The Journal of Immunology

Human Plasmacytoid Dendritic Cells Display and Shed B Cell Maturation Antigen upon TLR Engagement

Elisabeth Schuh, Andrea Musumeci, Franziska S. Thaler, Sarah Laurent, Joachim W. Ellwart, Reinhard Hohlfeld, Anne Krug and Edgar Meinl

J Immunol 2017; 198:3081-3088; Prepublished online 10 March 2017;
doi: 10.4049/jimmunol.1601746
http://www.jimmunol.org/content/198/8/3081

The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Human Plasmacytoid Dendritic Cells Display and Shed B Cell Maturation Antigen upon TLR Engagement

Elisabeth Schuh,* Andrea Musumeci,† Franziska S. Thaler,* Sarah Laurent,* Joachim W. Ellwart,‡ Reinhard Hohlfeld,*,§ Anne Krug,† and Edgar Meinl*

The BAFF-APRIL system is best known for its control of B cell homeostasis, and it is a target of therapeutic intervention in autoimmune diseases and lymphoma. By analyzing the expression of the three receptors of this system, B cell maturation Ag (BCMA), transmembrane activator and CAML interactor, and BAFF receptor, in sorted human immune cell subsets, we found that BCMA was transcribed in plasmacytoid dendritic cells (pDCs) in both blood and lymphoid tissue. Circulating human pDCs contained BCMA protein without displaying it on the cell surface. After engagement of TLR7/8 or TLR9, BCMA was detected also on the cell surface of pDCs. The display of BCMA on the surface of human pDCs was accompanied by release of soluble BCMA (sBCMA); inhibition of γ-secretase enhanced surface expression of BCMA and reduced the release of sBCMA by pDCs. In contrast with human pDCs, murine pDCs did not express BCMA, not even after TLR9 activation. In this study, we extend the spectrum of BCMA expression to human pDCs. sBCMA derived from pDCs might determine local availability of its high-affinity ligand APRIL, because sBCMA has been shown to function as an APRIL-specific decoy. Further, therapeutic trials targeting BCMA in patients with multiple myeloma should consider possible effects on pDCs. ***The Journal of Immunology*****, 2017, 198: 3081–3088.**

The two ligands BAFF and APRIL, along with their three receptors, B cell maturation Ag (BCMA), transmembrane activator and CAML interactor (TACI), and BAFF receptor (BAFF-R), are best known for regulating B cell homeostasis (1), but there is evidence that the function of this system exceeds B cell biology (2). This system is a therapeutic target: an mAb to BAFF, belimumab, has been approved in systemic lupus erythematosus (SLE) (3); the soluble receptor atacicept is currently tested in SLE (4), but unexpectedly worsened multiple sclerosis (5, 6); and depleting Abs against the receptors BAFF-R and BCMA are under development for treatment of lymphoma (7). BCMA promotes myeloma growth in the bone marrow (BM) (8), and a recent clinical trial showed that T cells targeting BCMA with a chimeric Ag receptor cause remission in multiple myeloma (9).

In this study, we analyzed whether the three receptors BAFF-R, TACI, and BCMA are found in human blood on immune cell subsets other than B cells. We found that human plasmacytoid dendritic cells (pDCs) transcribe *BCMA*. pDCs comprise ∼0.5% of PBMCs in human blood (10). They develop in the BM, circulate in blood, and reside in lymphoid organs, which they enter from the blood via high endothelial venules (11). pDCs selectively express TLR7 and TLR9, which sense viral RNA and bacterial DNA (12, 13). Upon TLR engagement they produce enormous amounts of IFN-α and proinflammatory cytokines (14–16). We found that circulating human pDCs contain BCMA intracellularly, but do not expose it on the surface in the resting state. Upon engagement of TLR7/8 and TLR9, however, they displayed BCMA also on the surface membrane. This membrane display of BCMA by pDCs was accompanied by release of soluble BCMA (sBCMA), which was mediated by the γ-secretase. Because sBCMA functions as a decoy for APRIL (17), this has immunoregulatory implications. Our finding of BCMA on pDCs is of direct relevance for therapeutic strategies targeting the BAFF-APRIL system, and in particular for therapeutic trials targeting BCMA-expressing cells in patients with multiple myeloma (9, 18).

Materials and Methods

Cell preparation and gating strategy

Buffy coats were obtained from healthy, adult volunteers by venipuncture after written informed consent according to local ethics policy guidelines of the Ludwig-Maximilian University in accordance with the Declaration of Helsinki. PBMCs were separated from buffy coats by Pancoll (Pan Biotech, Aidenbach, Germany) density gradient centrifugation. Single-cell suspensions were prepared from tonsillar tissue by gentle mechanical disruption and sieving. pDCs from whole PBMCs and from tonsillar tissue were isolated by direct magnetic labeling with anti–BDCA-4 (CD304)–conjugated microbeads (positive selection, BDCA-4 cell isolation kit; Miltenyi Biotec, Bergisch Gladbach, Germany) and enriched by using a magnetic cell sorting device (Miltenyi Biotec). pDC purity routinely

*Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, LMU Munich, 82152 Planegg-Martinsried, Germany; †Institute for Immunology, Biomedical Center, LMU Munich, 82152 Planegg-Martinsried, Germany; ‡Helmholtz Center, Institute of Molecular Immunology, 81675 Munich, Germany; and §Munich Cluster for Systems Neurology (SyNergy), LMU Munich and Technical University Munich, 81377 Munich, Germany

ORCID: 0000-0002-2497-0699 (A.M.).

Received for publication October 11, 2016. Accepted for publication February 13, 2017.

This work was supported by Deutsche Forschungsgemeinschaft Grant SFB TR128 (TP B08) to E.M. and R.H. and Grant SFB 1054 (TP A06) to A.K. and A.M., Munich Cluster for Systems Neurology (SyNergy) Grant ExC 1010, the Clinical Competence Network for Multiple Sclerosis, the Centers of Excellence in Neurodegeneration (CoEN), and the Verein zur Therapieforschung für Multiple Sklerose-Kranke.

E.S. performed human experiments, analyzed data, and wrote the paper; A.M. performed mouse experiments and analyzed the data; J.W.E. performed cell sorting; R.H., F.S.T., S.L., A.K., and E.M. contributed to development of the study concept and study supervision, performed analysis and interpretation of data, and assisted with writing, reviewing, and editing of the manuscript. All authors discussed the results and commented on the manuscript.

Address correspondence and reprint requests to Dr. Edgar Meinl, Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals of LMU Munich, Großhaderner Straße 9, 82152 Martinsried-Planegg, Germany. E-mail address: edgar.meinl@med.uni-muenchen.de

The online version of this article contains supplemental material.

Abbreviations used in this article: BAFF-R, BAFF receptor; BCMA, B cell maturation Ag; BM, bone marrow; CpG-A, type A CpG; CpG-B, type B CpG; CpG ODN, CpG-containing ODN; CYC, *cyclophilin A*; GSI, γ-secretase inhibitor; MHCII, MHC class II; ODN, oligodeoxynucleotide; PC, plasma cell; pDC, plasmacytoid dendritic cell; qPCR, quantitative PCR; sBCMA, soluble BCMA; SLE, systemic lupus erythematosus; TACI, transmembrane activator and CAML interactor.

exceeded >90% as determined by surface staining with FITC-conjugated anti–BDCA-2 (CD303 mAb, clone AC144; Miltenyi Biotec) and biotinylated anti-CD123 mAb (clone 7G3; Becton Dickinson, San Jose, CA), as well as appropriate secondary Abs. Similarly, B cells, T cells, and monocytes were isolated from whole PBMCs by direct magnetic labeling with anti-CD19–, anti-CD3–, and anti-CD14–conjugated microbeads (Miltenyi Biotec), respectively.

Murine pDCs were identified by surface staining with PE-Cy7–conjugated anti-CD11c (clone N418), Brilliant Violet 650–conjugated anti-MHC class II (MHCII) (clone M5/114.15.2), brilliant violet 605–conjugated anti-B220 (clone RA3-6B2; all mAbs from BioLegend, San Diego, CA), eFluor450-conjugated anti-BST2 (clone eBio927; eBioscience/Affymetrix, San Diego, CA), and Alexa Fluor 647–conjugated anti–Siglec H (produced in our laboratory from the 440c hybridoma). Allophycocyanin-Cy7–conjugated anti-CD19 (clone 1D3; from BD Biosciences, San Jose, CA) was used to exclude B cells.

Cell sorting

In some experiments human pDCs labeled with FITC-conjugated anti-BDCA-2$^+$ (mAb; clone AC144; Miltenyi Biotec) were, after the MACS, additionally FACS-sorted using MoFlo (Beckman Coulter, Brea, CA). Purity of BDCA-2$^+$ pDCs after cell sorting routinely was ~99% (Supplemental Fig. 1A–C). For murine pDC RNA isolation, freshly isolated BM from type B CpG (CpG-B)–treated and untreated mice was stained as described earlier, and CD11c$^+$ MHCII$^+$ SiglecH^{high} B220^{high} cells were sorted directly into lysis buffer using a FACSAria Fusion (BD Biosciences).

Quantitative PCR

RNA from human blood- and tissue-derived cell subpopulations including pDCs, B cells, T cells, monocytes, and whole mononuclear cells were isolated using the RNeasy Micro Kit (Qiagen, Venlo, the Netherlands). In addition, RNA from murine BM-derived pDCs and murine myeloma cells was isolated using the RNeasy Micro Kit (Qiagen). cDNA was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). For real-time PCR, TaqMan assays (Applied Biosystems) for human *cyclophilin A* (*CYC*), murine *Gapdh*, human *IFN-α1*, human *MCL-1*, and murine *Bcma* were used in combination with the TaqMan PCR Core Reagent Kit (Applied Biosystems). Primers for human *BCMA*, *TACI*, and *BAFF-R* were used as described previously (19). Samples were run as duplicates in MicroAmp Optical 96-well reaction plates (Applied Biosystems) in a 7900HT Fast Real-Time PCR System (Applied Biosystems). Data were analyzed using SDSv2.3 software (Applied Biosystems).

Culture of human pDCs

pDCs were cultured for 72 h on 96-well culture plates in culture medium containing RPMI 1640 (Sigma-Aldrich, St. Louis, MO), 10% FBS (Biochrom AG, Berlin, Germany), 100 U/ml penicillin + 100 μg/ml streptomycin (Life Technologies, Munich, Germany), 1% nonessential amino acids (Life Technologies), 1 mM sodium-pyruvate (Life Technologies), and 2 mM L-glutamine (Pan Biotech). Isolated pDCs were seeded at a concentration of 8×10^5 cells/ml. pDCs were either activated with unmethylated type A CpG (CpG-A) oligodeoxynucleotide (ODN) 2216 (5 μg/ml; Enzo Life Sciences, Framingdale, NY), CpG-B ODN 2006 (2.5 μg/ml; InvivoGen, San Diego, CA), Resiquimod (R848; 1 μg/ml; Sigma-Aldrich), recombinant human IL-3 (25 ng/ml; R&D Systems, Minneapolis, MN), or cocultured with irradiated CD40L-expressing mouse L cells (5×10^4 cells/ml). DAPT (1 μM; Merck Calbiochem, Darmstadt, Germany), a γ-secretase inhibitor (GSI), was added 12 h before supernatants were collected. Every 24 h, if not indicated otherwise, a sample of cells was harvested for flow cytometric analysis and culture supernatants were collected for measurement. The human myeloma cell line JK-6L, the Burkitt's lymphoma cell line Raji, and the murine myeloma cell line J558L were used as positive controls in FACS and quantitative PCR (qPCR) experiments for BAFF-R, BCMA, and TACI (17, 20, 21).

To analyze the effects of APRIL on the transcription of *MCL-1*, we stimulated human blood-derived pDCs with CpG-A (0.5 ng/ml; a half-maximal concentration based on previous experiments) for 48 h after treatment with 500 ng/ml APRIL (Mega-April; Adipogen, San Diego, CA) for 24 h. The GSI DAPT was added 12 h before cells were harvested for qPCR measurements of *MCL-1*.

Flow cytometry

Surface expression of BCMA on human blood- and tissue-derived pDCs was determined by flow cytometry on FACSVerse (Becton Dickinson) using A7D12.2 (IgG2b isotype) (17) (kindly provided by Biogen Idec, Cambridge, MA), the secondary Ab Alexa Fluor 647 (IgG2b isotype; Life Technologies), and the BDCA-2 (Miltenyi), as well as the appropriate isotype control. FlowJo software (FlowJo, Ashland, OR) was used for data analysis. Murine pDCs were identified as described earlier. Murine BCMA was detected by surface staining with biotinylated anti-BCMA (polyclonal goat IgG; R&D Systems) and Alexa Fluor 488–conjugated streptavidin (Molecular Probes). Viable cells were discriminated using propidium iodide (Thermo Fisher Scientific, Darmstadt, Germany) or Fixable Viability Dye eFluor 506 (eBioscience).

Cell lysates

Cells were lysed on ice for 1 h in NP-40 lysis buffer (150 mM NaCl, 50 mM Tris [pH 7.5], 1% Nonidet P-40) containing complete protease inhibitor mixture (Sigma-Aldrich). Total protein concentration was measured by bicinchoninic acid assay (Thermo Fisher Scientific).

ELISA

Cell culture supernatants were centrifuged (2× at 400 × *g* for 10 min and 20,000 × *g* for another 20 min). sBCMA from cell culture supernatants or cell lysates was detected by ELISA (BCMA/TNFRSF17 ELISA Duoset; R&D Systems). IFN-α was measured using the VeriKine Human IFN-α ELISA Kit (R&D Systems).

Murine pDCs and treatment with a GSI

pDCs from mice were analyzed in two ways. In a first set of experiments, mice were injected s.c. with either 50 μg of CpG-B (ODN 1826; Eurofins Genomics, Ebersberg, Germany): DOTAP (Roth, Karlsruhe, Germany) resuspended in PBS or PBS alone. After 72 h, animals were euthanized, and spleen and BM were collected. Single-cell suspensions from each tissue were treated with 1 μM LY-411575 (Sigma-Aldrich) and 1 μM DAPT for 20 h or left untreated. In a second set of experiments, primary murine BM cells were stimulated in vitro with 1 μM CpG-B (ODN 1826) for 24, 48, or 72 h. Two different GSIs, DAPT and LY-411575, were added for the last 20 h of culture. Subsequently these cultures were stained for BCMA expression. pDCs were identified as CD11c$^+$ BST2$^+$ B220$^+$ cells after exclusion of CD19$^+$ MHCII$^+$ cells. The murine myeloma cell line J558L was used as a positive control for BCMA expression and was also treated with GSIs. All experimental procedures involving mice were performed in accordance with the regulations of the local government authority.

Statistics

Statistical significance was assessed with Prism Software (GraphPad) by unpaired *t* test analysis.

Results

Human pDCs transcribe BCMA

We sorted different immune cell subsets from human PBMCs, namely BDCA-2$^+$ pDCs, CD3$^+$ T cells, CD14$^+$ monocytes, and CD19$^+$ B cells, and analyzed the transcript level of *BCMA*, *TACI*, and *BAFF-R* using qPCR. Thereby we found that circulating pDCs transcribe *BCMA* (Fig. 1A). In contrast with CD19$^+$ B cells, human pDCs show only little or no expression of *TACI* and *BAFF-R* (Fig. 1B, 1C). Human pDCs in tonsils also transcribed *BCMA* (Supplemental Fig. 2A).

pDCs in human blood carry the BCMA protein, but do not display BCMA on the cell surface

Next, we analyzed BCMA surface expression on BDCA-2$^+$ pDCs by FACS. We found that pDCs directly isolated ex vivo did not express detectable amounts of BCMA protein on the cell surface; this was seen with pDCs in blood (Fig. 1D) and tonsils (Supplemental Fig. 2B). The human myeloma cell line JK-6L was used as a positive control for the BCMA staining (Fig. 1D). Cell lysates from whole PBMCs, BDCA-2$^+$ pDCs, CD3$^+$ T cells, CD19$^+$ B cells, and CD14$^+$ monocytes were tested for intracellular BCMA protein content by ELISA to determine whether pDCs contain BCMA protein. In comparison with all other blood cell subpopulations, pDCs showed the highest level of BCMA protein (Fig. 1E).

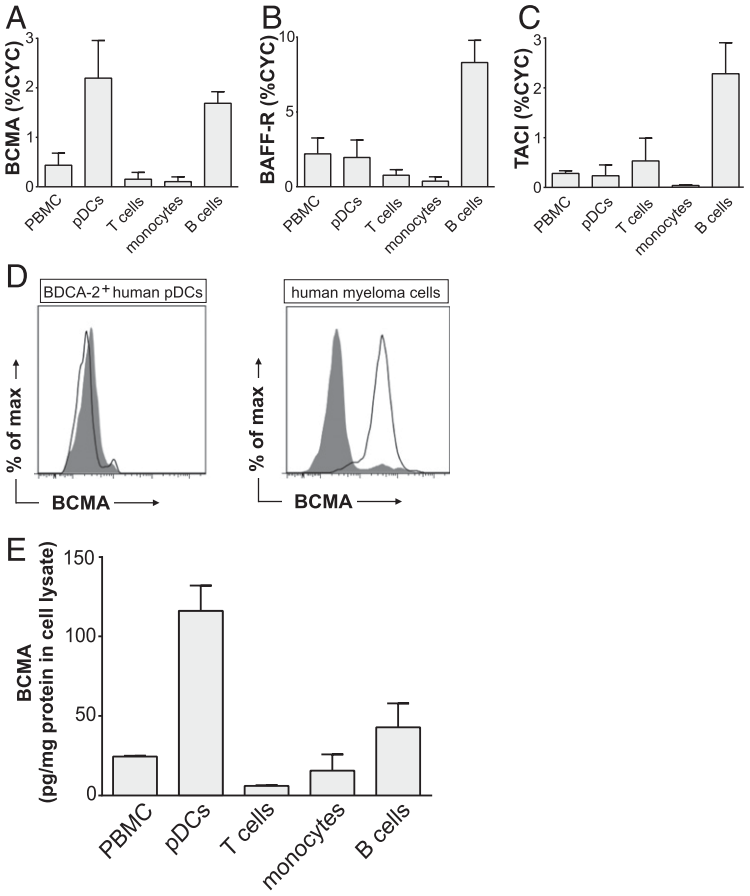

FIGURE 1. BCMA transcript and protein in blood-derived pDCs. (**A**–**C**) The expression levels of *BCMA*, *BAFF-R*, and *TACI* were analyzed by qPCR in PBMCs and sorted immune cell subsets including pDCs (BDCA-2$^+$), T cells (CD3$^+$), B cells (CD19$^+$), and monocytes (CD14$^+$). Transcript levels of *BCMA*, *TACI*, and *BAFF-R* were determined in comparison with the housekeeping gene cyclophilin (% CYC). Combined data of three independent experiments with three donors are depicted (mean ± SEM). (**D**) Human pDCs (BDCA2$^+$ fraction in PBMCs) and human myeloma cells (J558L) were stained for BCMA expression (solid line). The closed graph represents the isotype control. One representative of six independent experiments is shown. (**E**) The BCMA contents of cell lysates from whole PBMCs, sorted BDCA-2$^+$ pDCs, CD3$^+$ T cells, CD19$^+$ B cells, and CD14$^+$ monocytes were determined by ELISA. BCMA protein content is shown in relation to total protein levels. pDCs show higher BCMA protein than PBMCs ($p < 0.05$), T cells ($p < 0.01$), monocytes ($p < 0.01$), as well as B cells ($p < 0.05$). Combined data of four independent experiments with different donors are shown (mean ± SEM; unpaired *t* test).

Human pDCs display BCMA on the cell surface after TLR engagement

We analyzed whether activation of pDCs had an effect on BCMA expression on the cell surface. We sorted pDCs from peripheral blood and activated them with the TLR9 ligand CpG-A (ODN 2216), the TLR7/8 ligand R848, IL-3, or by cocultivation with irradiated CD40L-expressing mouse fibroblasts. This showed that pDCs display BCMA on the cell surface after stimulation with CpG-A (ODN 2216) and R848, whereas IL-3 or CD40L had only little effects on BCMA expression on the cell surface (Fig. 2A). In contrast with BCMA, the other receptors for BAFF and APRIL, BAFF-R and TACI, were not induced on human pDCs by any of these stimuli (Supplemental Fig. 3A, 3B). The human myeloma cell line JK-6L and Raji cells derived from Burkitt's lymphoma were used as a positive control for the BCMA, BAFF-R, and TACI (Fig. 1D, Supplemental Fig. 3C).

To analyze whether the enhanced surface expression of BCMA after TLR-mediated activation was accompanied by transcriptional induction, we quantified the mRNA for *BCMA* by qPCR after stimulation with CpG at different time points. We used *IFN-α1* as a positive control. Although transcripts for *IFN-α1* were rapidly induced, we did not detect induction of *BCMA* (Supplemental Fig. 4).

pDCs shed sBCMA, which is mediated by the γ-secretase

We found previously that the γ-secretase directly sheds BCMA from plasma cells (PCs) (17). We now analyzed the shedding of BCMA from pDCs using FACS and ELISA as readout systems. We found that the surface expression of BCMA on pDCs (Fig. 2A) is accompanied by the appearance of sBCMA in the supernatant of these cells (Fig. 2A). Thus, we saw a significant release of sBCMA after treatment with CpG-A (ODN 2216) and R848 ($p < 0.05$, unpaired *t* test). IL-3 had no effect, and coculture with irradiated CD40L-expressing mouse fibroblast slightly enhanced surface expression, but did not significantly induce sBCMA release into the supernatant (Fig. 2A).

To study the role of the γ-secretase in shedding of BCMA from pDCs, we applied the GSI DAPT. We stimulated pDCs with CpG-A ODN 2216 and cultured them for 24, 48, or 72 h in the presence or absence of the GSI DAPT that was added 12 h before supernatants were harvested. Production of sBCMA was strongly decreased in the presence of DAPT (Fig. 2B). In parallel to the decrease of sBCMA in the cell culture supernatant, we observed an increase of BCMA surface expression after DAPT treatment as analyzed by FACS (Fig. 2B).

Because BCMA is a survival receptor for PCs in the BM (22) via induction of the antiapoptotic protein Mcl-1 (23), we tested whether the expression of *MCL-1* was modulated in activated human pDCs treated with APRIL. Thereby we noted a high basal expression of *MCL-1* that was not further enhanced under our culture conditions by APRIL. Specifically, we found in human blood-derived pDCs after stimulation with CpG-A for 48 h and APRIL for the final 24 h in the presence or absence of the GSI DAPT the following expression levels of *MCL-1* (mean % CYC ± SEM of two experiments): nonstimulated, 17.9 ± 0.1%; CpG-A, 19.4 ± 1.1%; CpG-A+APRIL, 19.8 ± 0.4%; CpG-A+DAPT+APRIL, 21.5 ± 10.4%; APRIL, 20.8 ± 5.6%; and DAPT, 18.5 ± 2.4%.

CpG-A, CpG-B, and R848 induce the release of sBCMA from pDCs, but differ in induction of IFN-α

To assess the effects of different CpG oligonucleotides on pDCs with regard to sBCMA release and IFN-α production, we treated primary human pDCs isolated from peripheral blood either with the TLR9 ligands CpG-A (ODN 2216) and CpG-B (ODN 2006) or

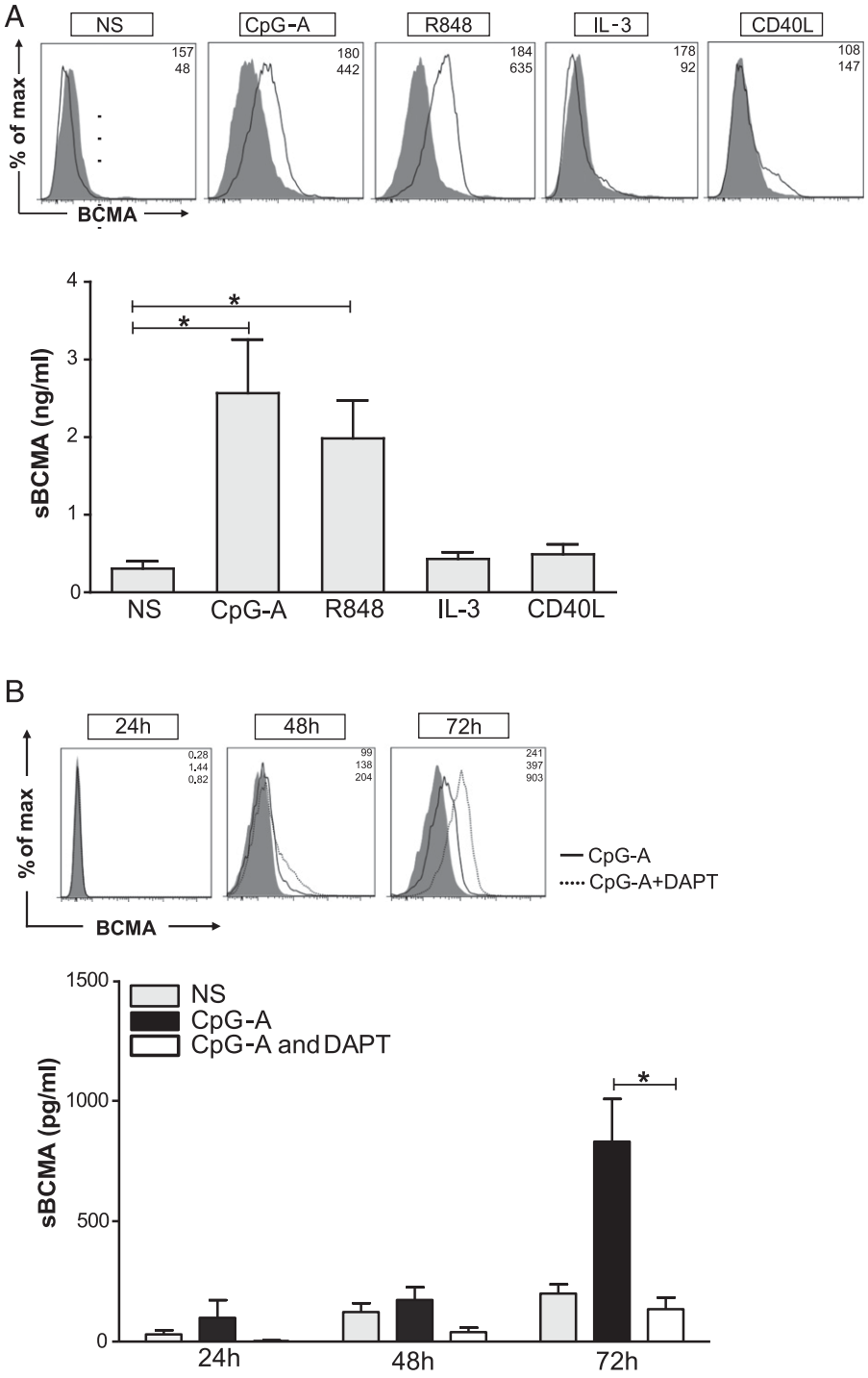

FIGURE 2. pDCs display BCMA on the cell surface and release sBCMA upon TLR activation, mediated by the γ-secretase. Human pDCs were sorted from PBMCs and cultured as indicated. Surface expression of BCMA was analyzed by FACS; filled gray histograms represent the isotype control, and solid black lines the BCMA surface expression. sBCMA in the culture supernatant was determined by ELISA. (**A**) pDCs were either cultured unstimulated (NS) or were activated with CpG-A, R848, IL-3, or CD40L for 3 d. Three experiments with three different donors were performed. For surface staining one representative experiment and for supernatant analysis the summary of all three experiments is shown. Closed histograms represent the isotype control, and solid black lines the BCMA expression. Numbers in the histograms indicate the mean fluorescence intensity (MFI) of the isotype control (upper number) and after stimulation with the indicated activator (lower number). Mean ± SEM; $*p < 0.05$, unpaired *t* test. (**B**) Human sorted pDCs were activated with CpG-A in the presence or absence of the GSI DAPT or cultured without exogenous stimulus (NS). Closed histograms represent the isotype control of DAPT-treated cells, solid black lines show the BCMA expression, whereas dotted black lines show the BCMA expression in the presence of DAPT. Numbers in the histograms indicate the MFI of the isotype control (upper number) and after stimulation with CpG in the absence (middle number) or presence (lower number) of the GSI DAPT. For BCMA surface staining one representative from three independent experiments is shown. For supernatant analysis combined data of three independent experiments are shown (mean ± SEM). $*p < 0.05$, unpaired *t* test.

the TLR7/8 ligand R848. We found that CpG-A strongly induced type-I IFN production as determined by ELISA, whereas CpG-B and R848 did not induce high amounts of IFN-α. In contrast, CpG-A, CpG-B, as well as R848 induced sBCMA release to a similar extent, with concentrations in the cell culture supernatants of around 2.0 ng/ml (Fig. 3).

Murine spleen and BM pDCs do not display or shed BCMA

We analyzed whether not only human, but also murine, pDCs display BCMA on the surface after TLR engagement, because this would allow elaborating the functional relevance of BCMA on pDCs in vivo. We performed two sets of experiments. First, we analyzed BCMA expression in pDCs from the BM and spleen of CpG-B–treated and untreated mice in the presence or absence of the GSI LY-411575. We analyzed surface expression by flow cytometry gating on $CD11c^+$ $MHCII^+$ $SiglecH^{high}$ $B220^{high}$ pDCs (Fig. 4A) and determined the transcript levels of *Bcma* by quantitative RT-PCR using pDCs sorted from the BM of CpG-B–treated mice. Thereby we found that murine BM-pDCs neither expressed BCMA on the surface nor contained relevant amounts of *Bcma* mRNA, in contrast with murine myeloma cells (Fig. 4B, 4C).

Second, we obtained primary BM cells from mice, and activated them in vitro with CpG-B for different time periods in the presence or absence of the GSIs DAPT or LY-411575. Activation of pDCs may downregulate Siglec H (24). Therefore, murine pDCs were identified as $CD11c^+BST2^+B220^+$ cells after exclusion of $CD19^+$

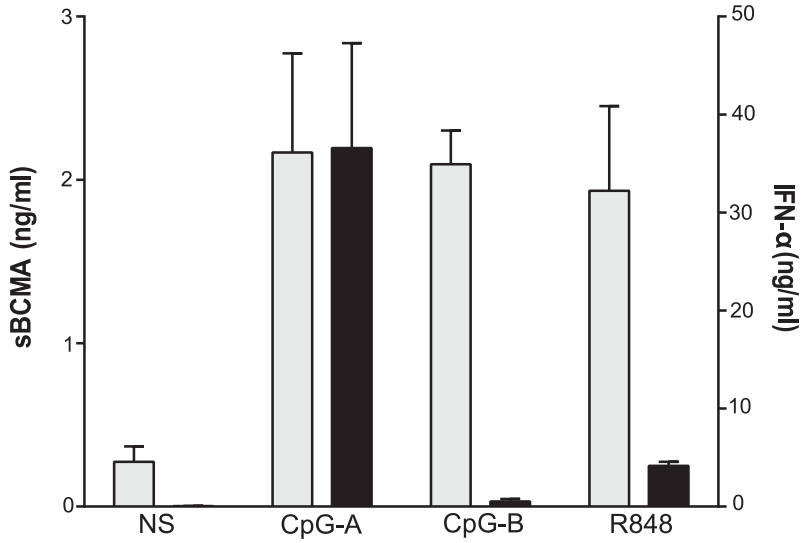

FIGURE 3. Effects of CpG-A, CpG-B, and R848 on the release of sBCMA and IFN-α from pDCs. Human purified pDCs were left unstimulated in culture (NS) or activated with CpG-A, CpG-B, or R848 for 3 d. The amount of sBCMA (light gray bars) and IFN-α (black bars) in the supernatant was determined by ELISA. Combined data of three independent experiments are depicted and represent three independent blood donations (mean ± SEM).

MHCII$^+$ cells (Fig. 4D). Although murine BM pDCs upregulated CD86 expression after CpG-B treatment (data not shown), BCMA expression was not detectable, even after treatment with GSIs (Fig. 4E). In contrast, our positive control murine myeloma cells showed enhanced levels of BCMA on the cell surface after exposure to the GSIs (Fig. 4F). We conclude that, in contrast with human pDCs, murine pDCs do not display detectable amounts of BCMA on the cell surface even after TLR9 activation and γ-secretase inhibition.

Discussion

In this study, we extend our current understanding of the BAFF-APRIL system beyond the B cell compartment by showing that human pDCs display and shed BCMA.

pDCs are key players in maintaining the balance between an efficient defense and preventing autoimmune responses; they have been implicated in immune tolerance, inflammation, and are part of the microenvironment of tumors (25–28). One of the most prominent features of pDCs is their production of IFN-I in response to TLR activation (29–31). We report in this article that, in response to TLR7/8 or TLR9 engagement, pDCs display BCMA on their surface.

BCMA is a survival receptor for PCs in the BM (22) via induction of the antiapoptotic protein MCL-1 (23). We observed a strong basal expression of *MCL-1* in pDCs, which in our culture conditions was not further enhanced by treatment with APRIL in the presence or absence of a GSI. BCMA is also displayed by multiple myeloma cells and mediates their survival (8, 9, 32). Therefore, strategies with mAbs that deplete BCMA-expressing cells are in clinical development (18, 32). Recently, it was reported that chimeric Ag receptor T cells targeting BCMA caused remission in multiple myeloma patients (9). We show in this study that blood-derived pDCs display BCMA on their surface upon TLR engagement. We propose therefore that effects on pDCs should be considered in therapies targeting BCMA. In multiple myeloma patients the BM constitutes a microenvironment that promotes tumor cell growth and immunosuppression (8). pDCs participate in this environment and support myeloma growth and survival (33). pDCs derived from the BM of multiple myeloma patients, but not from controls, have been reported to express BCMA (32) and to respond to APRIL with enhanced survival (8).

We found that the surface display of BCMA on pDCs is accompanied by its shedding. Shedding of the extracellular part of membrane receptors is a general immunoregulatory mechanism (34). The shedding of BCMA not only regulates the amount of surface display of this receptor, but in addition generates a soluble variant, sBCMA. pDC-derived sBCMA may fine-tune the local availability of APRIL in a microenvironment because sBCMA acts as decoy receptor for APRIL in vitro (17). This may be particularly relevant in the BM, where pDCs (~0.4% of the nuclear cells) (35) are more abundant than PCs (~0.2%). The BM constitutes a survival niche for long-lived PCs (36), and APRIL is one of the critical survival factors for PCs in this environment (37). Because sBCMA functions as a decoy for APRIL (17), sBCMA released by pDCs might modulate the survival niche for PCs in the BM.

The biochemical mechanism of shedding of different BAFF and APRIL receptors is different. We have previously shown that ADAM-10 sheds TACI from activated B cells (20), and that the γ-secretase releases BCMA from PCs (17). In this article, we report that the γ-secretase sheds BCMA from pDCs; this can be explained by the ubiquitous expression of the γ-secretase (38, 39) and the facilitation of γ-secretase–mediated shedding of BCMA by the shortness of the extracellular domain of BCMA (17). sBCMA may also function as a potential biomarker in immunopathologies such as multiple sclerosis and SLE (17). Further, sBCMA is elevated and of prognostic value in multiple myeloma (40). Based on our findings, sBCMA should now be analyzed in a malignancy that is derived from pDCs, blastic pDC neoplasm (41).

We noted that both CpG-A and CpG-B were potent inducers of sBCMA release from pDC, whereas only CpG-A was able to induce great amounts of type-I IFNs. CpG-containing ODNs (CpG ODNs) are short ssDNA molecules that can be used as synthetic analogs to potently induce type-I IFN production in pDCs (42). Not all of the known CpG ODNs can induce pDCs to produce type-I IFNs (7). CpG ODNs themselves are classified into two main groups: D-type, now known as CpG-A ODNs; and K-type, now known as CpG-B ODNs (43, 44). CpG-A ODNs can efficiently stimulate IFN-α production by pDCs (45–48), whereas CpG-B ODNs are a weak inducer of IFN-α (43, 49). Both CpG-A and CpG-B ODNs can stimulate pDCs to produce proinflammatory cytokines (e.g., TNF) and upregulate costimulatory molecule expression, although CpG-B ODNs appear to be more potent for induction of CD80, CD86, MHC class II, and chemokine IL-8 (50). In contrast with CpG-A, CpG-B ODNs also induce B cell proliferation and Ab production efficiently, thereby linking innate and adaptive immunity (44, 49). Our results suggest that induction of BCMA

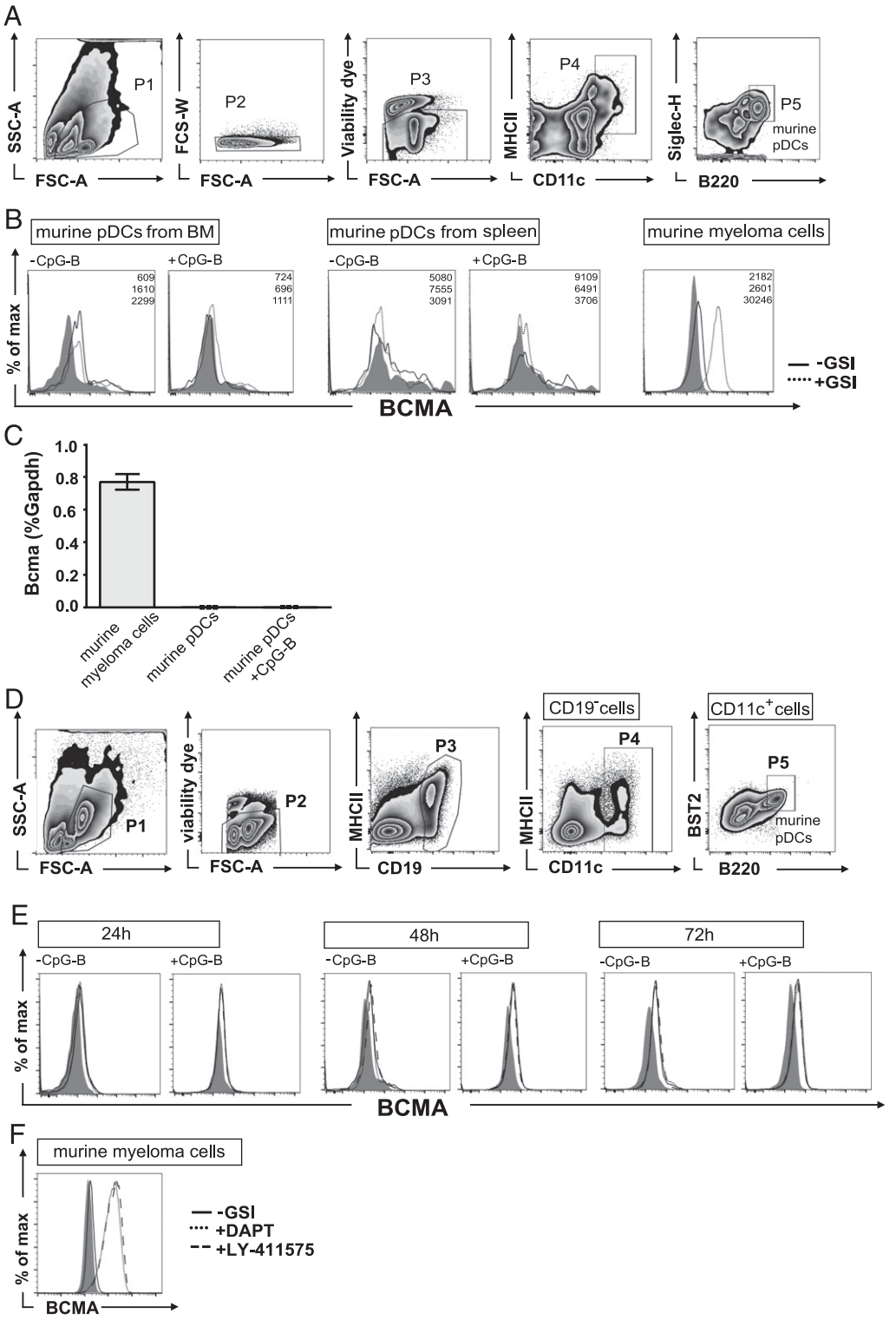

FIGURE 4. Murine pDCs do not display BCMA on the surface after TLR engagement. (**A**–**C**) Murine pDCs were activated with CpG-B in vivo for 72 h. BM cells and splenocytes were then cultured in the presence or absence of the GSIs LY-411575 and DAPT (both 1 μM) for 20 h. (A) We gated on singlets (P2) and living cells (P3) from all lymphocytes (P1). Murine pDCs were then identified by gating on the $CD11c^+$ $MHCII^+$ $B220^+$ $SiglecH^+$ fraction (P4 and P5). (B) Membrane expression of BCMA was determined by FACS. Filled gray histograms represent the isotype control, and solid lines represent staining with anti-BCMA. Dotted lines indicate treatment with the GSI LY-411575, and black lines show samples treated without GSI. Numbers in the histograms indicate the mean fluorescence intensity (MFI). (C) The transcript levels of *Bcma* were determined from sorted murine BM-pDCs, with or without stimulation with CpG-B. Combined data from murine pDCs of three mice with mean ± SEM are shown. Murine myeloma cells served as a positive control for *Bcma* expression. (**D** and **E**) In a second experimental approach, primary murine BM cells were cultured in medium containing 1% Flt3l-containing supernatant (2.5×10^6 cells/ml) in the presence or absence of 1 μM CpG-B for the indicated time periods with or without addition of GSIs and analyzed by flow cytometry. (D) Gating strategy for pDCs: doublets and dead cells (P2) from all lymphocytes (P1) are excluded. Murine pDCs were identified by excluding $CD19^+MHCII^+$ (P3) cell fraction first and then gating on $CD11c^+BST2^+B220^+$ (P4 and P5) cells. (E) Flow cytometry analysis for BCMA surface expression was performed after 24, 48, and 72 h of stimulation with CpG-B in the absence (solid line) or in the presence of the GSIs LY-411575 (dashed line) or DAPT (dotted line). (**F**) Murine myeloma cells served as a positive control. Filled gray histograms represent the isotype control.

expression does not require the delayed kinetic of TLR9 activation that mediates the strong type-I IFN response triggered by CpG-A (51).

We have analyzed the presence of BCMA on murine pDCs. Although human and murine PCs display and shed BCMA similarly (17), we found that BCMA is produced only by human, but not by mouse, pDCs at detectable levels. This is in harmony with a previous study that did not detect surface BCMA on murine pDCs, although in this previous work the surface expression of BCMA on pDCs was not analyzed after TLR9 activation (52). Nonetheless, it cannot be excluded that murine pDCs may express BCMA under conditions not tested in this study, or maybe murine pDCs from another organ could express it. Although mice are a valuable model system to learn about immunological mechanisms relevant for humans, species differences between mice and human have to be considered when planning further functional experiments (53).

Taken together, this study extends the complexity of the BAFF-APRIL system, which is critically involved in a variety of diseases and serves as a drug target. The expression of BCMA by human pDCs is of direct relevance for clinical trials targeting BCMA in patients with multiple myeloma (7, 9) and for therapies targeting the BAFF-APRIL system in autoimmune diseases (54, 55).

Acknowledgments

We thank the blood donors who participated in this study and we also thank Dr. E. Beltran and Dr. N. Kawakami for comments on the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

1. Mackay, F., and P. Schneider. 2009. Cracking the BAFF code. *Nat. Rev. Immunol.* 9: 491–502.
2. Vincent, F. B., D. Saulep-Easton, W. A. Figgett, K. A. Fairfax, and F. Mackay. 2013. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. *Cytokine Growth Factor Rev.* 24: 203–215.
3. Stohl, W., S. Metyas, S. M. Tan, G. S. Cheema, B. Oamar, D. Xu, V. Roschke, Y. Wu, K. P. Baker, and D. M. Hilbert. 2003. B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum.* 48: 3475–3486.
4. Isenberg, D., C. Gordon, D. Licu, S. Copt, C. P. Rossi, and D. Wofsy. 2015. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). [Published erratum appears in 2016 Ann. Rheum. Dis. 75: 946.] *Ann. Rheum. Dis.* 74: 2006–2015.
5. Kappos, L., H. P. Hartung, M. S. Freedman, A. Boyko, E. W. Radü, D. D. Mikol, M. Lamarine, Y. Hyvert, U. Freudensprung, T. Plitz, and J. van Beek, ATAMS Study Group. 2014. Atacicept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial. *Lancet Neurol.* 13: 353–363.
6. Hoffmann, F., and E. Meinl. 2014. B cells in multiple sclerosis: good or bad guys?: an article for 28 May 2014 - World MS Day 2014. *Eur. J. Immunol.* 44: 1247–1250.
7. Croft, M., C. A. Benedict, and C. F. Ware. 2013. Clinical targeting of the TNF and TNFR superfamilies. *Nat. Rev. Drug Discov.* 12: 147–168.
8. Tai, Y. T., C. Acharya, G. An, M. Moschetta, M. Y. Zhong, X. Feng, M. Cea, A. Cagnetta, K. Wen, H. van Eenennaam, et al. 2016. APRIL and BCMA promote human multiple myeloma growth and immunosuppression in the bone marrow microenvironment. *Blood* 127: 3225–3236.
9. Ali, S. A., V. Shi, I. Maric, M. Wang, D. F. Stroncek, J. J. Rose, J. N. Brudno, M. Stetler-Stevenson, S. A. Feldman, B. G. Hansen, et al. 2016. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood* 128: 1688–1700.
10. Lande, R., and M. Gilliet. 2010. Plasmacytoid dendritic cells: key players in the initiation and regulation of immune responses. *Ann. N. Y. Acad. Sci.* 1183: 89–103.
11. Sozzani, S., W. Vermi, A. Del Prete, and F. Facchetti. 2010. Trafficking properties of plasmacytoid dendritic cells in health and disease. *Trends Immunol.* 31: 270–277.
12. Heil, F., H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner, and S. Bauer. 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303: 1526–1529.
13. Bauer, S., C. J. Kirschning, H. Häcker, V. Redecke, S. Hausmann, S. Akira, H. Wagner, and G. B. Lipford. 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc. Natl. Acad. Sci. USA* 98: 9237–9242.
14. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur. J. Immunol.* 31: 3026–3037.
15. Proietto, A. I., M. O'Keeffe, K. Gartlan, M. D. Wright, K. Shortman, L. Wu, and M. H. Lahoud. 2004. Differential production of inflammatory chemokines by murine dendritic cell subsets. *Immunobiology* 209: 163–172.
16. Jego, G., A. K. Palucka, J. P. Blanck, C. Chalouni, V. Pascual, and J. Banchereau. 2003. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 19: 225–234.
17. Laurent, S. A., F. S. Hoffmann, P. H. Kuhn, Q. Cheng, Y. Chu, M. Schmidt-Supprian, S. M. Hauck, E. Schuh, M. Krumbholz, H. Rübsamen, et al. 2015. γ-Secretase directly sheds the survival receptor BCMA from plasma cells. *Nat. Commun.* 6: 7333.
18. Tai, Y. T., and K. C. Anderson. 2015. Targeting B-cell maturation antigen in multiple myeloma. *Immunotherapy* 7: 1187–1199.
19. Krumbholz, M., D. Theil, T. Derfuss, A. Rosenwald, F. Schrader, C. M. Monoranu, S. L. Kalled, D. M. Hess, B. Serafini, F. Aloisi, et al. 2005. BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. *J. Exp. Med.* 201: 195–200.
20. Hoffmann, F. S., P. H. Kuhn, S. A. Laurent, S. M. Hauck, K. Berer, S. A. Wendlinger, M. Krumbholz, M. Khademi, T. Olsson, M. Dreyling, et al. 2015. The immunoregulator soluble TACI is released by ADAM10 and reflects B cell activation in autoimmunity. *J. Immunol.* 194: 542–552.
21. Rennert, P., P. Schneider, T. G. Cachero, J. Thompson, L. Trabach, S. Hertig, N. Holler, F. Qian, C. Mullen, K. Strauch, et al. 2000. A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. *J. Exp. Med.* 192: 1677–1684.
22. O'Connor, B. P., V. S. Raman, L. D. Erickson, W. J. Cook, L. K. Weaver, C. Ahonen, L. L. Lin, G. T. Mantchev, R. J. Bram, and R. J. Noelle. 2004. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med.* 199: 91–98.
23. Peperzak, V., I. Vikström, J. Walker, S. P. Glaser, M. LePage, C. M. Coquery, L. D. Erickson, K. Fairfax, F. Mackay, A. Strasser, et al. 2013. Mcl-1 is essential for the survival of plasma cells. *Nat. Immunol.* 14: 290–297.
24. Puttur, F., C. Arnold-Schrauf, K. Lahl, G. Solmaz, M. Lindenberg, C. T. Mayer, M. Gohmert, M. Swallow, C. van Helt, H. Schmitt, et al. 2013. Absence of Siglec-H in MCMV infection elevates interferon alpha production but does not enhance viral clearance. *PLoS Pathog.* 9: e1003648.
25. Liu, C., Y. Lou, G. Lizée, H. Qin, S. Liu, B. Rabinovich, G. J. Kim, Y. H. Wang, Y. Ye, A. G. Sikora, et al. 2008. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. *J. Clin. Invest.* 118: 1165–1175.
26. Irla, M., N. Küpfer, T. Suter, R. Lissilaa, M. Benkhoucha, J. Skupsky, P. H. Lalive, A. Fontana, W. Reith, and S. Hugues. 2010. MHC class II-restricted antigen presentation by plasmacytoid dendritic cells inhibits T cell-mediated autoimmunity. *J. Exp. Med.* 207: 1891–1905.
27. Martinelli, E., C. Cicala, D. Van Ryk, D. J. Goode, K. Macleod, J. Arthos, and A. S. Fauci. 2007. HIV-1 gp120 inhibits TLR9-mediated activation and IFN-alpha secretion in plasmacytoid dendritic cells. *Proc. Natl. Acad. Sci. USA* 104: 3396–3401.
28. Menon, M., P. A. Blair, D. A. Isenberg, and C. Mauri. 2016. A regulatory feedback between plasmacytoid dendritic cells and regulatory B cells is aberrant in systemic lupus erythematosus. *Immunity* 44: 683–697.
29. Swiecki, M., and M. Colonna. 2015. The multifaceted biology of plasmacytoid dendritic cells. *Nat. Rev. Immunol.* 15: 471–485.
30. Ganguly, D., S. Haak, V. Sisirak, and B. Reizis. 2013. The role of dendritic cells in autoimmunity. *Nat. Rev. Immunol.* 13: 566–577.
31. Reizis, B., A. Bunin, H. S. Ghosh, K. L. Lewis, and V. Sisirak. 2011. Plasmacytoid dendritic cells: recent progress and open questions. *Annu. Rev. Immunol.* 29: 163–183.
32. Tai, Y. T., P. A. Mayes, C. Acharya, M. Y. Zhong, M. Cea, A. Cagnetta, J. Craigen, J. Yates, L. Gliddon, W. Fieles, et al. 2014. Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. *Blood* 123: 3128–3138.
33. Chauhan, D., A. V. Singh, M. Brahmandam, R. Carrasco, M. Bandi, T. Hideshima, G. Bianchi, K. Podar, Y. T. Tai, C. Mitsiades, et al. 2009. Functional interaction of plasmacytoid dendritic cells with multiple myeloma cells: a therapeutic target. *Cancer Cell* 16: 309–323.
34. Levine, S. J. 2008. Molecular mechanisms of soluble cytokine receptor generation. *J. Biol. Chem.* 283: 14177–14181.
35. Nizzoli, G., J. Krietsch, A. Weick, S. Steinfelder, F. Facciotti, P. Gruarin, A. Bianco, B. Steckel, M. Moro, M. Crosti, et al. 2013. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* 122: 932–942.
36. Manz, R. A., A. E. Hauser, F. Hiepe, and A. Radbruch. 2005. Maintenance of serum antibody levels. *Annu. Rev. Immunol.* 23: 367–386.
37. Kometani, K., and T. Kurosaki. 2015. Differentiation and maintenance of long-lived plasma cells. *Curr. Opin. Immunol.* 33: 64–69.
38. Kopan, R., and M. X. Ilagan. 2004. Gamma-secretase: proteasome of the membrane? *Nat. Rev. Mol. Cell Biol.* 5: 499–504.
39. Selkoe, D. J., and M. S. Wolfe. 2007. Presenilin: running with scissors in the membrane. *Cell* 131: 215–221.
40. Sanchez, E., M. Li, A. Kitto, J. Li, C. S. Wang, D. T. Kirk, O. Yellin, C. M. Nichols, M. P. Dreyer, C. P. Ahles, et al. 2012. Serum B-cell maturation antigen is elevated in multiple myeloma and correlates with disease status and survival. *Br. J. Haematol.* 158: 727–738.

41. Chaperot, L., N. Bendriss, O. Manches, R. Gressin, M. Maynadie, F. Trimoreau, H. Orfeuvre, B. Corront, J. Feuillard, J. J. Sotto, et al. 2001. Identification of a leukemic counterpart of the plasmacytoid dendritic cells. *Blood* 97: 3210–3217.
42. Marshak-Rothstein, A., and I. R. Rifkin. 2007. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu. Rev. Immunol.* 25: 419–441.
43. Kadowaki, N., S. Antonenko, and Y. J. Liu. 2001. Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c- type 2 dendritic cell precursors and CD11c+ dendritic cells to produce type I IFN. *J. Immunol.* 166: 2291–2295.
44. Verthelyi, D., K. J. Ishii, M. Gursel, F. Takeshita, and D. M. Klinman. 2001. Human peripheral blood cells differentially recognize and respond to two distinct CPG motifs. *J. Immunol.* 166: 2372–2377.
45. Krug, A., S. Rothenfusser, V. Hornung, B. Jahrsdörfer, S. Blackwell, Z. K. Ballas, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur. J. Immunol.* 31: 2154–2163.
46. Vollmer, J., R. Weeratna, P. Payette, M. Jurk, C. Schetter, M. Laucht, T. Wader, S. Tluk, M. Liu, H. L. Davis, and A. M. Krieg. 2004. Characterization of three CpG oligodeoxynucleotide classes with distinct immunostimulatory activities. *Eur. J. Immunol.* 34: 251–262.
47. Hornung, V., S. Rothenfusser, S. Britsch, A. Krug, B. Jahrsdörfer, T. Giese, S. Endres, and G. Hartmann. 2002. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J. Immunol.* 168: 4531–4537.
48. Gerosa, F., A. Gobbi, P. Zorzi, S. Burg, F. Briere, G. Carra, and G. Trinchieri. 2005. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J. Immunol.* 174: 727–734.
49. Hanabuchi, S., N. Watanabe, Y. H. Wang, Y. H. Wang, T. Ito, J. Shaw, W. Cao, F. X. Qin, and Y. J. Liu. 2006. Human plasmacytoid predendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). *Blood* 107: 3617–3623.
50. Kerkmann, M., S. Rothenfusser, V. Hornung, A. Towarowski, M. Wagner, A. Sarris, T. Giese, S. Endres, and G. Hartmann. 2003. Activation with CpG-A and CpG-B oligonucleotides reveals two distinct regulatory pathways of type I IFN synthesis in human plasmacytoid dendritic cells. *J. Immunol.* 170: 4465–4474.
51. Kim, S., V. Kaiser, E. Beier, M. Bechheim, M. Guenthner-Biller, A. Ablasser, M. Berger, S. Endres, G. Hartmann, and V. Hornung. 2014. Self-priming determines high type I IFN production by plasmacytoid dendritic cells. *Eur. J. Immunol.* 44: 807–818.
52. Tezuka, H., Y. Abe, J. Asano, T. Sato, J. Liu, M. Iwata, and T. Ohteki. 2011. Prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IgA induction. *Immunity* 34: 247–257.
53. Mestas, J., and C. C. Hughes. 2004. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* 172: 2731–2738.
54. Stohl, W., J. L. Scholz, and M. P. Cancro. 2011. Targeting BLyS in rheumatic disease: the sometimes-bumpy road from bench to bedside. *Curr. Opin. Rheumatol.* 23: 305–310.
55. Vincent, F. B., E. F. Morand, P. Schneider, and F. Mackay. 2014. The BAFF/APRIL system in SLE pathogenesis. *Nat. Rev. Rheumatol.* 10: 365–373.