

not after vaccination. Moreover, MuV-specific CD8⁺ T cells in mumps cases show a persistent polyfunctionality, which is not seen after vaccination. A prospective study in a mumps outbreak setting, investigating the MuV-specific T-cell responses before and after MuV exposure, could provide evidence whether the long-lived polyfunctional T-cell profile could serve as a correlate of protection.

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A comparative analysis of human bone marrow–resident and peripheral memory B cells



To the Editor:

While plasma cells are well-established bone marrow (BM) long-term residents with unique features,¹ only a few studies indicate memory B (memB) cells as persisting in BM and other

lymphoid organs. Little is known about phenotypic and functional differences between circulating and tissue-resident memB cells. No major differences were seen for memB cells from spleen, tonsils, BM, and peripheral blood (PB) regarding maturation and activation status, migratory traits, adhesion marker, or the death receptor CD95.² Thus, it was deduced that phenotypically similar memB cells recirculate between the different organs. However, differences were seen for ICOS-L, CD62L, ITGβ7, and CD31 in this study, suggesting tissue-specific functions, which have not been analyzed in detail yet. In another study, BM-resident memory T cells have been characterized with distinct functional features compared with their circulating counterparts, particularly exhibiting a more resting state.³ To elucidate any differences between human BM-resident memB cells and their circulating counterparts, we now performed a comprehensive comparative analysis using paired BM and PB samples.

Detailed methodology is provided in the [Methods](#) section in this article's Online Repository at www.jacionline.org. Briefly, paired BM diaphysis/femur head and PB samples were obtained from 58 patients (mean age, 62 years; 50% men, 50% women) undergoing hip joint replacement surgery and allocated to different analyses including microarray, cytometric analyses, and cell culture experiments. The study was approved by the Charité Berlin local ethics committee (EA1/269/14). After isolation of PBMCs and bone marrow mononuclear cells (BMCs), cells were cultured in 1.5 mL Iscove Modified Dulbecco Medium (IMDM)/10% FCS/1% penicillin/streptomycin in 12-well plates at 37°C and 5% CO₂ for 5 hours for cytokine detection (TNF-α and IL-6) or 6 days for Annexin-V and Ki-67 assays. Stimulations were performed by either heat-killed formalin-fixed *Staphylococcus aureus* Cowan strain I (1:10,000 diluted; Merck Millipore, Darmstadt, Germany), 6 μg/mL CpG (ODN 2006; InvivoGen, San Diego, Calif), and 1 μg/mL pokeweed mitogen (Sigma-Aldrich, München, Germany) or 1 μg/mL human recombinant CD40L (Biolegend, Fell, Germany) and 10 μg/mL F(ab')₂ antihuman IgM + IgG (Affymetrix eBioscience, Frankfurt am Main, Germany). After 1 hour of stimulation, 7.5 μg/mL brefeldin A (Sigma-Aldrich) was added to the culture for the remaining 4 hours to facilitate detection of intracellular TNF-α and IL-6. Intracellular α-defensin 1-3 (DEFA1-3) expression was measured immediately after cell isolation. For inducing DEFA1-3 expression, lymphocytes were cultured with PB- or BM-derived serum and CD45^{hi}CD15⁺ cells for 18 hours at 37°C, 5% CO₂. Flow cytometric analyses were carried out using the LSRII Fortessa Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ) (see [Table E1](#) and [Fig E1](#) in this article's Online Repository at www.jacionline.org).

Switched and nonswitched memory B cells from 3 paired PBMC and BMMC (diaphysis plus femur head) samples were sorted. After RNA isolation, microarray hybridization and bioinformatic analysis were performed (see [Table E2](#) in this article's Online Repository at www.jacionline.org).

An altered B cell subset distribution was apparent among mature B cells with reduced percentages of CD27⁺IgD⁺ nonswitched memory B cells as well as increased percentages of CD27[−]IgD[−] double negative B cells and CD38^{hi}CD27^{hi} plasma cells in BM when compared to PB ([Fig 1, A](#)). Our data provide evidence that BM memB cells are more resting compared with their circulating counterparts. This was

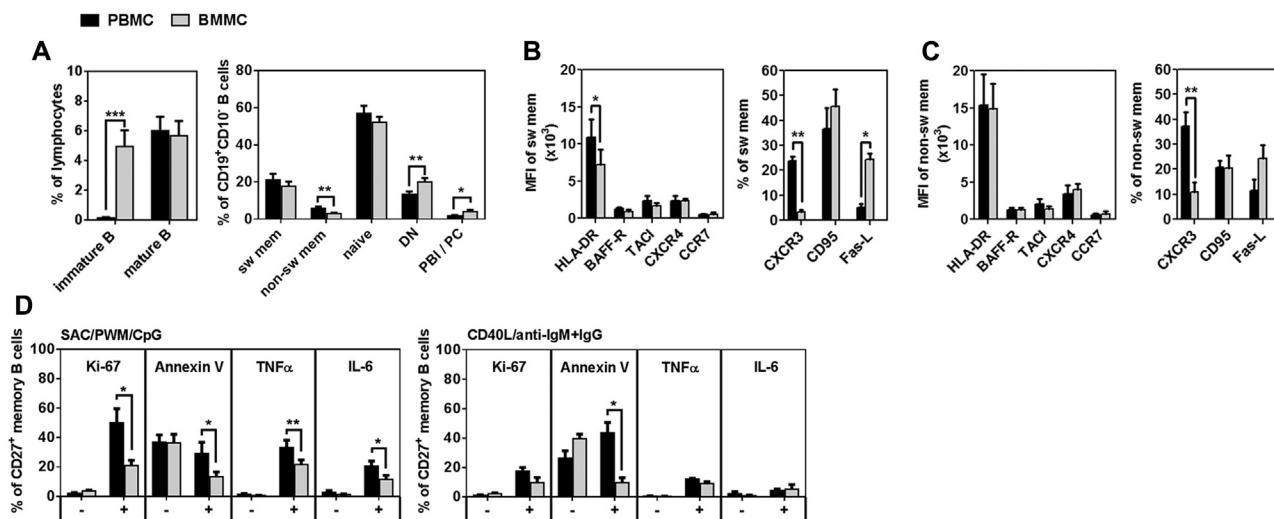


FIG 1. Paired PBMCs/BMMCs were analyzed with flow cytometry for (A) the percentage of CD10⁺ immature/CD10⁻ mature cells within total lymphocytes (n = 14) and CD27⁺IgD⁻ switched memory (sw mem), CD27⁺IgD⁺ nonswitched memory (non-sw mem), CD27⁻IgD⁺ naive, CD27⁻IgD⁻ double-negative (DN) B cells, CD38^{hi}CD27^{hi} plasmablasts (PBI) or plasma cells (PC) within mature B cells (n = 14) (B and C). The mean fluorescence intensity (MFI) expression and the percentage of indicated molecules on CD27⁺IgD⁺ nonswitched and CD27⁺IgD⁻ switched memB cells (n = 5-8). D, Unstimulated (-)/stimulated (+) cells with SAC/pokeweed mitogen/CpG or CD40L/anti-IgM+IgG were quantified for proliferation (Ki-67⁺, n = 7-9), apoptosis (Annexin V⁺, n = 7), and cytokine release (TNF-α⁺ and IL-6⁺, n = 5-8) of memB cells. BAFF-R, B-cell activating factor receptor; CXCR4, C-X-C chemokine receptor type 4; SAC, *Staphylococcus aureus* Cowan strain I; TACI, transmembrane activator and CAML interactor. Mean ± SEM values are given.

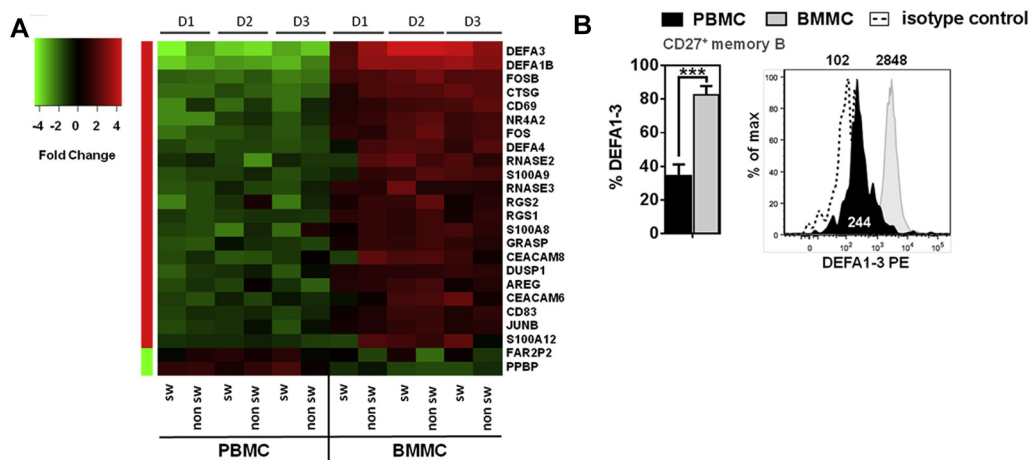


FIG 2. RNA of switched (sw) and nonswitched (non sw) memB cells from paired human PBMC and BMMC samples of 3 different donors (D1-D3) was used for microarray analysis. A, The gene expression pattern for indicated differentially expressed genes as heatmap based on the Linear Model for Microarray Analysis (LIMMA) algorithm. B, The DEFA1-3 protein expression in CD27⁺ memB cells (n = 20) as percentage and mean fluorescence intensity. PE, Phycoerythrin. Mean ± SEM values are given.

indicated by reduced HLA-DR and CXCR3, but increased Fas-ligand expression on BM cells (Fig 1, B and C). Moreover, upon reactivation, BM memB cells displayed less proliferation and apoptosis, as well as reduced production of the inflammatory cytokines TNF-α and IL-6 (Fig 1, D). Transcriptome analyses revealed a significantly higher amount of α-defensin (DEFA) transcripts in BM memB cells (Fig 2, A) and accordingly an increased DEFA1-3 protein expression (Fig 2, B). This molecule class belongs to the antimicrobial peptides functioning as part of innate immunity.⁴ Few studies

have indicated DEFA1-3 expression by adaptive immune cells. An upregulation was found in PB T cells of patients with drug-induced subcutaneous diseases,⁵ as well as in CD8⁺ T cells from long-term HIV-infected nonprogressors whose cells secrete DEFA1-3 upon stimulation.⁶ This state of affairs contributes to the anti-HIV-1 activity of CD8 antiviral factor. Low DEFA1-3 expression in PB memB cells could be induced by addition of BM serum (see Fig E2, A, in this article's Online Repository at www.jacionline.org), or by BM CD45^{hi}CD15⁺ cells, highly expressing DEFA (Fig E2, B).

We proposed that DEFA1-3 RNA- and protein-rich exosomes are released from these CD15⁺DEFA1-3^{high} cells into the serum and subsequently taken up by BM memB cells. Exosomes are small vesicles released from the surface of many cell types into body fluids carrying mRNA, microRNA, or DNA as well as proteins.⁷ Our hypothesis was supported by the finding that significant amounts of DEFA1 and DEFA3 mRNA as well as protein were detectable in exosomes isolated from BM but not in those from PB serum (Fig E2, C and D). Importantly, the addition of the exosome uptake inhibitor cytochalasin-D to the coculture assay with CD15⁺DEFA1-3^{high} cells profoundly reduced the DEFA1-3 expression in PB-derived memB cells (Fig E2, E). Cytochalasin-D disrupts the actin polymerization and thereby prevents exosome uptake by target cells.⁸ This suggests that the induction of DEFA1-3 expression in memB cells is indeed exosome-dependent and provides first evidence for an exosome-related communication between cells of the innate and adaptive immune system in the BM by mediating DEFA1-3 transfer. Whether or not BM memB cells are inherently capable of DEFA mRNA transcription and translation without the help of exosomes cannot be determined from our data and needs further investigation.

Functionally, supernatants from BMMCs showed a higher bactericidal activity against *Streptococcus pneumoniae* than did those from PB (see Fig E3, A, in this article's Online Repository at www.jacionline.org), which is potentially attributable to higher numbers of CD15⁺DEFA1-3^{high} cells and DEFA1-3^{high} BM memB cells. Inactivation of DEFA with the α 1-proteinase inhibitor⁹ resulted in reduced killing activity in almost 40% of functionally positive BMMC supernatants (Fig E3, B). BMMC supernatants were defined as functionally positive if bacterial growth was inhibited by at least 1 log. Because of a lack of specific neutralizing antibodies targeting DEFA1-3, we could not exclusively inhibit these antimicrobial peptides in the bacterial killing assay. In consequence, our data do not prove that DEFA is responsible for bactericidal activity in the BM, but rather indicate their significant contribution, along with other antimicrobial molecules, to bacterial killing. Preliminary data also suggest a higher DEFA expression in splenic memB cells, which could hint at a similar role in antimicrobial activity as proposed for the BM (data not shown).

In summary, our study provides evidence that BM-residing memB cells differ phenotypically and functionally from those in PB. We show evidence that the BM contains niches for resting memB cells and suggest a novel, essentially innate mechanism by which memB cells could contribute to BM immunity.

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METHODS

Sample preparation and flow cytometry

Before isolation of PBMCs/BMMCs, bone pieces were incubated for 10 minutes in IMDM medium/10% FCS/1% penicillin/streptomycin (Merck Millipore) at room temperature under constant agitation followed by cell filtration with a 100- μ m cell strainer. PBMCs/BMMCs were isolated from heparin PB samples and bone diaphysis/femur heads with density gradient centrifugation (Biocoll separating solution; Merck Millipore). Flow cytometric analysis was performed at the LSRII Fortessa Flow Cytometer (Becton Dickinson) configured with a blue (488 nm, 100 mW), yellow-green (561 nm, 100 mW), red (639 nm, 40 mW), violet laser (404 nm, 50 mW) and ultraviolet laser (355 nm, 60 mW), enabling the detection of up to 18 colors simultaneously. Sorted cells from PB or BM were measured at 4°C at the Aria II-SORP Flow Cytometer (BD Biosciences) equipped with a blue (488 nm, 100 mW), yellow-green (561 nm, 50 mW), red (639 nm, 40 mW), violet laser (408 nm, 50 mW) and 15 fluorescence channels. Both instruments allow precise laser focusing with a cuvette flow cell gel coupled to the collection optics. Details about detection optics/filter configurations are depicted in Fig E4. Data were evaluated with the FlowJo-9.8.5 software (TreeStar, Ashland, Ore). Absolute cell numbers were determined with differential PB counts. Dead cells were excluded by propidium iodide (Biolegend) or LD Pacific Blue/Orange (Thermo Fisher Scientific, Darmstadt, Germany) staining. For intracellular staining, the fixation/permeabilization buffer set (DEFA1-3, IL-6, TNF- α) and the Foxp3/Transcription Factor Staining Buffer Set (Ki-67) were used according to manufacturer's instructions (Affymetrix eBioscience). Apoptosis was assessed with the Annexin-V FITC Kit (BD Biosciences, Heidelberg, Germany).

Microarray analysis

RNA was extracted with the RNeasy Micro Kit with prior addition of β -mercaptoethanol and additional DNase I digestion according to manufacturer's instructions (Qiagen, Hilden, Germany). RNA integrity was checked by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif). RNA (2 ng) was used for coupled cDNA synthesis and amplification process with the RNA Ovation Pico WTA System V2 (NuGEN, San Carlos, Calif). To generate biotin-labeled targets, 5.5 μ g cDNA per reaction was used in the Encore Biotin Module (NuGEN) and added to a hybridization cocktail for hybridization to the Human Gene 2.0 ST Array according to the manufacturer's GeneChip Hybridization Wash and Stain Kit containing eukaryotic control cRNA and a control oligonucleotide B2 (Affymetrix). Hybridization, staining, and washing processes were achieved at 45°C and 60 rpm for 16 hours in the GeneChipHybridization Oven 640 and the GeneChip Fluidics Station 450 (Affymetrix). The scanning was carried out with the GeneChip Scanner 3000 7G (Affymetrix). All Affymetrix HGU-133plus2 mRNA-array samples passed an initial quality control based on the R-package "arrayQualityMetrics," version 3.24.0.^{E1} Raw intensity expression values were background corrected, log2-transformed, and quantile normalized with the GCRMA algorithm of the R "oligo" package version 1.32.0.^{E2} A linear contrast model was fit to the normalized data using the R package "limma" version 3.24.15^{E3} to identify differentially expressed transcripts. R version was 3.2.2 and bioconductor version was 3.1. In addition, a gene set enrichment analysis (GSEA) showed comparable results to LIMMA, based on the R-package GSEABase (Morgan M, Falcon S, Gentleman R, 2016, GSEABase: Gene set enrichment data structures and methods. R package version 1.36.0., data not shown). In Table E2, the arithmetic mean of combined log differences (nonswitched and switched memB cells of all 3 patients per gene) is displayed.

Exosome analysis

BM and PB serum was freshly prepared by centrifugation. Exosomes were isolated from BM- and PB-derived serum samples with the Total Exosome

Isolation Reagent, followed by RNA extraction with the Total Exosome RNA and Protein Isolation Kit according to manufacturer's instructions (Thermo Fisher Scientific). cDNA was generated with the high-capacity cDNA reverse transcription kit (Applied Biosystems). The following thermal cycler program at a GeneAmp PCR System 9700 (Applied Biosystems) was applied: 10 minutes 25°C, 120 minutes 37°C, 5 minutes 85°C. PCR reactions were performed in 96-well MicroAmpOptical plates (Applied Biosystems) in a final volume of 11 μ L composed of 2 μ L cDNA, 4.25 μ L Diethyl pyrocarbonate (DEPC)-treated water, 6.25 μ L TaqManUniversal PCR Master Mix (Applied Biosystems), and 0.5 μ L 6-carboxyfluorescein (FAM)-labeled DEFA1 (Hs00414018_m1) and DEFA3 (Hs00234383_m1) primer pair/probe sets (Thermo Fisher Scientific). 18S rRNA was used as normalizer gene (primer pair/probe sets: Thermo Fisher Scientific). The following PCR program was used: 1 $\times$ (2 minutes 50°C), 1 $\times$ (10 minutes 95°C), 40 $\times$ to 50 $\times$ (15 seconds 95°C, 1 minute 60°C). PCR was performed at a 7500 Real-Time PCR System (Applied Biosystems).

Bacterial killing assay

PBMCs and BMMCs were cultured for 1 day at 37°C, 5% CO₂, in IMDM medium supplemented with ITS-G (5 μ g/mL Insulin, 5 μ g/mL Transferrin, 5 ng/mL Sodium-Selenite; Thermo Fisher Scientific) followed by freezing of supernatants. 1 $\times$ 10² colony-forming units of wild-type serotype 2 *Streptococcus pneumoniae* (strain D39/NCTC 7466) were incubated with IMDM + 0.1% FCS medium only, or with 5 μ g/mL cathelicidin antimicrobial peptide LL-37 (Innovagen AB, Lund, Sweden) as positive control, or with respective PBMC and BMMC supernatants for 0, 3, and 6 hours in a total volume of 100 μ L, 96-well plates (Sarstedt, Nümbrecht, Germany) at 37°C, 5% CO₂. For α -defensin inhibition, supernatants of PBMCs and BMMCs were preincubated with 1 mg/mL alpha 1-proteinase inhibitor (Sigma-Aldrich) for 1 hour at 37°C, 5% CO₂, before addition to the bacteria. Subsequently bacteria were plated on Columbia Agar plates with 5% sheep PB (BD Biosciences), cultured for 1 day at 37°C, and then quantified for colony-forming units.

ELISA

Supernatants of *Staphylococcus aureus* Cowan strain I/CpG/pokeweed mitogen-stimulated PBMCs and BMMCs were freeze-dried at day 6 after stimulation. DEFA1-3 concentration was determined with a human DEFA1-3 ELISA kit including a monoclonal biotinylated antibody and a streptavidin-peroxidase conjugate (Hycult Biotech, Beutelsbach, Germany) according to manufacturer's instructions. The measurement was done at a Tecan Sunrise microplate reader with Magellan-Data Analysis Software 6.4 (Tecan Group, Männedorf, Switzerland).

Statistics

The 2-sided Mann-Whitney *U* and the Wilcoxon matched-pairs signed-ranks test were applied. Values were expressed as mean $\pm$ SEM. Statistical analysis was performed with the GraphPad Prism 6 software (La Jolla, Calif). *P* values of .05 or less were considered statistically significant.

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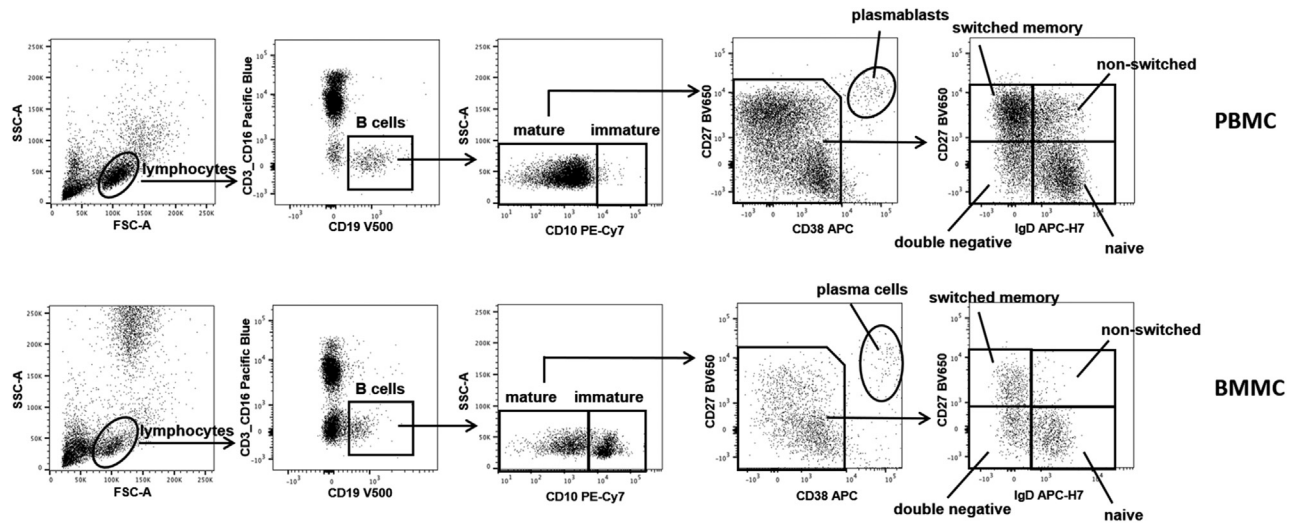


FIG E1. Flow cytometric identification of human B-cell subsets within PB and BM. Shown is the gating strategy for the flow cytometric identification of B-cell subsets within PBMCs (upper row) and BMMCs (lower row). Lymphocytes were first gated for $CD3^+CD16^-CD19^+$ B cells, which were further distinguished for $CD10^-$ mature and $CD10^+$ immature B cells. Within $CD10^-$ mature B cells, $CD38^{hi}CD27^{hi}$ plasmablasts/plasma cells were separated from other mature B-cell subsets, including $CD27^+IgD^-$ switched memory, $CD27^+IgD^+$ nonswitched memory, $CD27^-IgD^+$ naive, and $CD27^-IgD^-$ double-negative B cells. APC, Allophycocyanin; FSC-A, Forward scatter-area; PE, phycoerythrin; SSC-A, side scatter-area.

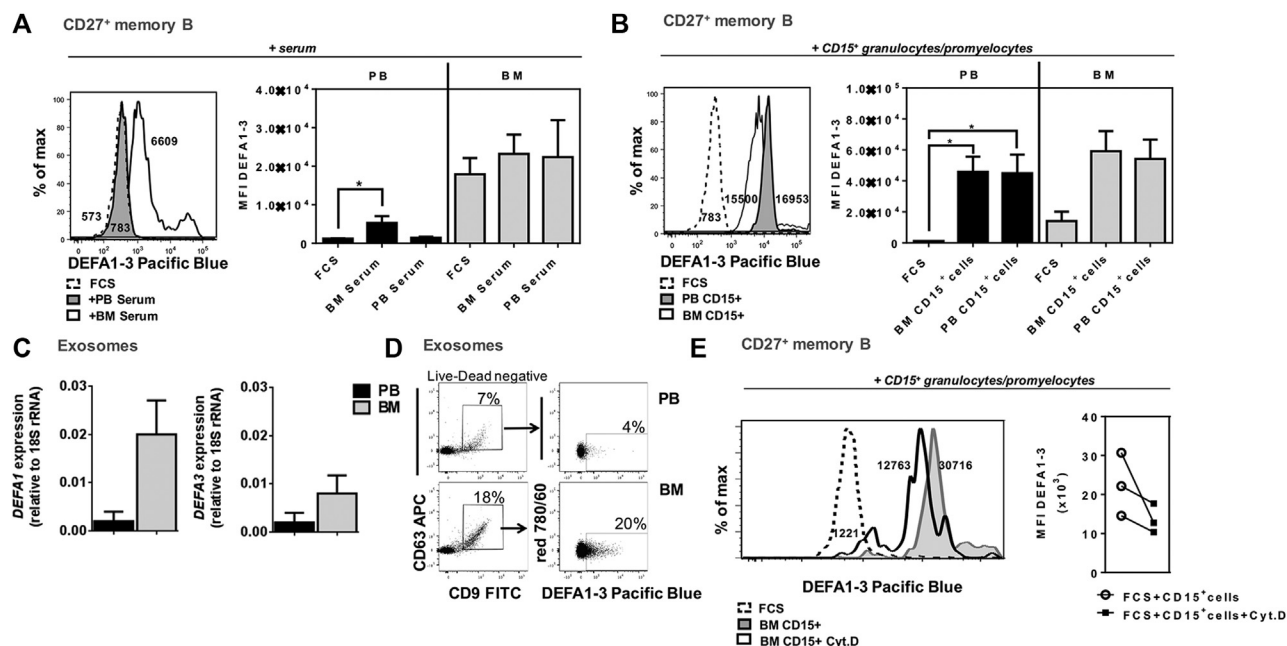


FIG E2. BM-derived serum and CD45^{hi}CD15⁺ cells induce a high DEFA1-3 expression in memB cells. **A**, cDNA expression levels of *DEFA1* and *DEFA3* were determined in exosome samples isolated from PB (black bars, $n = 5$) or BM (gray bars, $n = 5$) with quantitative RT-PCR. The relative expression to 18S rRNA is given. **B**, Exosome preparations from PB (upper graphs) and BM (lower graphs) were analyzed with flow cytometry. Exosomes were identified as Live-Dead[−]CD63⁺CD9⁺ particles (left) and analyzed for intracellular expression of DEFA1-3 (right). Shown is a representative experiment out of 4. **C** and **D**, $(1 \text{ to } 2) \times 10^5$ sorted CD19⁺/CD3⁺ lymphocytes from PB (black bars, $n = 6$) and BM (gray bars, $n = 3$ -6) were cultured in 300 μ L IMDM medium supplemented either with 20% FCS and (Fig E2, **B**) with 5% FCS + 15% BM- or PB-derived serum or (Fig E2, **C**) with 5% FCS + BM- or PB-derived $(1 \text{ to } 2) \times 10^5$ -sorted CD45^{hi}CD15⁺ cells. After 18 hours of incubation at 37°C, 5%CO₂, the DEFA1-3 expression in CD19⁺CD27⁺ memB cells was determined by flow cytometry. Mean values of MFI $\pm$ SEM are given. **E**, For exosome transfer inhibition, sorted BM CD45^{hi}CD15⁺ granulocytes and PB CD19⁺/CD3⁺ lymphocytes were separately preincubated with 20 μ M cytochalasin-D (Sigma-Aldrich) for 40 minutes at 37°C, 5% CO₂. Afterwards, CD19⁺/CD3⁺ lymphocytes from PB were cultured for 18 hours with 20% FCS or 20% FCS + BM-derived CD45^{hi}CD15⁺ cells in the presence or absence of cytochalasin-D (Cyt.D; $n = 3$). The histograms show the DEFA1-3 expression as % of maximum for memB cells after culture with FCS (dashed line), with FCS + CD15⁺ cells (gray filled), and with FCS + CD15⁺ + Cyt.D (white filled). The graph on the right depicts the MFI values obtained from the 3 experiments for the DEFA1-3 expression by memB cells after incubation with FCS + CD15⁺ cells (circles) or with FCS + CD15⁺ cells + Cyt.D (squares). Differences between PBMCs and BMMCs or between 20% FCS control and other stimulations were statistically evaluated with the Wilcoxon matched-pair signed-rank test. *APC*, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *MFI*, mean fluorescence intensity. * $P \leq .05$.

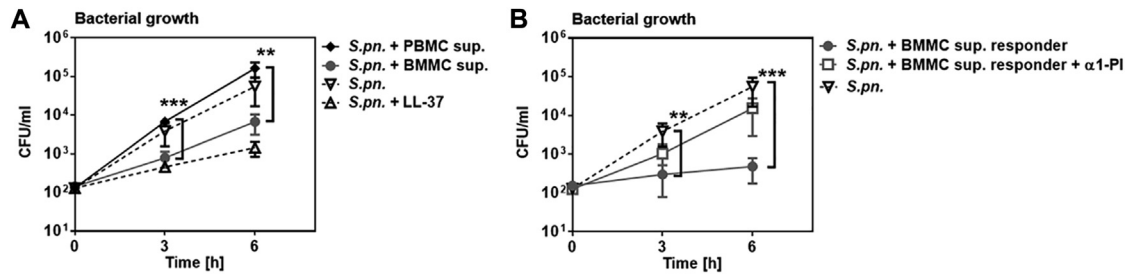


FIG E3. Higher antimicrobial activity by BMMCs than PBMCs supernatants. **A**, Growth of *Streptococcus pneumoniae* (*S.pn.*) was measured for indicated time points in the presence of supernatants (sup.) from cultured BMMCs (circles) and PBMCs (rhombuses) compared with mock. Antimicrobial LL-37 (5 μ g/mL) served as positive control (triangle). **B**, BMMC responders (circles), who mitigated the growth of *S.pn.* by at least 1 log compared with control, from Fig E3, A ($n = 13$ of 17), were mock treated (triangle) or incubated with 1 mg/mL alpha 1-proteinase inhibitor (α 1-PI, squares; $n = 8$) and growth of *S.pn.* was analyzed against control showing a neutralization of the antimicrobial activity in 3 of 8 BMMC supernatants. Shown is the number of colony-forming units (CFU/mL) as mean $\pm$ SEM values (PB: $n = 13$, BM: $n = 17$, controls: $n = 5$). Differences between PBMC or BMMC supernatants were statistically evaluated with the Wilcoxon matched-pair signed-rank test (Fig E3, A) and with the unpaired Mann-Whitney test (Fig E3, B). ** $P \leq .01$, *** $P \leq .001$.

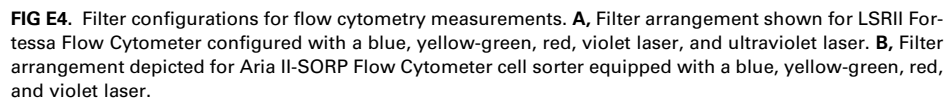


TABLE E1. Fluorochrome-conjugated monoclonal antihuman antibodies for flow cytometry

Antibody	Clone	Company
B-cell subsets		
CD19 V500	HIB19	BD Biosciences
CD19 FITC	HIB19	Biolegend
CD27 BV650	O323	Biolegend
CD27 PE	M-T271	BD Biosciences
IgD-APC H7	IA6-2	BD Biosciences
CD38 APC	HIT2	Biolegend
CD10 PE-Cy7	HI10a	Biolegend
CD24 PerCP Cy5.5	ML5	BD Biosciences
IgM PerCP Cy5.5	MHM-88	Biolegend
T cells		
CD3 Pacific Blue	UCHT1	BD Biosciences
CD3 PerCP Cy5.5	OKT3	Biolegend
Granulocytes, NK cells		
CD15 FITC	HI98	Biolegend
CD16 PE-Cy7, Pacific Blue	3G8	Biolegend
CD45 AF700	HI30	Biolegend
CD56 PE	HCD56	Biolegend
Functional marker		
HLA-DR PerCP-Cy5.5	L243	Biolegend
BAFF-R FITC	11C1	Biolegend
TACI PE	1A1	Biolegend
CXCR3 FITC	49801	R&D Systems
CXCR4 PE	12G5	Biolegend
CCR7 FITC	150503	R&D Systems
CD95 FITC	DX2	Biolegend
Fas-L FITC	SB93a	Abcam
DEFA1-3 Biotin	D21	Hycult Biotech
Ki-67 FITC	REA183	Miltenyi Biotec
TNF- α FITC	MAB11	Affymetrix eBioscience
IL-6 PE	MQ2-6A3	BD Biosciences
Exosomes		
CD9 FITC	HI9a	Biolegend
CD63 APC	H5C6	Biolegend
Isotype controls		
IgG1 κ Biotin	MOPC-21	Hycult Biotech
IgG1 κ FITC	MOPC-21	Biolegend
IgG1 κ PE	MOPC-21	BD Biosciences
IgG2a/ κ PE	MOPC-173	Biolegend
IgG2a/ κ PerCP-Cy5.5	MOPC-173	Biolegend
IgG2b/ κ FITC	MPC-11	Biolegend
Streptavidin conjugates		
Streptavidin PE		BD Biosciences
Streptavidin Pacific Blue		Thermo Fisher Scientific

CD, Cluster of differentiation; V500, violet 500; BV650, brilliant violet 650; APC-H7, allophycocyanin-H7; PE-Cy7, phycoerythrin-cyanin7; PerCP Cy 5.5, peridinin chlorophyll protein-cyanin 5.5; NK, natural killer cells; AF700, Alexa Fluor 700; HLA-DR, human leukocyte antigen-DR; BAFF-R, B cell activating factor receptor; TACI, transmembrane activator and CAML interactor; CXCR3/4, C-X-C chemokine receptor type 3/4; CCR7, C-C chemokine receptor type 7; Fas-L, Fas ligand.

TABLE E2. Differentially expressed genes (log-fold change) of memB cells from BM compared to PB

Upregulated genes					Downregulated genes				
DEFA3	7.18	EGR1	1.84	AZU1	1.16	FASLG	−0.01	NDUFB6	−1
DEFA1B	6.21	MPO	1.82	ZNF331	1.15	IPW	−0.01	ADPRM	−1.01
FOSB	4.24	IL-6	1.81	BEX1	1.15	SORD	−0.01	PRICKLE2-A S3	−1.01
CTSG	3.89	SIK1	1.7	IER5	1.14	NSUN6	−0.02	CEP19	−1.01
CD69	3.37	NR4A1	1.68	MMP8	1.13	ZNF280D	−0.02	SORD	−1.02
NR4A2	3.37	NFKBIA	1.68	PPP1R15B	1.12	HLA-DRB3	−0.02	GRK4	−1.03
FOS	3.34	SCARNA10	1.67	HBB	1.12	HLA-DRB4	−0.03	WEE1	−1.04
DEFA4	3	DUSP10	1.62	ARL4A	1.11	LTB	−0.04	LTB	−1.04
RNASE2	2.74	LTF	1.58	PTGR2	1.1	PTGR2	−0.05	MORC3	−1.06
S100A9	2.59	IPW	1.57	MS4A3	1.08	HLA-DRA	−0.06	CCT6P1	−1.07
RNASE3	2.56	KLF6	1.43	RASGEF1B	1.07	SPDYA	−0.06	ZNF280D	−1.1
RGS2	2.52	CSRP1	1.41	IFRD1	1.07	HLA-DRA	−0.06	DLEU2L	−1.11
RGS1	2.5	DUSP5	1.4	DDX3X	1.07	ZNF280D	−0.07	ANXA2P2	−1.11
S100A8	2.49	TMEM88	1.39	SDCBP	1.06	SMAD4	−0.12	RAP2A	−1.13
GRASP	2.4	OR2L1P	1.37	DDIT3	1.05	HBG2	−0.16	IQGAP2	−1.15
CEACAM8	2.34	KDM6B	1.33	TSPYL2	1.05	KLHL42	−0.17	SMAD4	−1.16
DUSP1	2.27	ZNF165	1.33	CXCR4	1.04	IPW	−0.17	PDIA3P1	−1.17
AREG	2.24	SRGN	1.32	ECRP	1.03	CCT6P1	−0.19	HGB2	−1.17
CEACAM6	2.22	SPDYA	1.31	CRISP3	1.03	SORD	−0.21	FAR2P2	−1.17
CD83	2.14	TRAJ58	1.3	TNFRSF13C	0.81	MORC3	−0.23	RBM12B-AS1	−1.19
JUNB	2.07	CLC	1.3	HLA-DRB1	0.45	HLA-DRA	−0.27	IGHD	−1.21
JUN	2.04	NSUN6	1.28	HLA-DRB5	0.34	FAS	−0.29	CXorf31	−1.25
SLC2A3	2.04	AHSP	1.26	SMAD4	0.33	IPW	−0.29	KLHL42	−1.42
AREG	2.01	CAMP	1.25	CCR7	0.27	TNFRSF13B	−0.32	RAD51D	−1.44
NR4A3	2.01	PER1	1.24	TNF	0.24	ZNF280D	−0.33	SNHG19	−1.73
S100A12	2	CSTA	1.24	IGHD	0.21	MORC3	−0.47	FAR2P2	−2.14
RGCC	1.99	HBD	1.22	HLA-DRB1	0.13	CXCR3	−0.56	PPBP	−2.3
YPEL5	1.99	PTGER4	1.22	HLA-DRB4	0.11	MORC3	−0.57		
ELANE	1.97	GADD45B	1.2	HLA-DRB3	0.06	SORD	−0.62		
PRTN3	1.9	KMT2E-A S1	1.2	GRK4	0.01	CD1C	−0.68		
PPP1R15A	1.85	SCARNA5	1.18			CD80	−0.73		