

Letter to the Editor

Inhibition of human B-cell development into plasmablasts by histone deacetylase inhibitor valproic acid

To the Editor:

Histone deacetylase (HDAC) inhibitors, including the well-tolerated antiepileptic drug valproic acid (VPA),¹ are an emerging class of anticancer drugs that possess tumor-selective cytotoxicity by interfering with differentiation,¹ cell cycle, and apoptosis.² At lower concentrations, HDAC inhibitors exploit immunomodulatory activity. In particular, VPA ameliorates the pathogenesis of experimental autoimmune encephalomyelitis,^{E1} decreases the incidence and severity of collagen-induced arthritis,^{E2} and attenuates inflammation in autoimmune neuritis.^{E3} *In vitro* treatment of human dendritic cells with VPA impairs their migratory capability and potential to effectively activate T cells.³ Furthermore, HDAC inhibition suppresses T-cell proliferation,^{E4} increases T_H2 cytokine production,^{E5} and induces suppressive function of regulatory T cells.^{E6} Humoral immune responses against bacteria are maintained by immunoglobulin-secreting plasma cells and memory B cells. Antigen-driven B-cell proliferation, differentiation, affinity maturation, and immunoglobulin isotype switching depend on costimulatory signals, such as interaction between CD40 and CD40L and stimulation of cytokine receptors such as IL-21 receptor (IL-21R). In mammals, 18 HDAC isoforms have been identified. VPA inhibits class I HDACs, including HDAC1 and HDAC2, and class IIa HDACs.¹ Conditional deletion of HDAC1 and HDAC2 in mouse pre-BI cells leads to a complete block in differentiation because pre-B cells stop cell division and undergo apoptosis.⁴ Mature mouse B cells conditionally lacking HDAC1 and HDAC2 do not proliferate and die in response to mitogens,⁴ pointing to the importance of HDACs for the regulation of B-cell development.

Despite of the wide range of HDAC activities and applications of HDAC inhibitors, little is known on their effect on human B cells. We therefore analyzed human B-cell activation and differentiation by activating naive and memory B-cell subsets with CD40L and IL-21 in the presence of the HDAC inhibitor VPA used at low therapeutic concentration of 0.25 mmol/L (42 mg/L). During 9 days of cultivation, naive B cells gradually differentiated into plasmablasts that expressed high levels of CD27 and CD38. HDAC inhibition by VPA delayed and reduced the expression of these plasmablast markers (Fig 1, A). A less dramatic effect was observed after activating switched memory B cells. Their development was delayed at early time points but was comparable to controls after 9 days (see Fig E1, A and B, in this article's Online Repository at www.jacionline.org). VPA treatment of naive but not of memory B cells reduced the absolute number of CD27^{high}CD38^{high} plasmablasts in culture (Fig 1, B and C, respectively) in a dose-dependent manner (see Fig E1, C). Consistently, the analysis of early activation events in response to CD40L and IL-21 in the presence of VPA showed impaired blast formation (see Fig E2, A and B, in this article's Online Repository at www.jacionline.org) and decreased upregulation of CD69 (see Fig E2, C and D), a lectin involved in retention of lymphocytes in lymphoid organs.^{E7} Furthermore, induction of the MHC class II protein HLA-DR (see Fig E2, E and F) was observed. In contrast,

VPA did not modify the expression of the costimulatory molecule CD86 (see Fig E2, G and H). In addition, B-cell receptor signaling measured by calcium influx was not modulated by VPA (see Fig E2, I). Similar results were obtained by analyzing short-lived plasma cell development of murine splenocytes (data not shown).

Serum immunoglobulins are maintained by long-lived plasma cells and memory B cells,^{E8} whereas protective immunoglobulins against newly encountered microbial pathogens are produced by short-lived plasmablasts differentiating from naive B cells on antigen encounter.^{E9} Because VPA treatment reduced the number of plasmablasts that develop from naive B cells, culture supernatant fluids also contained less IgM, IgG, and IgA (Fig 1, D-F), whereas the concentration of IgG and IgA in supernatant fluids of VPA-treated memory B cells was comparable with those of untreated cells (Fig 1, G and H). Class switch recombination to IgG and IgA is mediated through activation-induced cytidine deaminase encoded by *AICDA*.^{E10} VPA treatment did not change the expression of *AICDA* in activated naive B cells (see Fig E3, A, in this article's Online Repository at www.jacionline.org), indicating that the reduced amount of switched immunoglobulins is due to the block in plasmablast development.

Although the mechanism is still unclear, in activated B cells differentiation is strictly linked to proliferation.^{E11} Inhibition of HDACs in multiple myeloma cells, a prototypic plasma cell tumor, leads to apoptosis and cell cycle arrest because of increased expression of the cyclin-dependent kinase inhibitor proteins p21 and p27.² Naive B cells treated with 0.25 mmol/L VPA maintained their viability (Fig 2, A; see also Fig E4, A, in this article's Online Repository at www.jacionline.org) but showed reduced proliferation both in response to CD40L and IL-21 stimulation (Fig 2, B; see also Fig E4, B-D) as well as in response to TLR9 activation (Fig 2, C; see also Fig E4, E). Similarly, VPA treatment inhibited T-cell proliferation induced by CD3 and CD28 stimulation (see Fig E4, F and G). In activated B cells, CD27^{high} and CD38^{high} cells accumulated in the pool of cells that underwent more rounds of proliferation. HDAC inhibition led to a reduction in CD27^{high} and CD38^{high} cells in the proportion of cells that underwent more divisions (Fig 2, D and E; see also Fig E4, H and I). The reduced proliferation of VPA-treated naive B cells did not result from increased expression of p21 and p27 (data not shown). Collectively, these data show that inhibition of HDACs by 0.25 mmol/L VPA does not impair the survival of human naive B cells but inhibits the proliferation induced on B-cell activation.

Plasma cell differentiation is mediated by a network of transcription factors either induced (BLIMP1, XBP1, IRF4) or repressed (BCL6, PAX5) after B-cell activation.^{E12} VPA did not change the expression pattern of these genes determining the fate of developing B cells (see Fig E3, B-F; Table E1 in this article's Online Repository at www.jacionline.org), suggesting that expression of fate-determining genes can occur independently of B-cell proliferation.

Our data show that VPA neither modified CD40 protein expression (see Fig E5, A and B, in this article's Online Repository at www.jacionline.org) nor IL-21R mRNA and protein expression (see Fig E5, C-E). Because IL-21R signaling via the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is required for differentiation of immunoglobulin-secreting plasma cells,⁵ we investigated mRNA and protein expression of

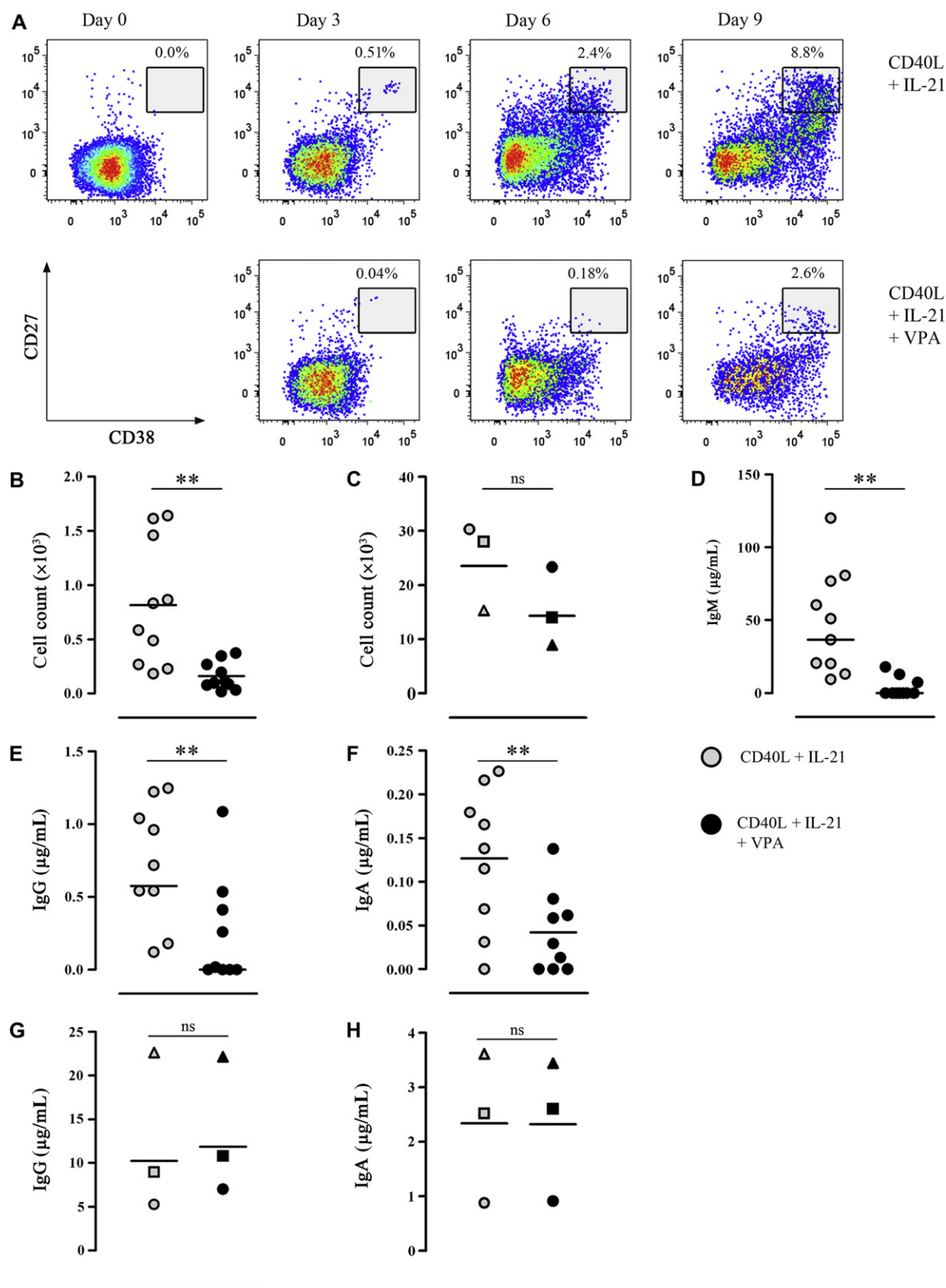


FIG 1. B-cell phenotype and immunoglobulin secretion. Cells were stimulated as indicated. **A**, Naive B cells. Percentage of CD27^{high}CD38^{high} cells within live CD19⁺ cells is indicated. Absolute counts of CD27^{high}CD38^{high} cells of activated naive (**B**) and memory (**C**) B cells 9 days after activation. IgM (**D**), IgG (**E** and **G**), and IgA (**F** and **H**) concentrations in culture supernatant fluids of naive (**D-F**) and memory (**G** and **H**) B cells 9 days after stimulation. Each symbol represents one independent experiment; ns, Not significant. ***P* < .01.

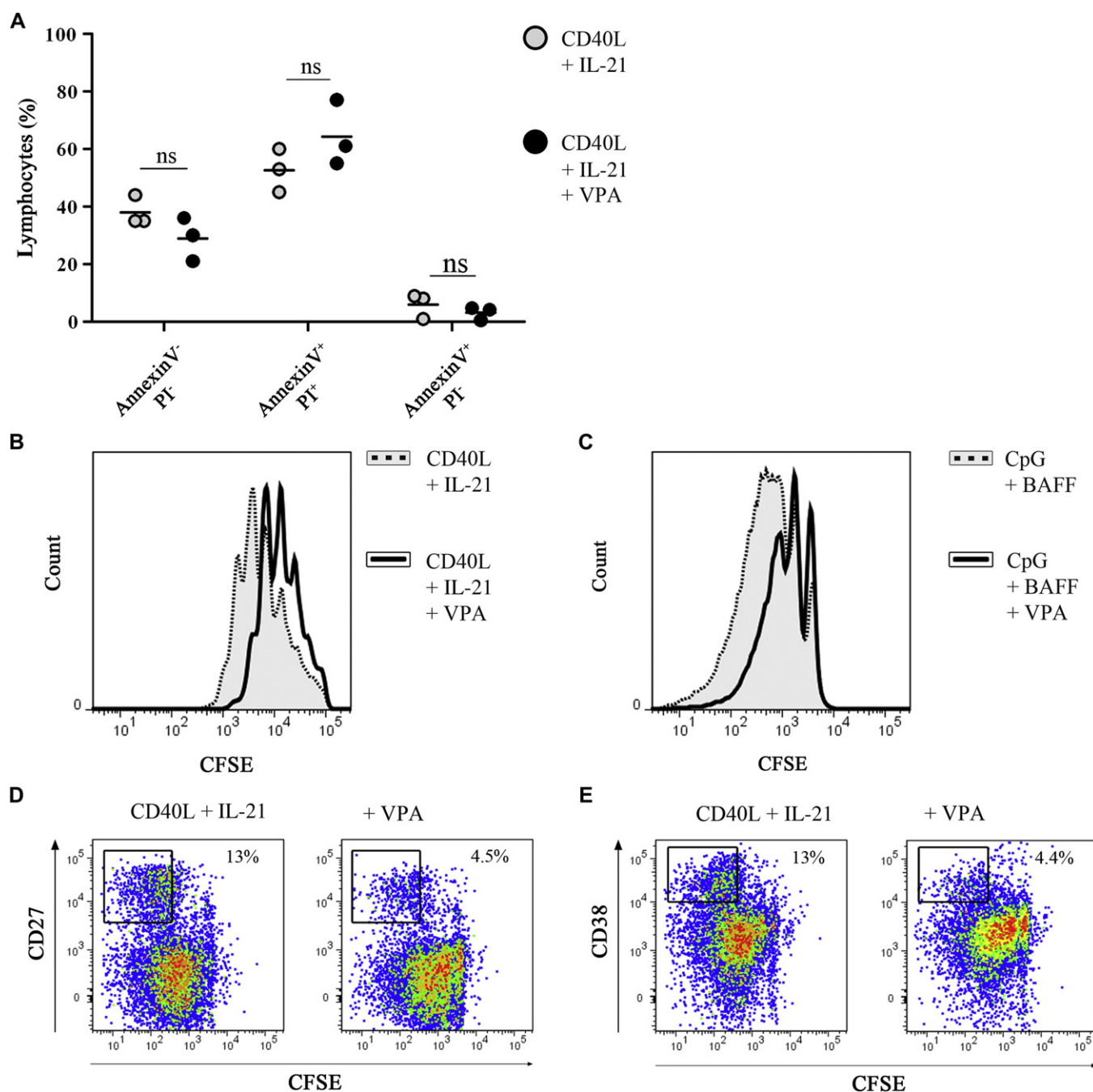


FIG 2. B-cell viability and proliferation. Naive B cells were stimulated as indicated for 6 days. **A**, Percentages of live (AnnexinV⁻PI⁻), dead (AnnexinV⁺PI⁺) and apoptotic (AnnexinV⁺PI⁻) cells determined. **B** and **C**, B cell proliferation was analyzed by CFSE labeling. CD27 (**D**) and CD38 (**E**) expression within CFSE-labeled CD19⁺ cells. **A**, Each symbol represents one independent experiment; **B**, **D**, and **E** are representative for 6 independent experiments; **C** is representative for 3 independent experiments; *BAFF*, B-cell activating factor; *ns*, not significant.

JAK3, STAT3, and STAT5, as well as their activated phosphorylated forms but detected comparable expression in VPA-treated and untreated B cells (see Fig E5, F-J). In addition, B cells stimulated with CD40L and IL-21 for 4 to 6 days maintained their ability to respond to IL-21 stimulation as shown by comparable JAK3, STAT3, and STAT5 phosphorylation in both conditions (see Fig E5, K and L). Taken together, these data show that HDAC inhibition with VPA does not interfere with the

IL-21R-dependent activation of the JAK-STAT signaling pathway in B cells.

Collectively, our data show that the HDAC inhibitor VPA inhibits differentiation of naive B cells into immunoglobulin-secreting plasmablasts by interfering with B-cell proliferation. In contrast, reactivation and differentiation of memory B cells as well as immunoglobulin secretion is not affected by VPA. Published reports show transitory IgA and IgG₄ hypogammaglobulinemia in

epileptic children,⁶ panhypogammaglobulinemia in a newborn,⁷ and unaltered immunoglobulin levels in adults⁸ treated with VPA. Taken together these support the concept that, although memory B cells present in adults are not affected, naive B cells that predominantly constitute the B-cell compartment in younger children are inhibited in their development. Therefore, chronic VPA treatment may induce immunodeficiency against newly encountered microbial pathogens. Furthermore, specific vaccination responses in patients chronically treated with VPA may be impaired. In these patients serum immunoglobulin levels will be maintained by long-lived plasma cells and memory B cells not affected by the treatment. In addition, a recent clinical study showed that the HDAC inhibitor ITF2357 (givinostat) ameliorates the autoimmune disorder juvenile idiopathic arthritis.⁹ In particular, in systemic lupus erythematosus 60% of the autoantibodies against nuclear antigens are produced by autoreactive short-lived plasma cells.¹⁰ Thus, our data on the effect of VPA on the generation of plasmablasts shown here provide a rationale for the use of HDAC inhibitors in treatment of autoimmune diseases.

Additional information is available (see this article's **Methods** section and References in the Online Repository at www.jacionline.org).

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Anne-Kathrin Kienzler, MSc^{a,b,*}

Marta Rizzi, MD, PhD^{a,*}

Maike Reith, MSc^a

Stephen L. Nutt, PhD^c

Hermann Eibel, PhD^a

From ^athe Centre of Chronic Immunodeficiency (CCI), University Medical Center; ^bthe Faculty of Biology, Albert-Ludwigs-University Freiburg, Germany; and ^cThe Walter

and Eliza Hall Institute of Medical Research, Parkville, Australia. E-mail: hermann.eibel@uniklinik-freiburg.de.

*These authors contributed equally to this work.

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METHODS

In vitro cell culture

Sodium valproate (VPA) was purchased from Sigma-Aldrich (St Louis, Mo) and dissolved in PBS. Buffy coats were purchased from the blood bank of the University Medical Center Freiburg. Human naive ($CD3^{-}CD19^{+}CD27^{-}IgG^{-}IgA^{-}$) and memory ($CD3^{-}CD19^{+}CD27^{+}IgG^{+}IgA^{+}$) B cells (1.5×10^5 cells/mL) that had undergone fluorescence-activated sorting (FACS) were stimulated with CD40L and IL-21 as described,^{E13} or 500 nmol/L CpG ODN 2006 (Apara Biosciences, Baden-Württemberg, Germany) and 100 ng/mL recombinant human B-cell activating factor in the presence of 0.25 mmol/L VPA when indicated in enriched Iscoves medium supplemented with 10% FCS, 1 μ g/mL insulin, 2.5 μ g/mL apo-transferrin, 0.1% fatty acid supplement, 1% nonessential amino acids, 2 mmol/L glutamine, and 1 μ g/mL reduced glutathione. Starting the culture with equal number of B cells, the absolute number of plasmablasts after 9 days was determined by counting the events in the $CD27^{high}CD38^{high}$ gate by flow cytometry. To analyze T-cell proliferation 1×10^6 peripheral blood mononuclear cells (PBMCs)/mL were stimulated with 0.25 μ g/mL anti-human CD3 (clone UCHT1; BD, Franklin Lakes, NJ) and 0.25 μ g/mL anti-human CD28 (clone CD28.2; BD) in the presence of 0.25 mmol/L VPA when indicated in enriched Iscoves medium. The human chronic lymphocytic leukemia cell line MEC1 was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). MEC1 cells were stimulated with IL-21 in the presence of 1 mmol/L VPA when indicated for 4 days in Iscoves medium supplemented with 10% FCS.

Flow cytometry

Phenotype of human B cells was determined by flow cytometry with the following antibodies: CD3 FITC, CD69 FITC, CD19 AmCyan, CD19 APC-H7, CD27 PE, CD86 PE, CD38 PE-Cy7, CD38 PerCP-Cy5.5, IL21R APC (all BD), CD19 Alexa 647 (Invitrogen, Carlsbad, Calif), CD27 PerCP-Cy5.5 (BioLegend, San Diego, Calif), IgG DyLight 649, IgA DyLight 649 (Jackson ImmunoResearch Laboratories Inc, West Grove, Pa). Anti-human HLA-DR (L243) was biotinylated with the use of the Lightning-Link Biotin Conjugation Kit (Type A; Invivo Biosciences, Cambridge, United Kingdom). The biotinylated antibody was detected with streptavidin allophycocyanin (APC; BD). The mouse anti-human CD40 antibody (AbD Serotec, Oxford, United Kingdom) was detected with anti-mouse IgG₁-PE (Caltag Laboratories, Burlingame, Calif). Phenotype of T cells was determined by flow cytometry with the following antibody: CD4 PerCP-Cy5.5 (BD). Dead cell exclusion was performed by DAPI (4',6-diamidino-2-phenylindole) staining. Lymphocyte proliferation was monitored by CFSE (carboxyfluorescein diacetate, succinimidyl ester; Molecular Probes, Eugene, Ore) labeling. To measure apoptosis, B cells were stained with AnnexinV APC and propidium iodide (PI; eBioscience, San Diego, Calif), according to the manufacturer's instructions. To measure intracellular calcium after B-cell receptor stimulation, 2.5×10^6 PBMCs/mL were cultured with 0.25 mmol/L VPA when indicated in enriched Iscoves medium for 36 hours. PBMCs were then incubated with 0.5 μ mol/L Fluo-4 AM, 1 μ mol/L Fura red, and 0.1% Pluronic (Molecular Probes) in Iscoves medium without FCS for 30 minutes at 37°C. Staining for CD19 was performed for 15 minutes on ice. The concentration of intracellular-free calcium was recorded as the ratio of fluorescence of Fluo-4/Fura red by using a FACSCanto II (BD). After recording a baseline for 1 minute, stimulation with 10 μ g/mL anti-human IgM (F(ab')₂; Southern Biotech, Birmingham, Ala) was performed and recorded for six minutes. Ionomycin (2 μ g/mL; Sigma-Aldrich) served as a positive control. The data acquired were analyzed with FlowJo software (TreeStar Inc, Ashland, Ore) by gating on $CD19^{+}$ cells and visualized as median values with Gaussian smoothing of the curve by using the kinetics tool.

Determination of immunoglobulin concentrations

Immunoglobulins in culture supernatant fluids were measured by ELISA. In brief, 96-well plates (Maxisorp; Nunc, Naperville, Ill) were coated with anti-human immunoglobulin mix (Jackson ImmunoResearch

Laboratories Inc) in bicarbonate buffer. Bound immunoglobulins were detected with alkaline phosphatase-conjugated anti-human IgM, IgG, or IgA (Jackson ImmunoResearch Laboratories Inc) and developed with p-nitrophenyl phosphate (Sigma-Aldrich) in diethanolamine buffer. Immunoglobulin concentrations were calculated from human IgM, IgG, or IgA standards (N Protein Standard SL; Siemens, Waltham, Mass) processed in parallel.

Quantitative PCR

RNA was extracted with TRIZOL reagent (Invitrogen) and quantified with the Agilent 2100 Bioanalyzer with the use of the RNA Nano Series II Kit (Agilent Technologies, Palo Alto, Calif). cDNA synthesis was performed with equal amount of RNA with the use of SuperScript III reverse transcriptase (Invitrogen) and random hexamer primers (Amersham Pharmacia Biotech, Piscataway, NJ). Quantitative PCR was performed with the TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, Calif) and a LightCycler 480 (Roche, Indianapolis, Ind). The following primer/probe sets were used: *PRDM1*, *BCL6*, *IRF4*, *PAX5*, *XBPI*, *AICDA*, *IL21R*, *JAK3*, *STAT3*, *STAT5A*, and *STAT5B* (see Table E1), final concentrations were 600 nmol/L for primers and 200 nmol/L for probes (Eurogentec, Seraing, Belgium). The following amplification program was used: 2 minutes at 50°C and 10 minutes at 95°C followed by 45 cycles that consisted of 15 seconds at 95°C and 1 minute at 58°C. Relative expression was calculated with the $2^{-\Delta C_q}$ method with all mRNA levels standardized to the reference *RPLPO* (Applied Biosystems).

Western blot analysis

Cell lysates were prepared with lysis buffer that contained 50 mmol/L HEPES pH 7, 250 mmol/L NaCl, 5 mmol/L EDTA, 1% NP40, 10 μ mol/L sodium pervanadate, Phosphatase Inhibitor Cocktail 2 (Sigma-Aldrich), and Complete Protease Inhibitor (Roche). Incubation with antibodies specific for JAK3, STAT3, STAT5, phospho-JAK3 (Y980/981), phospho-STAT3 (Y705), phospho-STAT5 (Y694) (all Cell Signaling, Danvers, Mass), and β -actin (Sigma-Aldrich) was performed in PBS/5% BSA/0.1% Tween, followed by incubation with respective horse-raddish peroxidase (HRPO)-coupled antibodies (Jackson ImmunoResearch Laboratories Inc or Sigma-Aldrich). Finally, membranes were incubated in SuperSignal West Femto Maximum Sensitivity Substrate or in SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Waltham, Mass).

Statistics

Statistics was performed with the paired (if $n \geq 3$) or unpaired (if $n = 2$) 2-tailed *t* test. Calculations were performed with GraphPad Prism (GraphPad Software Inc, San Diego, Calif).

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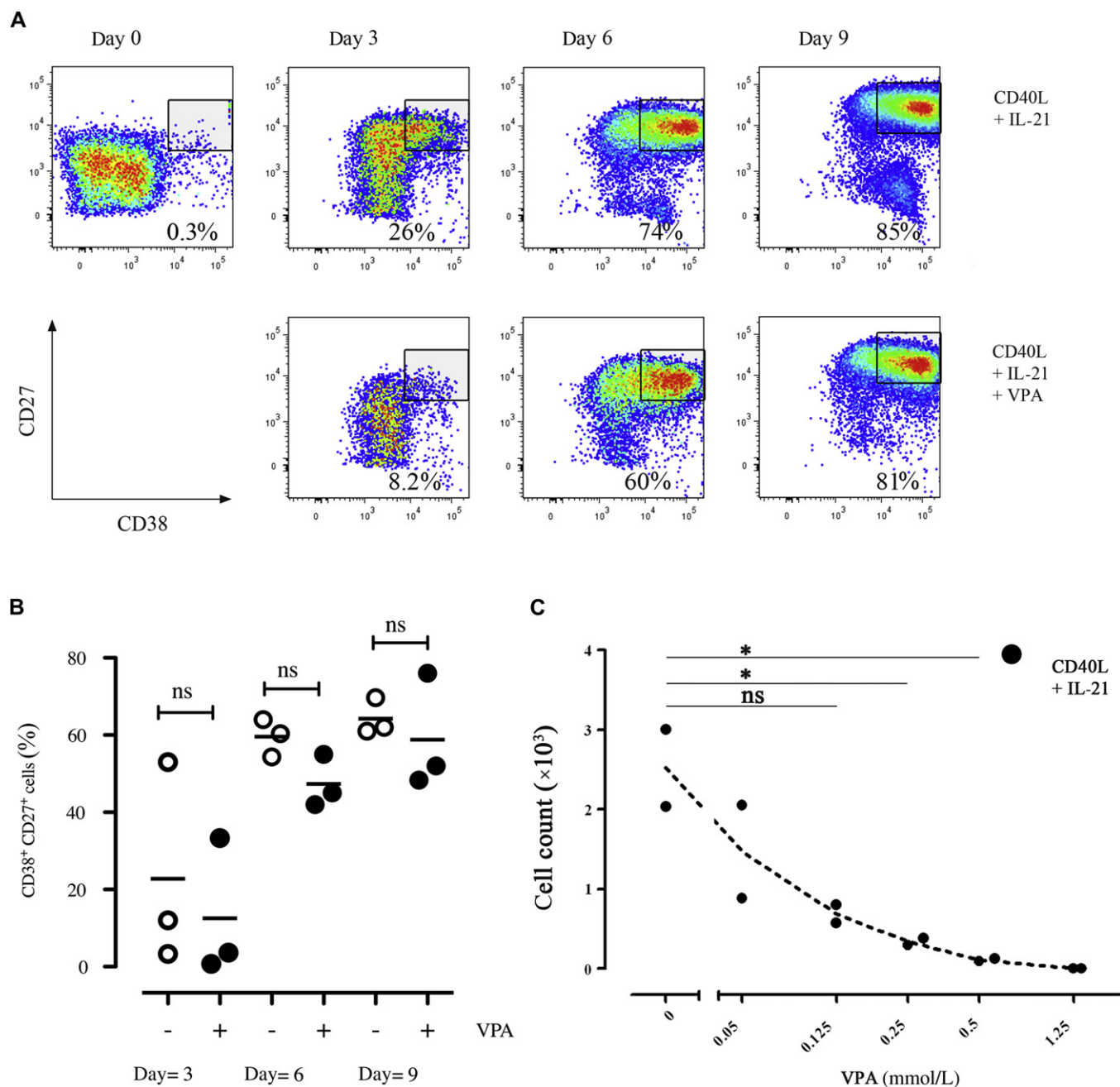


FIG E1. Memory B-cell differentiation into plasmablasts is not influenced by VPA treatment, whereas naive B-cell differentiation is affected in a dose-dependent manner. **A**, Representative flow cytometric analysis of human memory B cells stimulated with CD40L and IL-21 in the presence or absence of VPA as indicated. Percentage of CD27^{high}CD38^{high} cells within live CD19⁺ cells is shown. **B**, Percentage of CD27^{high}CD38^{high} cells from 3 different donors activated and analyzed as shown in **A**. **C**, Naive B cells were stimulated as indicated. Absolute count of CD27^{high}CD38^{high} cells 6 days after activation is plotted on the y axis. Each symbol represents one independent experiment; ns, Not significant; * $P < .05$.

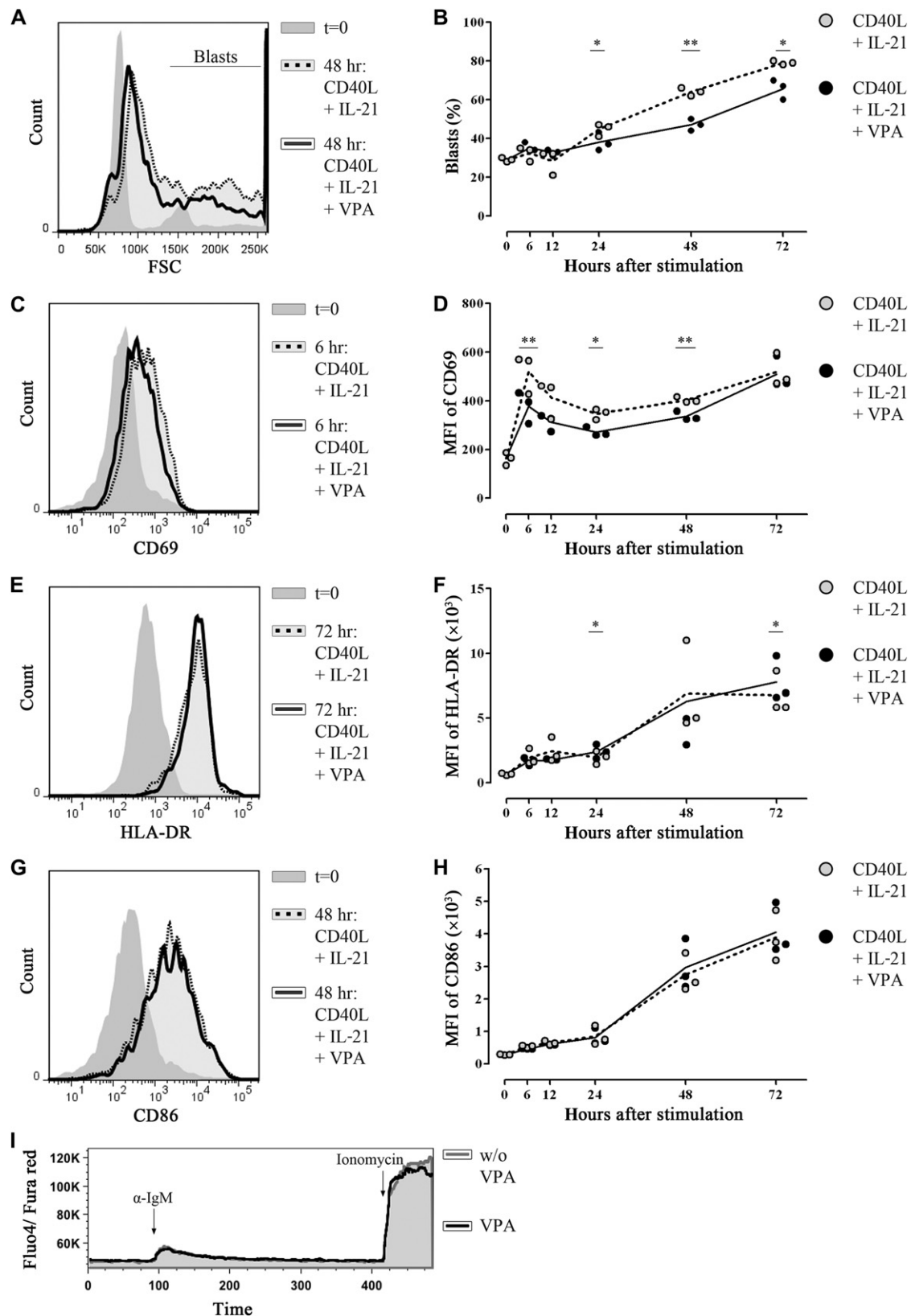


FIG E2. VPA inhibits blast formation and early expression of CD69, induces MHC class II expression, but does not influence CD86 expression and calcium influx on BCR stimulation. Naive B cells were stimulated as indicated. **A** and **B**, Blast formation was determined by analyzing cell size by forward scatter in flow cytometry. Expression of the early activation markers CD69 (**C** and **D**), HLA-DR (**E** and **F**), and CD86 (**G** and **H**) analyzed by flow cytometry. **A**, **C**, **E**, and **G** are representative FACS plots; (**B**, **D**, **F**, and **H**) depict the median

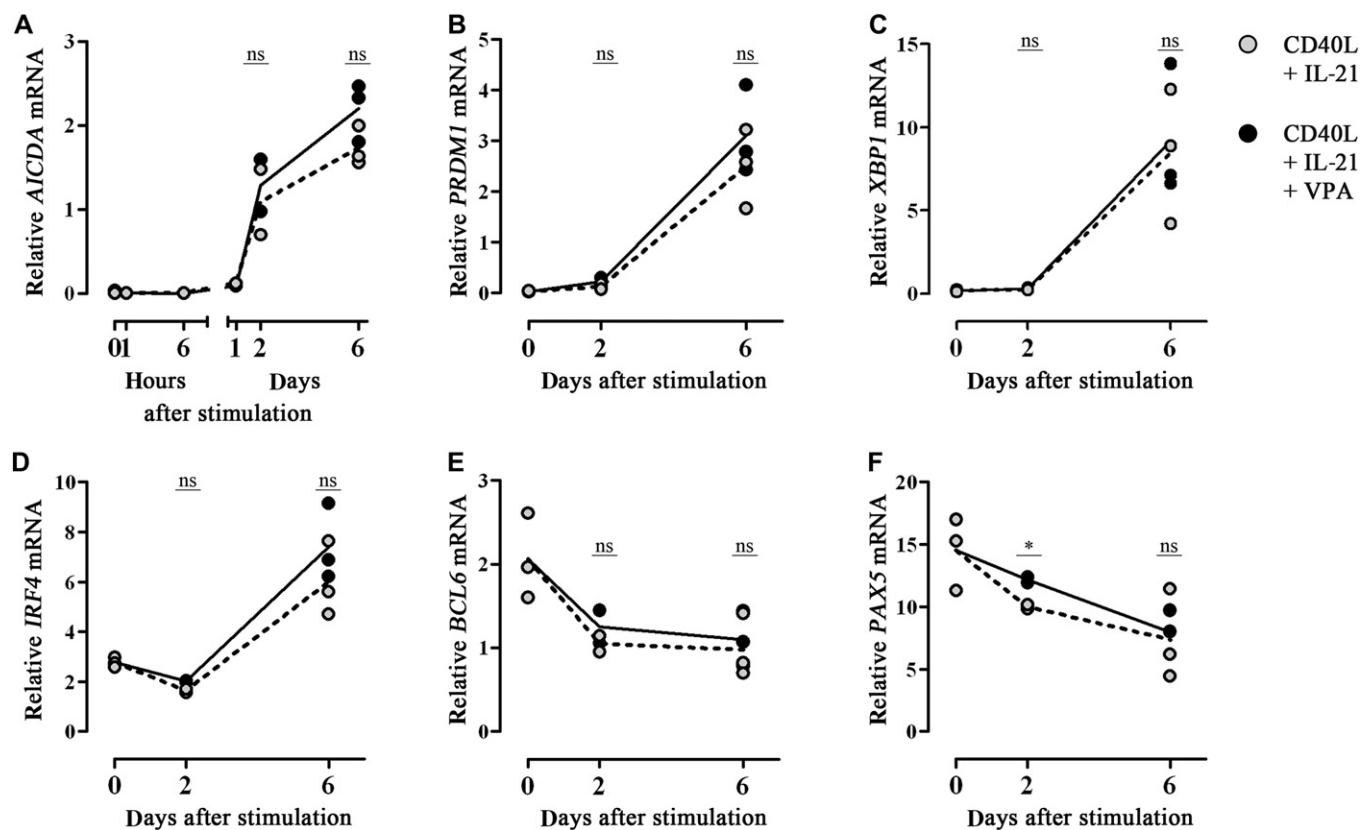


FIG E3. No variation in expression of B-cell fate-determining genes in the presence of VPA. Naive B cells were activated as indicated. mRNA levels of *AICDA* (A), *PRDM1* (B), *XBPI* (C), *IRF4* (D), *BCL6* (E), and *PAX5* (F) were determined by quantitative PCR at indicated time points. Each symbol represents one independent experiment. *AICDA*, Activation-induced cytidine deaminase; *PRDM1*, PR domain containing 1, with ZNF domain; *XBPI*, X-box binding protein 1; *IRF4*, interferon regulatory factor 4; *BCL6*, B-cell CLL/lymphoma 6; *PAX5*, paired box 5. * $P < .05$.

fluorescence intensity over time. Each symbol represents one independent experiment. I, Calcium influx on BCR stimulation of VPA-treated compared with untreated cells. Shown is 1 representative of 2 independent experiments. BCR, B-cell receptor; FSC, forward scatter; HLA-DR, human leukocyte antigen DR; FACS, fluorescence-activated cell sorting; MFI, median fluorescence intensity; ns, not significant. * $P < .05$. ** $P < .01$.

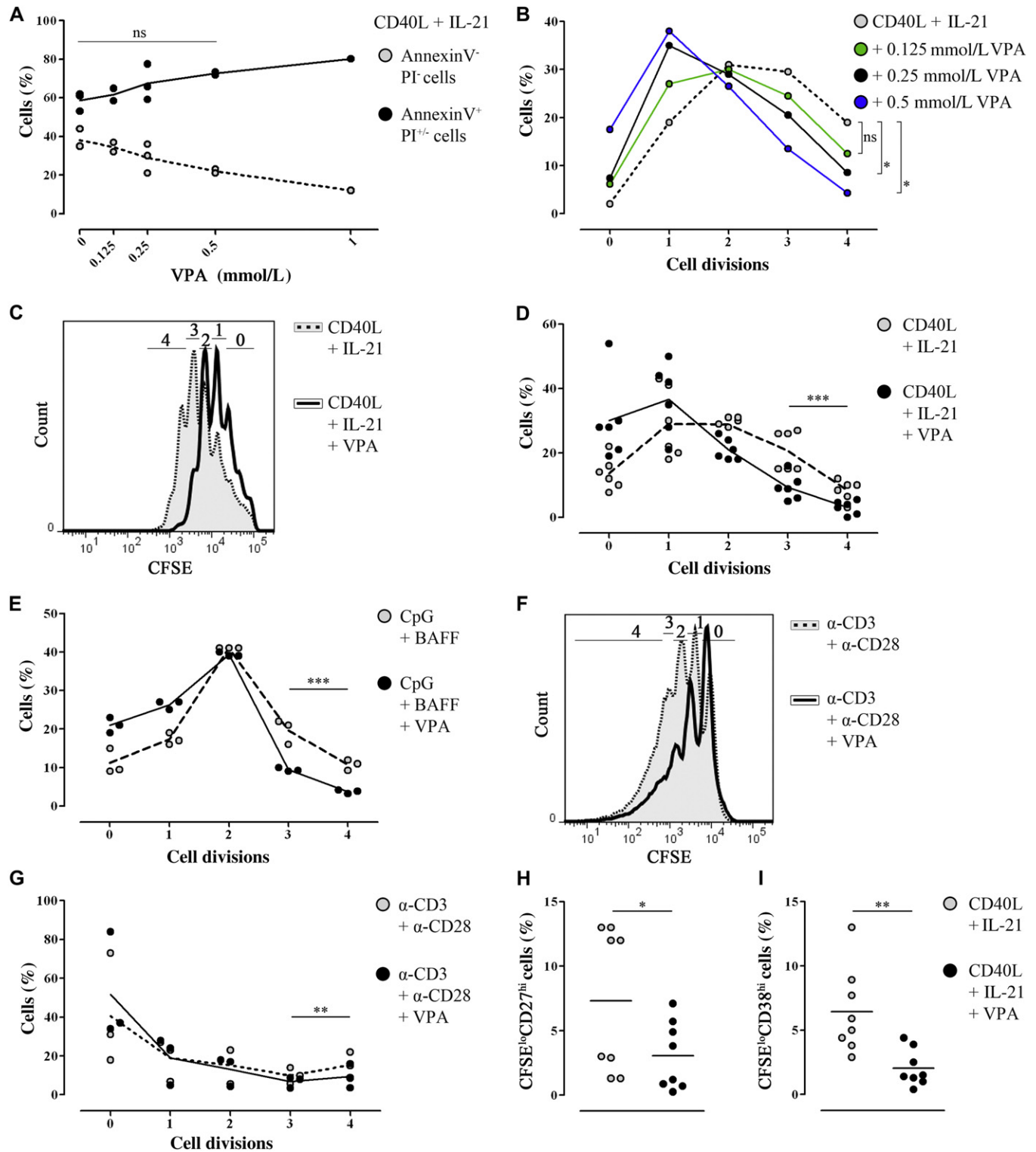


FIG E4. VPA at a dose of 0.25 mmol/L does not induce apoptosis, but inhibits B- and T-cell proliferation. (A-E, H, and I) Naive B cells or PBMCs (F and G) were stimulated as indicated for 6 days. **A**, Percentages of live (AnnexinV⁻PI⁻) and dead (AnnexinV⁺PI⁺) cells, including apoptotic (AnnexinV⁺PI⁺) cells. **B-G**, Lymphocyte proliferation was analyzed by CFSE labeling. **B**, B-cell proliferation in the presence of escalating doses of VPA. **C** and **F**, Representative FACS plot showing the gating strategy for statistical analysis of proliferation. **D**, **E**, and **G**, Percentage of cells that have undergone indicated numbers of divisions. Statistics was performed for cells that have undergone 3 or more divisions. Percentage of CFSE^{low} and CD27^{high} (H) or CD38^{high} (I) cells gated on CD19⁺ cells for 8 independent experiments. In A, D, E, and G-I each symbol represents 1 independent experiment; in B each symbol represents the mean of 2 independent experiments. PI, Propidium iodide; α -, anti-; BAFF, B-cell activating factor; lo, low expression; hi, high expression; ns, not significant. * $P < .05$. ** $P < .01$. *** $P < .001$.

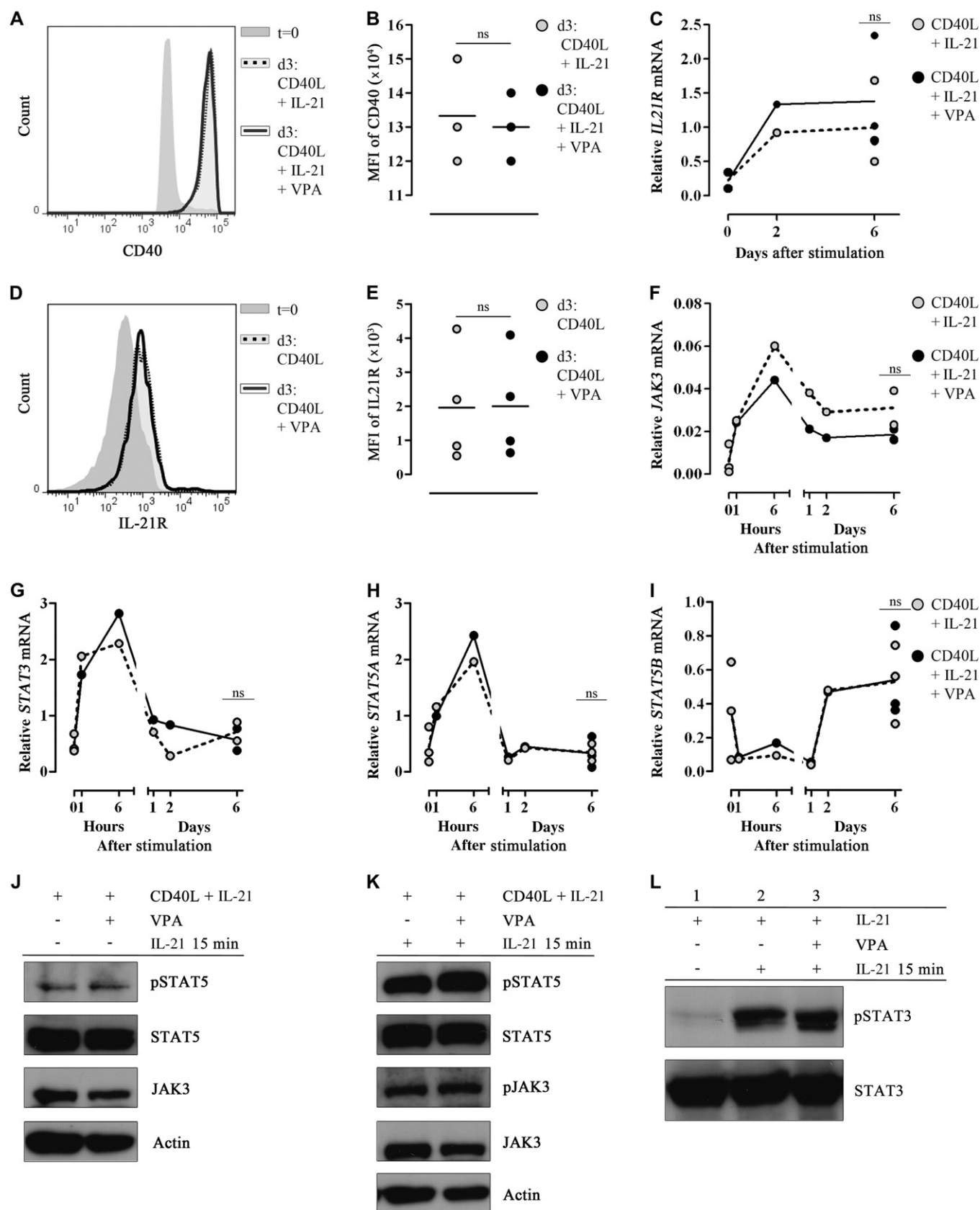


FIG E5. CD40 and IL-21R expression and JAK-STAT signaling in B cells are not modulated by VPA. (A-K, Native B cells were stimulated as indicated.) **A** and **B**, Cell surface expression of CD40. **A**, Representative FACS plot. **B**, MFI of CD40 in 3 independent experiments. **C-E**, IL-21R expression. **C**, Variation of relative IL-21R

mRNA expression over time in culture. **D**, Representative FACS plot. **E**, MFI of IL-21R in 4 independent experiments. Relative mRNA levels of *JAK3* (**F**), *STAT3* (**G**), *STAT5A* (**H**), and *STAT5B* (**I**) over time in culture. Each symbol represents one independent experiment. **J**, Cells were lysed after 6 days in culture either directly, or after 15 minutes (**K**) of pulse stimulation with IL-21. Lysates were analyzed by Western blot analysis with the use of antibodies specific for JAK3 and STAT5 or their phosphorylated forms, and for β -actin. **L**, MEC1 cells were cultured as indicated. Cells were lysed after 4 days either directly (*lane 1*) or after 15 minutes of pulse stimulation with IL-21 (*lanes 2-3*). Lysates were analyzed by Western blot analysis with the use of an antibody specific for STAT3 or its phosphorylated form. (*p*)*JAK3*, (phospho) Janus kinase 3; (*p*)*STAT3*, 5A, 5B, (phospho) signal transducer and activator of transcription 3, 5 isoform A and B; *t*=0, before activation; *d3*, 3 days after activation; FACS, fluorescence-activated cell sorting; MFI, median fluorescence intensity; *ns*, not significant.

TABLE E1. Primer and probe sequences used in quantitative PCR

Gene name	Ref seq	Amplicon size (bp)	Forward primer	Reverse primer	Probe
<i>AICDA</i>	NM_020661.2	90	5'-TCCTTTTCACTGGACTTTGGT-3'	5'-TAGGTCCCAGTCCGAGATGTAG-3'	5'-CTTCGCAATAAGAACGGCTGCCAC-3'
<i>BCL6</i>	NM_001706.4	75	5'-ATGGGAGAGACCCAGTCTGA-3'	5'-GCAGTCACA CTCATTGCAGAA-3'	5'-AGATTCTAGCTGTGAGAACGGGGCCTT-3'
<i>IL21 receptor</i>	NM_021798.3	104	5'-ATCACAGACCAGTCTGGCAAC-3'	5'-CCTGAGAAGGTCACAGTCACG-3'	5'-AGCTTTCTCCTGGCTGAGAGCATCAAGC-3'
<i>IRF4</i>	NM_001195286.1	99	5'-ACCTGCAAGCTCTTTGACAC-3'	5'-AAAGCATAGAGTCACCTGGAATC-3'	5'-CTTGTCAGAGCTGCAAGCGTTTGCT-3'
<i>JAK3</i>	NM_000215.3	113	5'-AGGCTGCTGGAGGAAGTG-3'	5'-CTGCGGCGGAGAACATAG-3'	5'-CCA 8CA 7TC 8GG 5CT 8TG CC-3'
<i>PAX5</i>	NM_016734.1	104	5'-ATCATCCGGACAAAAGTACAGC-3'	5'-GTGCTCACCGAGGACACC-3'	5'-CACAGCATAGTGCCACTGGCTCCGT-3'
<i>PRDM1</i>	NM_001198.3	122	5'-GACTTTGCAGAAAGGCTTCACT-3'	5'-ACATTCTTTGGGCAGAGTTCAT-3'	5'-CAATGATGAATCTCACACAAACACAGAGCAGT-3'
<i>RPLPO</i>	NM_053275.3	105	Purchased from Applied Biosystems		
<i>STAT3</i>	NM_139276.2	116	5'-CACAATCTACGAAGAATCAAGCAG-3'	5'-GCAGTCTGTAGAAGGCGTGAT-3'	5'-TCTTCAGAGCAGGTATCTTGAGAAGCCAAT-3'
<i>STAT5A</i>	NM_003152.3	110	5'-CAGAGGCTGGTCCGAGAA-3'	5'-AGTCGCAGCTCCTCAAATGT-3'	5'-CCAACAATTGCAGCTCTCCGGCT-3'
<i>STAT5B</i>	NM_012448.3	81	5'-CTCCAAATACTACACACCAGTTCC-3'	5'-TGCTTGATCTGTGGCTTCAC-3'	5'-CGAGTCTGCTACTGCTAAAGCTGTTGATGGA-3'
<i>XBPI</i>	NM_005080.3	136	5'-CCTGGTTGCTGAAGAGGAG-3'	5'-GATGTTCTGGAGGGGTGAC-3'	5'-CGGAAGCCAAGGGGAATGAAGT-3'

AICDA, Activation-induced cytidine deaminase; *BCL6*, B-cell CLL/lymphoma 6; *IL21*, interleukin 21; *IRF4*, interferon regulatory factor 4; *JAK3*, Janus kinase 3; *PAX5*, paired box 5; *PRDM1*, PR domain containing 1, with ZNF domain; *RPLPO*, ribosomal protein, large, P0; *STAT*, signal transducer and activator of transcription; *XBPI*, X-box binding protein 1.