT2) or HB7), CD40 (clone 5C3), CD43 (clone 1G10), CD45 (clones 2D1 and HI30), CD49d (ITGa4, clone 9F10), CD49e (ITGa5, 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 (Igk, 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 eBiosciences (San Diego, CA); CD38 (clone HI72) from ExBio (Vestec, Czech Republic); CD43 (clone TP1/36) from Immunostep (Salamanca, Spain); IgM (polyclonal rabbit Ab), Ig $\lambda$  (polyclonal rabbit Ab), and Ig $\kappa$  (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 PB samples or mononuclear cells obtained by Ficoll-hypaque density gradient centrifugation were labeled with Abs conjugated with different fluorochromes (2.10<sup>6</sup> cells in 100µL/test) as described.<sup>(1)</sup> In some experiments, T and NK 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  $\geq 2.10^5$  cells showing CD19 and/or CD38 expression and low-to-intermediate sideward light scatter (SSC) values (where B lymphocytes and PC are located). In multiparameter phenotyping, we used a first gating on CD38<sup>high</sup> cells because we have checked that CD38<sup>high</sup> cells comprise all and only

cytoplasmic Ig PC in 10 PB samples. Multiparameter phenotyping of B lymphocytes was performed on gated CD19<sup>+</sup>CD20<sup>+</sup> 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).<sup>(2)</sup>

#### *Cell isolation and purification.*

PC subpopulations, CD20<sup>-</sup>CD38<sup>++</sup>CD138<sup>-</sup> cells and CD20<sup>-</sup>CD38<sup>++</sup>CD138<sup>+</sup> cells, were sorted with a FACSAria flow cytometry to perform cytospins. The purity of the sorted cells was  $\geq$ 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-*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 <.05 were considered to be associated with statistical significance.

## References

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