Published in final edited form as:

Nat Immunol. 2002 September ; 3(9): 822–829. doi:10.1038/ni829.

DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL

Mikhail B. Litinskiy[1], **Bernardetta Nardelli**[2], **David M. Hilbert**[2], **Bing He**[1], **Andras Schaffer**[1], **Paolo Casali**[1], and **Andrea Cerutti**[1]

[1]Division of Molecular Immunology, Department of Pathology and Laboratory Medicine, Joan and Sanford I.Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021, USA

[2]Human Genome Sciences, 9410 Key West Avenue, Rockville, MD 20850, USA

Abstract

Immunoglobulin (Ig) class-switch DNA recombination (CSR) is thought to be highly dependent upon engagement of CD40 on B cells by CD40 ligand on T cells. We show here that dendritic cells up-regulate BLyS and APRIL upon exposure to interferon-α, interferon-γ or CD40 ligand. In the presence of interleukin 10 (IL-10) or transforming growth factor-β, BLyS and APRIL induce CSR from C_μ to C_γ and/or C_α, genes in B cells, whereas CSR to C_ε requires IL-4. Secretion of class-switched antibodies requires additional stimulation by B cell antigen receptor engagement and IL-15. By eliciting CD40-independent Ig class switching and plasmacytoid differentiation, BLyS and APRIL critically link the innate and adaptive immune responses.

Antibody diversity is critical for the generation of a protective immune response. Early B cell precursors generate antigen recognition diversity by assembling immunoglobulin (Ig) variable-region V(D)J exons from individual variable (V), diversity (D) and joining (J) gene segments through recombination-activating gene 1 (RAG-1) and RAG-2 proteins[1]. Mature B cells functionally diversify the antibody repertoire through two antigen-dependent processes that usually occur in the germinal center (GC) of secondary lymphoid organs. These are Ig V(D)J gene somatic hypermutation, which increases the antibody affinity for antigen by introducing point mutations within the V(D)J exon, and Ig heavy chain (H) class switching, which modulates the antibody effector functions by substituting the constant region of IgM with that of IgG, IgA or IgE[2–4]. Hypermutated and class-switched GC B cells either give rise to long-lived memory B cells or terminally differentiate to plasma cells, which secrete large amounts of antibodies.

Class switching is mediated by class switch DNA recombination (CSR). CSR replaces the Ig heavy chain constant region C_μ gene with a targeted C_γ, C_α or C_ε gene by recombining the switch region μ (S_μ), a highly repetitive DNA segment 5′ of C_μ, with an analogous S_γ, S_α or

Correspondence should be addressed to A C. (acerutti@med.cornell.edu).

Competing interest statement

The authors declare competing financial interests: see the *Nature Immunology* website (http://www.nature.com/natureimmunology) for details.

S_ε region 5′ of the targeted C_H gene[2,4]. Most antigens, including complex viral and bacterial proteins, elicit CSR in GC B cells by up-regulating the tumor necrosis factor (TNF) family member CD40 ligand (CD40L) on $CD4^+$ T cells[2,5]. By activating the I_H promoter, a regulatory DNA sequence that includes a noncoding I_H exon located 5′ of each S region, engagement of CD40 on B cells by CD40L induces germline I_H-C_H transcription, which increases the accessibility of the targeted S region to the CSR machinery[2,4]. This as yet elusive enzymatic complex includes activation-induced cytidine deaminase (AID), a B cell–specific and CD40-inducible RNA-editing enzyme, and initiates CSR by promoting deletion of intervening IgH DNA between S_μ and the targeted S region[4,6].

CSR is thought to be highly dependent on CD40L, as IgG, IgA and IgE production is severely impaired in the X-linked hyper-IgM syndrome[7]. However, viral glycoproteins and bacterial polysaccharides can stimulate IgG and IgA production in the absence of CD40L-expressing $CD4^+$ T cells[2,8–11]. This implies the existence of CSR-inducing molecules different from CD40L. Unlike T cell–dependent (TD) class switching, T cell–independent (TI) class switching occurs in splenic marginal zone or intestinal lamina propria B cells and provides prompt protection against invading pathogens[10,11]. The requirements and modalities of TI class switching remain obscure. Antigen-presenting cells (APCs) interact with B cells to enhance IgG and IgA production[12]; this led us to hypothesize that APCs play a key role in the initiation of CD40-independent CSR.

APCs, including dendritic cells (DCs), monocytes and macrophages, express the B lymphocyte stimulator protein (BLyS, also known as BAFF, TALL-1, THANK and zTNF4)[13,14]. This TNF family member binds to three receptors selectively expressed by B cells, including transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI), B cell maturation antigen (BCMA) and BAFF receptor (BAFF-R also known as BR3)[15–19]. APCs express a second ligand, a proliferation-inducing ligand (APRIL), which binds with high affinity to BCMA and with lower affinity to TACI but not to BAFF-R[20,21]. Engagement of TACI, BCMA and BAFF-R by BLyS activates a CD40-like pathway that enhances B cell survival by up-regulating the antiapoptotic molecules NF-κB and Bcl-2[22]. Unlike CD40L deficiency, which impairs TD but not TI IgG and IgA responses[23,24], BLyS deficiency impairs both TD and TI IgG and IgA production[25]. A similar phenotype can be induced by overexpressing TACI-Ig and BCMA-Ig, two soluble decoy receptors that neutralize BLyS and APRIL[15–18,26,27]. Conversely, BLyS overexpression increases IgG, IgA and IgE and leads to a systemic lupus erythematosus (SLE)-like syndrome with kidney deposition of IgG and IgA[17,28]. Finally, APRIL overexpression enhances IgG production in response to TI but not TD antigens[29]. These findings indicate that BLyS and APRIL up-regulate class-switched Igs, but offer no clue as to whether this up-regulation stems only from the accumulation of terminally differentiated B cells or derives also from enhanced CSR in IgD^+ and/or IgM^+ B cells.

We show here that human DCs and monocytes up-regulate BLyS and APRIL upon stimulation with interferon-α (IFN-α), IFN-γ, lipopolysaccharide (LPS) or CD40L. In the presence of appropriate cytokines, BLyS and APRIL induce CD40-independent CSR to C_γ, C_α or C_ε in B cells. These cells further differentiate to plasmacytoid cells that secrete class-switched antibodies upon B cell antigen receptor (BCR) engagement and exposure to IL-15.

Results

BLyS and APRIL induce CSR

CSR generates an extrachromosomal reciprocal switch DNA recombination product, switch circle (SC), which includes the I_H promoter 5′ of the targeted C_H gene, the DNA segment between S_μ and the targeted S region, and C_μ (Fig. 1a). Under the influence of the I_H promoter, the SC transcribes a chimeric I-C_μ product, referred as circle transcript (CT)[30]. Together with AID, SCs and CTs constitute highly specific markers of ongoing CSR[30]. These markers were used to evaluate the CSR-inducing activity of BLyS and APRIL in human IgD^+ B cells, which, before stimulation, express unrearranged S_μ, S_γ, S_α and S_ε regions[2,31]. BLyS and APRIL were used together with cytokines with known CSR-inducing activity. These cytokines included interleukin 10 (IL-10), a B cell– and APC-derived cytokine that induces switching to IgG1, IgG3, IgA1 and IgA2[2,12]; IL-4, a T cell–derived cytokine that induces switching to IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 and IgE[31]; and transforming growth factor-β1 (TGF-β1), a B cell– and APC-derived cytokine that inudces switching to IgA1 and IgA2[2,12].

Compared to IgD^+ B cells incubated with medium or cytokines alone, IgD^+ B cells stimulated with recombinant soluble BLyS or APRIL alone contained low amounts of $S_\gamma1/2$-S_μ, $S_\gamma3$-S_μ and $S_\alpha1/2$-S_μ SCs (Fig. 1b) and weakly expressed $I_\gamma1$ and $I_\gamma2$ ($I_\gamma1/2$)-C_μ, $I_\gamma3$-C_μ and $I_\alpha1/2$-C_μ CTs as well as AID transcripts (Fig. 1c). Neutralizing antibodies to IL-10 or TGF-β prevented the appearance of SCs and CTs (data not shown), which suggested that BLyS and APRIL elicit CSR by inducing the secretion of IL-10 and TGF-β by B cells. When exposed to BLyS or APRIL and exogenous IL-10, IgD^+ B cells further up-regulated $S_\gamma1/2$-S_μ, $S_\gamma3$-S_μ and $S_\alpha1/2$-S_μ SCs, $I_\gamma1/2$-C_μ, $I_\gamma3$-C_μ and $I_\alpha1/2$-C_μ CTs and AID transcripts. In the presence of BLyS or APRIL and exogenous IL-4, IgD^+ B cells contained $S_\gamma1/2$-S_μ, $S_\gamma3$-S_μ, $S_\alpha1/2$-S_μ, $S_\gamma4$-S_μ and S_ε-S_μ SCs and expressed $I_\gamma1/2$-C_μ, $I_\gamma3$-C_μ $I_\alpha1/2$-C_μ $I_\gamma4$-C_μ and I_ε-C_μ CTs. Finally, IgD^+ B cells contained $S_\alpha1/2$-S_μ SCs and expressed $I_\alpha1/2$-C_μ CTs and AID transcripts upon exposure to BLyS or APRIL and exogenous TGF-β1 (Fig. 2).

Additional experiments compared the CSR-inducing activity of BLyS and APRIL with that of other TNF family members. IgD^+ B cells exposed to BLyS or APRIL and IL-4 up-regulated total S_γ-S_μ and S_α-S_μ SCs, although at lower amounts compared to IgD^+ B cells stimulated with CD40L and IL-4 (Fig. 3a). In contrast, IgD^+ B cells stimulated with TNF-α or lymphotoxin-α (LT-α), which are critically involved in the GC reaction[32], did not contain SCs. Finally, dose-response experiments showed that, when combined with IL-4, BLyS and APRIL induce CSR at concentrations as low as 1 ng/ml (Fig. 3b). These results indicated that, in the presence of appropriate cytokines, BLyS and APRIL induce CD40-independent CSR to C_γ, C_α or C_ε genes.

BLyS and APRIL up-regulate I_H-C_H transcripts

Germline I_H-C_H transcription increases the accessibility of the targeted S region 5′ of the C_H gene to the CSR machinery[2,4]. After CSR is completed, the targeted C_H gene is juxtaposed to the recombined V_HDJ_H exon to form a V_HDJ_H-C_H transcript that is subsequently translated into an Ig protein (Fig. 1). Compared to IgD^+ B cells incubated with IL-10 or IL-4

alone, IgD^+ B cells stimulated with BLyS alone up-regulated germline $I_\gamma1$-$C_\gamma1$, $I_\gamma2$-$C_\gamma2$, $I_\gamma3$-$C_\gamma3$ and $I_\alpha1$-$C_\alpha1$ transcripts as well as mature V_HDJ_H-$C_\gamma1$, V_HDJ_H-$C_\gamma2$, V_HDJ_H-$C_\gamma3$ and V_HDJ_H-$C_\alpha1$ transcripts (Fig. 4a). IgD^+ B cells exposed to APRIL alone expressed germline $I_\gamma1$-$C_\gamma1$, $I_\gamma2$-$C_\gamma2$ and $I_\alpha2$-$C_\alpha2$ transcripts as well as the corresponding mature transcripts. Compared to IgD^+ B cells stimulated with BLyS or APRIL alone, B cells incubated with BLyS or APRIL and IL-10 further up-regulated germline $I_\gamma1$-$C_\gamma1$, $I_\gamma2$-$C_\gamma2$, $I_\gamma3$-$C_\gamma3$ and $I_\alpha1$-$C_\alpha1$ or $I_\alpha2$-$C_\alpha2$ transcripts as well as the corresponding mature transcripts. Finally, IgD^+ B cells stimulated with BLyS or APRIL and IL-4 induced germline and mature transcripts corresponding to all seven human downstream $C_\gamma1$, $C_\gamma2$, $C_\gamma3$, $C_\gamma4$, $C_\alpha1$, $C_\alpha2$ and C_ε genes. Thus, in the presence of appropriate cytokines, BLyS and APRIL up-regulate multiple downstream I_H-C_H and V_HDJ_H-C_H transcripts in a CD40-independent manner.

BCR enhances BLyS- or APRIL-induced NF-κB

In addition to playing a central role in B cell proliferation and survival, NF-κB–Rel induces germline I_H-C_H transcription by activating the CD40 responsive element (RE) within the targeted I_H promoter[2,33]. Compared to IgD^+ B cells exposed to medium alone, IgD^+ B cells incubated with BLyS or APRIL up-regulated the binding of NF-κB-Rel to the CD40 RE of the germline $C_\gamma3$ gene promoter (Fig. 4b). NF-κB was also activated by BCR engagement, although to a lesser extent than BLyS or APRIL. Consistent with the critical role of antigen in TI antibody production[34] and with the ability of BLyS and APRIL to increase the proliferation and differentiation of BCR-activated B cells[14,35], BCR engagement enhanced the activation of NF-κB by BLyS or APRIL.

Further experiments established that BLyS and APRIL induce the nuclear translocation of similar NF-κB–Rel complexes, including p50–p65 and p50-c-Rel complexes (Fig. 4c). Smaller amounts of p50-p50 complexes were also detectable (data not shown). In BLyS-stimulated B cells, NF-κB activation was comparably inhibited by soluble TACI-Ig and BCMA-Ig decoy receptors (Fig. 4d), which bind to BLyS with high affinity[17,26]. In APRIL-stimulated B cells, NF-κB activation was inhibited by BCMA-Ig, which binds to APRIL with high affinity, and, to a lesser extent, by TACI-Ig, which binds to APRIL with lower affinity than BCMA-Ig[20,26]. As expected, a neutralizing antibody to soluble BLyS interfered with the activation of NF-κB induced by BLyS but not with that induced by APRIL. These experiments showed that both BLyS and APRIL activate NF-κB–Rel in human B cells. This activation was further up-regulated by BCR engagement and could be specifically inhibited by soluble BLyS and APRIL decoy receptors.

BLyS or APRIL and BCR induce IgG and IgA secretion

After exposure to BLyS and IL-4, up to 20% of IgD^+ B cells up-regulated surface IgG and IgA and concomitantly down-regulated surface IgM and IgD (Fig. 5a). These cells or B cells exposed to BLyS or APRIL alone did not secrete IgG and IgA (data not shown), unless further stimulated through BCR cross-linking (Fig. 5b). IgG and IgA production was further enhanced by IL-15 (Fig. 5b) or IL-2 (data not shown), two inducers of antibody secretion produced by APCs and T cells, respectively[36,37]. In BLyS-stimulated B cells, IgG and IgA secretion was comparably inhibited by soluble TACI-Ig and BCMA-Ig. In APRIL-stimulated B cells, IgG and IgA secretion was inhibited by BCMA-Ig and to a lesser extent

by TACI-Ig. Thus, BCR cross-linking and IL-15 are necessary to stimulate the secretion of class switched antibodies by BLyS- or APRIL-activated B cells.

IFN, CD40L or LPS induce BLyS and APRIL

After the capture of viral or bacterial antigens, DCs express IFN-α, a type I IFN that, in addition to possessing antiviral activity, critically modulates both TD and TI immune responses, including Ig class switching[38,39]. A similar effect is elicited by IFN-γ, a type II IFN expressed by several cell types, including APCs, natural killer (NK) cells and T cells[2,38]. Given the key roles of both IFN-α and IFN-γ in DC activation and differentiation[40–42], we hypothesized that IFNs modulate Ig class switching by up-regulating the expression of BLyS and APRIL in DCs. Compared to DCs incubated with medium alone, DCs incubated with IFN-α or IFN-γ up-regulated BLyS and APRIL transcripts, total BLyS and APRIL proteins, soluble BLyS as well as membrane-bound BLyS (Fig. 6). Similar up-regulation was induced by CD40L, another critical modulator of DC activation, maturation and survival[41]. Like DCs, monocytes up-regulated soluble and membrane-bound BLyS after stimulation with IFN-α, IFN-γ or bacterial LPS (Fig. 7a). These findings indicated that both innate and T cell–derived immune modulators enhance the expression of BLyS and APRIL.

DCs induce CSR through BLyS and APRIL

DCs play an key role in the modulation of CD40-dependent Ig class switching[12,41]. To verify whether APCs induce CD40-independent class switching through BLyS and APRIL, DCs or monocytes were first activated with CD40L, IFN-γ, IFN-α or LPS, then washed and exposed to IgD^+ B cells. Compared to IgD^+ B cells incubated with medium alone or control epithelial cells, IgD^+ B cells incubated with unstimulated DCs or unstimulated monocytes contained small amounts of $I_\gamma1/2\text{-}C_\mu$, $I_\gamma3\text{-}C_{\mu\_}$ and $I_\alpha1/2\text{-}C_\mu$ CTs and AID transcripts (Figs. 6d and 7b). All these markers of CSR were up-regulated by culturing IgD^+ B cells with activated DCs (Fig. 6d), activated monocytes (Fig. 7b) or culture fluids from monocytes incubated with IFN-γ (Fig. 7c). Activated DCs and monocytes induced CSR in a BLyS and APRIL–dependent but CD40-independent manner; up-regulation of CTs and AID was inhibited by BCMA-Ig and, to a lesser extent, by TACI-Ig or a neutralizing antibody to soluble BLyS (Figs. 6d and 7b,c). DC- and monocyte-induced CSR was also partially inhibited by neutralizing antibodies to IL-10 or TGF-β (data not shown), but not by a control mouse IgG1 or CD40-Ig, a soluble inhibitor of CD40L-CD40 interaction (Figs. 6d and 7b,c). These findings indicated that APCs elicit CD40-independent CSR to $C_\gamma$and C_α through both BLyS and APRIL.

DCs induce IgG and IgA through BLyS and APRIL

B cells undergoing terminal plasmacytoid differentiation up-regulate CD38 and syndecan-1 (also known as CD138), down-regulate CD20 and accumulate cytoplasmic Igs that are subsequently secreted[36]. In the absence of BCR engagement, IgD^+ B cells exposed to resting or activated DCs did not secrete IgG and IgA (data not shown). In the presence of BCR engagement, IgD^+ B cells incubated with CD40L-, IFN-γ– or IFN-α–activated DCs but not with resting DCs or control epithelial cells secreted IgG and IgA (Fig. 8a). This secretion occurred in a BLyS and APRIL–dependent but CD40-independent manner; it was

inhibited by BCMA-Ig and, to a lesser extent, by TACI-Ig or a neutralizing antibody to soluble BLyS, but not by a control IgG1 or CD40-Ig. DC-induced IgG and IgA secretion was also dependent on IL-15, as it was severely inhibited by a neutralizing antibody to this cytokine. In addition to secreting IgA and IgG, IgD^+ B cells exposed to activated DCs accumulated cytoplasmic IgA (Fig. 8b–d) and IgG (data not shown), down-regulated CD20 and up-regulated CD38 and syndecan-1 (Fig. 8e–g). Again, these plasmacytoid phenotypic traits were induced in a BLyS and APRIL–dependent but CD40-independent manner; they were inhibited by BCMA-Ig but not by CD40-Ig. Thus, APCs induced CD40-independent IgG and IgA secretion by expressing BLyS, APRIL and cytokines, including IL-15.

Discussion

We have shown that human DCs and monocytes up-regulate BLyS and APRIL upon activation by type I or II IFNs or CD40L. When exposed to BLyS or APRIL and appropriate cytokines, B cells undergo CD40-independent CSR to C_γ, $C_\alpha$or C_ε. In these cells, secretion of class-switched Igs requires BCR engagement. Our findings suggest that BLyS and APRIL belong to an evolutionary primitive pathway that, by mediating TI class switching, critically links the innate and adaptive immune responses.

Viral glycoproteins and bacterial polysaccharides induce IgM production as well as switching to IgG and IgA in the absence of $CD4^+$ T cells[8,9–11]. TI IgM and IgG production occurs mainly in $CD5^-$ B2 cells lodged in the splenic marginal zone and is critical in the clearance of blood-borne pathogens, as indicated by the high frequency of infections by encapsulated bacteria after splenectomy[9–11]. TI IgA production occurs in $CD5^+$ B1 cells in the intestinal lamina propria and is critical in the elimination of pathogens and prevention of the systemic penetration of dietary antigens and commensal bacteria[9–11,43]. Our findings indicate that TI class switching to IgG and IgA may be initiated by DCs and macrophages through BLyS and APRIL.

DCs and macrophages in the splenic marginal zone and intestinal mucosa sample and retain TI antigens[10,11,44]. Antigen capture would initiate TI Ig class switching by triggering the secretion of IFN-α[40–42], a type I IFN that up-regulates the expression of BLyS and APRIL in APCs. In the presence of IL-4, IL-10 or TFG-β produced by marginal zone or intestinal APCs, stromal cells and/or mast cells, BLyS and APRIL would induce CD40-independent CSR to C_γ, C_α and even C_ε[45] in IgD^+ and/or IgM^+ B cells. This model is consistent with published studies showing that IFN-α–activated DCs trigger TI class switching to all IgG subclasses in mice[38] and enhance IgG and IgA secretion in patients with SLE[39]. A similar DC-mediated BLyS and APRIL–dependent mechanism may also underlie the induction of TI class switching by IFN-γ or bacterial LPS[2,8,9]. Although it plays a central role in TI antibody production[8,34], BCR cross-linking is not required to initiate CSR in BLyS- or APRIL-activated B cells. Consistent with this, TI switching to IgA can occur without previous expression of surface IgM or IgD[46]. Yet, by up-regulating NF-κB, BCR engagement might enhance germline I_H-C_H transcription and CSR in B cells activated by BLyS and/or APRIL.

The relative contributions of TACI, BCMA and BAFF-R to activation of the CSR machinery remains elusive. Despite accumulating B cells, TACI-deficient mice show decreased serum IgA as well as impaired TI. but not TD, IgG and IgA responses[27]. Unlike these mice, BCMA-deficient mice lack an overt phenotype, which suggests a redundant role for BCMA[25]. In addition, BAFF-R–deficient mice and A/WySnJ mice, which express a disrupted BAFF-R, show fewer B cells as well as impaired TI IgG production[19,25]. Thus, TI CSR may result from the combined activation of TACI and BAFF-R. These CD40-like receptors induce nuclear translocation of NF-κB[15,47,48], which might activate I_γ and I_ε gene promoters by cooperating with IL-4 receptor–induced signal transducer and activator of transcription 6 (STAT6), and I_α gene promoters by cooperating with TGF-β receptor–induced Smad proteins[2]. When combined with IL-10, both BLyS and APRIL up-regulate germline $I_\gamma1$-$C_\gamma1$, $I_\gamma2$-$C_\gamma2$ and $I_\gamma3$-$C_\gamma3$ transcripts. However, although BLyS preferentially up-regulates $I_\alpha1$-$C_\alpha1$ transcripts, APRIL up-regulates mainly $I_\alpha2$-$C_\alpha2$ transcripts. This difference might stem from the activation of specific arrays of transcription factors by BLyS and APRIL. Like CD40-activated B cells[2], BLyS- or APRIL-activated B cells up-regulate $1_\gamma4$-$C_\gamma4$ and I_ε-C_ε transcripts only in the presence of IL-4.

B cells exposed to BLyS and APRIL do not secrete IgG and IgA unless stimulated through extensive BCR cross-linking. This would increase the specificity of a TI antibody response, as only B cells that are directly stimulated by the TI antigen would terminally differentiate into antibody-secreting plasmacytoid cells. Signals emanating from BCR, TACI, BCMA, BAFF-R and cytokine receptors would synergistically elicit proliferation and antibody production by cooperatively activating critical transcription factors, including NF-κB. Among the cytokines, IL-15 plays a key role in the differentiation of BLyS and APRIL–stimulated B cells to plasmacytoid cells. This cytokine is expressed by DCs and macrophages, signals through the β and γ receptor subunits of the IL-2 receptor and, like T cell–derived IL-2, costimulates antibody production in CD40-activated B cells[36,37].

Impaired IgG, IgA and IgE production in patients with the X-linked hyper-IgM syndrome[7] is thought to result from the interruption of CD40 signaling in B cells. By showing that CD40 engagement up-regulates CSR-inducing BLyS and APRIL in DCs, our findings suggest that CD40-CD40L interaction elicits Ig class switching by activating B cells both directly through CD40 and indirectly through BLyS and APRIL receptors. Consistent with this, TD IgG and IgA responses are severely decreased in BLyS-deficient mice[25]. The persistence of TI IgG and IgA production in CD40L-deficient mice[23,24] could be due to the ability of TI antigens to up-regulate BLyS and APRIL through IFN-α and LPS. Finally, by showing that BLyS and APRIL are up-regulated by T cell CD40L and IFN-γ, and—together with T cell IL-4—induce CSR to C_γ, C_α and C_ε genes, our findings further emphasize that BLyS and APRIL critically amplify TD Ig class switching. Thus, TACI-Ig, BCMA-Ig or antagonistic antibodies to BLyS and APRIL could be used to attenuate abnormal switching to IgG, IgA and/or IgE in autoimmune and allergic disorders, including SLE, rheumatoid arthritis and asthma.

Methods

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats purchased from the New York Blood Bank. IgD+ B cells were purified from PBMCs as described[33,49]. $CD14^+$ monocytes were magnetically sorted from PBMCs with a fluorescein (FITC)-conjugated antibody to CD14 (Serotec, Raleigh, NC) and anti-FITC microbeads (Miltenyi Biotec, Auburn, CA). To obtain $CD83^+$ DCs, purified monocytes were stimulated for 7 days with granulocyte monocyte-colony stimulating factor (GM-CSF, 50 ng/ml, R&D Systems, Minneapolis, MN), IL-4 (250 U/ml, Schering-Plough, Kenilworth, NJ) and monocyte-derived conditioned medium[50]. B cells (5.0×10^4) and DCs (2.5×10^4) or irradiated (4,000 rad) epithelial-like human embryonic kidney 293 cells (2.5×10^4) were cocultured in RPMI 1640 medium at a final volume of 200 μl.

Reagents

Human BLyS (Human Genome Sciences, Rockville, MD), APRIL MegaLigand (Alexis Biochemicals, San Diego, CA) and trimeric CD40L (Immunex, Seattle, WA) were used at 1 μg/ml. Human IL-10 (200 ng/ml, Schering-Plough), TGF-β (0.5 ng/ml), TNF-α (25 ng/ml), LT-α (25 ng/ml), IFN-α2a (50 U/ml), IFN-γ (50 U/ml, R&D Systems), IL-15 (100 ng/ml, Sigma, St. Louis, MO) and LPS from Escherichia Coli serotype 026:B6 (1 μg/ml, Sigma) were also used. Mouse IgG1 control MOPC-21 (Sigma), TACI-Ig, BCMA-Ig, the mouse IgG1 neutralizing 15C10 monoclonal antibody (mAb) to soluble BLyS (Human Genome Sciences) and CD40-Ig (Ancell, Bayport, MN) were used at 30 μg/ml. To simulate BCR engagement by a polyvalent TI antigen, B cells were exposed to 2 μg/ml of Immunobead Reagent (Irvine Scientifics, Camarillo, CA), a solid phase antibody against human Ig[33,49].

Flow cytometry and fluorescence microscopy

CD 14, CD20 (Sigma), CD3S (Pharmingen, San Diego, CA), CD83, syndecan-1 (Serotec) were detected with FITC- or phycoerythrin (PE)-conjugated antibodies. Membrane-bound BLyS was labeled with the biotinylated antibody 9B6 (Human Genome Sciences) and PE-conjugated streptavidin (PharMingen)[35]. Cells were acquired with a FACScalibur analyzer (Becton Dickinson Immunocytometry Systems, San Jose, CA). To visualize cellular nuclei and cytoplasmic IgA, cells were centrifuged, fixed in methanol and labeled with 4',6-diamidine-2'-phenylindole dihydrochloride (Boehringer Mannheim, Indianapolis, IN) and a FITC-conjugated antibody to IgA (Sigma), respectively. Slides were analyzed with a fluorescence microscope Zeiss Axioplan 2 (Atto Instruments, Rockville, MD).

ELISAs

Supernatants from DCs or monocytes (5×10^6/ml) were incubated overnight at 4°C on microplates coated with the capture mouse mAb 15C10 (IgG1) to human soluble BLyS (Human Genome Sciences). A biotinylated rabbit polyclonal antibody to mouse IgG (Human Genome Sciences), peroxidase streptavidin and the substrate 3,3′,5,5′-tetramethylbenzidine (Kirkegaard and Perry, Gaithersburg, MD) were used in sequential

steps[35]. Readings were done at 450 nm. IgM, IgG, IgA and IgE were detected as described[33,49].

Genomic DNA PCRs

Genomic DNA was extracted from B cells with the QIAmp DNA Mini Kit (Qiagen, Valencia, CA). Total S_γ-S_μ SCs as well as S_γ1/2-S_μ, S_γ3-S_μ, S_γ4-S_μ, S_α1/2-S_μ and S_ε-S_μ SCs were amplified from 500 ng of genomic DNA by a PCR strategy that included two nested forward primers that recognize I_H and the 5′ segment of S_γ, S_α or S_ε and two nested reverse primers that recognize distinct areas of the 3′ segment of S_μ[33,49]. Due to their high degree of sequence identity, the I_γ1 and I_γ2 regions as well as the I_α1 and I_α2 regions were amplified with common I_γ1/2 and I_α1/2 primers, respectively. The conditions were denaturation 1 min at 94 °C, annealing 1 min at 68 °C and extension 4 min at 72 °C for two rounds of 30 cycles. Before each PCR, DNA was denaturated for 5 min at 94 °C. The identity of PCR products with SCs was confirmed by DNA sequencing, β-actin was amplified as reported.

RT-PCRs

cDNA was reverse transcribed (RT) from 3 μg of total RNA[33,49]. PCRs were made semiquantitative by varying the number of amplification cycles and performing dilutional analysis so that there was a linear relationship between the amount of cDNA used and the intensity of the PCR product. I_γ1-C_γ1 (603 bp), I_γ2-C_γ2 (597 bp), I_γ3-C_γ3 (670 bp), I_γ4-C_γ4 (411 bp), I_α1-C_α1 (1,194 bp), I_α2-C_α2 (1,181 bp), V_HDJ_H-C_γ1 (~415 bp), V_HDJ_H-C_γ2 (~415 bp), V_HDJ_H-C_γ3 (~415 bp), V_HDJ_H-C_γ4 (~415 bp), V_HDJ_H-C_α1 (~900 bp), V_HDJ_H-C_α2 (~890 bp), V_HDJ_H-C_ε (~200 bp), β-actin (593 bp) and Igβ (220 bp) were RT-PCR-amphfied for 25 cycles as described[33,49]. I_ε-C_ε (409), AID (382 bp), BLyS (398 bp) and APRIL (417 bp) were PCR-amplified for 25 cycles with the following primer pairs. I_ε, forward 5′-GACGGGCCACACCATCCACAGGCACCAAATGGACGAC-3′ and C_ε, reverse 5′-CAGGACGACTGTAAGATCTTCACG-3′ AID forward 5′-TGCTCTTCCTC-CGCTACATCTC-3′ and AID reverse 5′-AACCTCATACAGGGGCAAAAGG-3′ BLyS forward 5′-CAGCTCCAGGAGAAGGCAACT-3′ and BLyS reverse 5′-CAAT-GCCAGCTGAATAGCAGG-3′ APRIL forward 5′-GCCAGCCTCATCTCCTTTCTTG-3′ and APRIL reverse 5′-TGGTTGCCACATCACCTCTGTCAC-3′. I_γ1/2-C_μ, (557 bp), I_γ3-C_μ (608 bp) I_γ4-C_μ (358 bp) I_α1/2-C_μ (666 bp) and I_ε-C_μ (408 bp) CTs were RT-PCR-amphfied for 25 cycles with the forward primers I_γ1/2 5′-GGGCTTCCAAGC-CAACAGGGCAGGACA-3′, I_γ3 5′-AGGTGGGCAGGCTTCAGGCACCGAT-3′, I_γ4 5′-TTGTCCAGGCCGGCAGCATCACCAGA-3′, I_α1/2 5′-CAGCAGCCCTCTTG-GCAGGCAGCCAG-3′ and I_ε, 5′-GAC GGGCCACACCATCCACAGGCACCAAATG-GACGAC-3′ together with the reverse primer C_μ 5'-GTTGCCGTTGGGGTGCTGGAC-3′. The PCR conditions were denaturation 1 min at 94 °C, annealing 1 min at 60 °C and extension 1 min at 72 °C.

Southern blots

PCR products were fractionated onto agarose gels, transferred overnight onto nylon membranes and hybridized with specific radiolabeled probes[33,49]. SCs were hybridized with a probe that recognized the recombined S_μ region. Hybridization products appeared smeary on gel electrophoresis because CSR did not target a consensus DNA sequence, but it

randomly occurred within a 1–10 kb S region. In each B cell, CSR yielded a single copy S_μ-S_x SC with a size that ranged from 500–4,000 bp. CTs were hybridized with a probe that encompassed nucleotides 1–250 of the first C_μ exon. Two $I_\gamma 1/2\text{-}C_\mu$ and $I_\alpha 1/2\text{-}C_\mu$ bands represented alternatively spliced forms, as reported[30].

Immunoblots and electrophoretic mobility shift assays

Total proteins were obtained as described[49], fractionated onto 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking, membranes were immunoblotted with antibodies to BLyS (Upstate Biotechnology, Lake Placid, NY), APRIL or actin (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were detected with an enhanced chemiluminescence detection system (Amersham, Little Chalfont, UK). Nuclear proteins were extracted as described[33,49] and incubated with radiolabeled double stranded oligonucleotide probes that encompassed the κB1 site of the germ line $C_\gamma 3$ gene promoter. DNA binding reactions and inhibition assays with antibodies to p65, c-Rel, RelB, p50 or p52 (Santa Cruz Biotechnology) were done as described[33,49].

Acknowledgments

We thank K. S. Picha (Immunex Corp.) for recombinant soluble CD40L and S. Narula (Schering-Plough) for IL-4 and IL-10. Supported by a New Investigator Grant from The Leukemia Research Foundation (to A. C), research grants from The SLE Foundation (to A. C. and P. C.) and grants from the National Institutes of Health AR 47872 (to A. C.), AR 40908 and AI 45011 (to PC).

References

1. Bassing CH, Swat W, Alt FW. The mechanism and regulation of chromosomal V(D)J recombination. Cell. 2002; 109(Suppl):45–55.
2. Stavnezer J. Antibody class switching. Adv. Immunol. 1996; 61:79–146. [PubMed: 8834495]
3. Papavasiliou FN, Schatz DG. Somatic hypermutation of immunoglobulin genes: merging mechanisms for genetic diversity. Cell. 2002; 109(Suppl):35–44.
4. Manis JP, Tian M, Alt FW. Mechanism and control of class-switch recombination. Trends Immunol. 2002; 23:31–39. [PubMed: 11801452]
5. MacLennan IC. Germinal centers. Annu. Rev. Immunol. 1994; 12:117–139. [PubMed: 8011279]
6. Okazaki I, Kinoshita K, Muramatsu M, Yoshikawa K, Honjo T. The AID enzyme induces class switch recombination in fibroblasts. Nature. 2002; 416:340–345. [PubMed: 11875397]
7. Durandy A, Honjo T. Human genetic defects in class-switch recombination (hyper-IgM syndromes). Curr. Opin. Immunol. 2001; 13:543–548. [PubMed: 11544001]
8. Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. Annu. Rev. Immunol. 1995; 13:655–692. [PubMed: 7612238]
9. Szomolanyi-Tsuda E, Welsh RM. T-cell-independent antiviral antibody responses. Curr. Opin.Immunol. 1998; 10:431–435. [PubMed: 9722919]
10. Cyster JG. B cells on the front line. Nature Immunol. 2000; 1:9–10. [PubMed: 10881164]
11. Fagarasan S, Honjo T. T-Independent immune response: new aspects of B cell biology. Science. 2000; 290:89–92. [PubMed: 11021805]
12. Fayette J, et al. Human dendritic cells skew isotype switching of CD40-activated naive B cells towards IgA1 and IgA2. J. Exp. Med. 1997; 185:1909–1918. [PubMed: 9166420]
13. Moore PA, et al. BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. Science. 1999; 285:260–263. [PubMed: 10398604]
14. Schneider P, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J. Exp. Med. 1999; 189:1747–1756. [PubMed: 10359578]

15. Yan M, et al. Identification of a receptor for BLyS demonstrates a crucial role in humoral immunity. Nature Immunol. 2000; 1:37–41. [PubMed: 10881172]
16. Thompson JS, et al. BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. J. Exp. Med. 2000; 192:129–135. [PubMed: 10880534]
17. Gross JA, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature. 2000; 404:995–999. [PubMed: 10801128]
18. Gross JA, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. Impaired B cell maturation in mice lacking BLyS. Immunity. 2001; 15:289–302. [PubMed: 11520463]
19. Thompson JS, et al. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. Science. 2001; 293:2108–2111. [PubMed: 11509692]
20. Hahne M, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. J. Exp. Med. 1998; 188:1185–1190. [PubMed: 9743536]
21. Rennert P, et al. 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. 2000; 192:1677–1684. [PubMed: 11104810]
22. Do RK, et al. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. J. Exp. Med. 2000; 192:953–964. [PubMed: 11015437]
23. Xu J, et al. Mice deficient for the CD40 ligand. Immunity. 1994; 5:423–431. [PubMed: 7882172]
24. Renshaw BR, et al. Humoral immune responses in CD40 ligand-deficient mice. J. Exp. Med. 1994; 180:1889–1900. [PubMed: 7964465]
25. Schiemann B, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science. 2001; 293:2111–2114. [PubMed: 11509691]
26. Yu G, et al. APRIL and TALL-1 and receptors BCMA and TACI: system for regulating humoral immunity. Nature Immunol. 2000; 1:252–256. [PubMed: 10973284]
27. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity. 2001; 14:573–582. [PubMed: 11371359]
28. Mackay F, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J. Exp. Med. 1999; 190:1697–1710. [PubMed: 10587360]
29. Stein JV, et al. APRIL modulates B and T cell immunity. J. Clin. Invest. 2002; 109:1587–1598. [PubMed: 12070306]
30. Kinoshita K, Harigai M, Fagarasan S, Muramatsu M, Honjo T. A hallmark of active class switch recombination: transcripts directed by I promoters on looped-out circular DNAs. Proc. Natl. Acad. Sci. USA. 2001; 98:12620–12623. [PubMed: 11606740]
31. Cerutti A, et al. CD40 ligand and appropriate cytokines induce switching to IgG, IgA, and IgE and coordinated germinal center and plasmacytoid phenotypic differentiation in a human monoclonal $IgM^{+}IgD^{+}$ B cell line. J. Immunol. 1998; 160:2145–2157. [PubMed: 9498752]
32. Matsumoto M, et al. Role of lymphotoxin and the type 1 TNF receptor in the formation of germinal centers. Science. 1996; 271:1289–1291. [PubMed: 8638112]
33. Cerutti A, et al. CD30 is a CD40-inducible molecule that negatively regulates CD40-mediated immunoglobulin class switching in non-antigen-selected human B cells. Immunity. 1998; 9:247–256. [PubMed: 9729045]
34. de Vinuesa CG, et al. Germinal centers without T cells. J. Exp. Med. 2000; 191:485–494. [PubMed: 10662794]
35. Nardelli B, et al. Synthesis and release of B-lymphocyte stimulator from myeloid cells. Blood. 2001; 97:198–204. [PubMed: 11133761]
36. Arpin C, et al. Generation of memory B cells and plasma cells *in vitro*. Science. 1995; 268:720–722. [PubMed: 7537388]
37. Armitage RJ, Macduff BM, Eisenman J, Paxton R, Grabstein KH. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. J. Immunol. 1995; 154:483–490. [PubMed: 7814861]

38. Le Bon A, et al. Type 1 interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells *in vivo*. Immunity. 2001; 14:461–470. [PubMed: 11336691]
39. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN-α in systemic lupus erythematosus. Science. 2001; 294:1540–1543. [PubMed: 11711679]
40. Siegal FP, et al. The nature of the principal type 1 interferon-producing cells in human blood. Science. 1999; 284:1835–1837. [PubMed: 10364556]
41. Banchereau J, et al. Immunobiology of dendritic cells. Annu. Rev. Immunol. 2000; 18:767–811. [PubMed: 10837075]
42. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell. 2001; 106:263–266. [PubMed: 11509174]
43. Macpherson AJ, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science. 2000; 288:2222–2226. [PubMed: 10864873]
44. Fagarasan S, Kinoshita K, Muramatsu M, Ikuta K, Honjo T. In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. Nature. 2001; 413:639–643. [PubMed: 11675788]
45. Life P, et al. T cell clones from an X-linked hyper-immunoglobulin (IgM) patient induce IgE synthesis *in vitro* despite expression of nonfunctional CD40 ligand. J. Exp. Med. 1994; 180:1775–1784. [PubMed: 7964460]
46. Macpherson AJ, et al. IgA production without μ or δ chain expression in developing B cells. Nature Immunol. 2001; 2:625–631. [PubMed: 11429547]
47. von Bulow GU, Bram RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. Science. 1997; 278:138–141. [PubMed: 9311921]
48. Xia XZ, et al. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. J. Exp. Med. 2000; 192:137–143. [PubMed: 10880535]
49. Cerutti A, et al. Dysregulation of $CD30^+$ T cells by leukemia impairs isotype switching in normal B cells. Nature Immunol. 2001; 2:150–156. [PubMed: 11175813]
50. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J. Exp. Med. 1994; 179:1109–1118. [PubMed: 8145033]

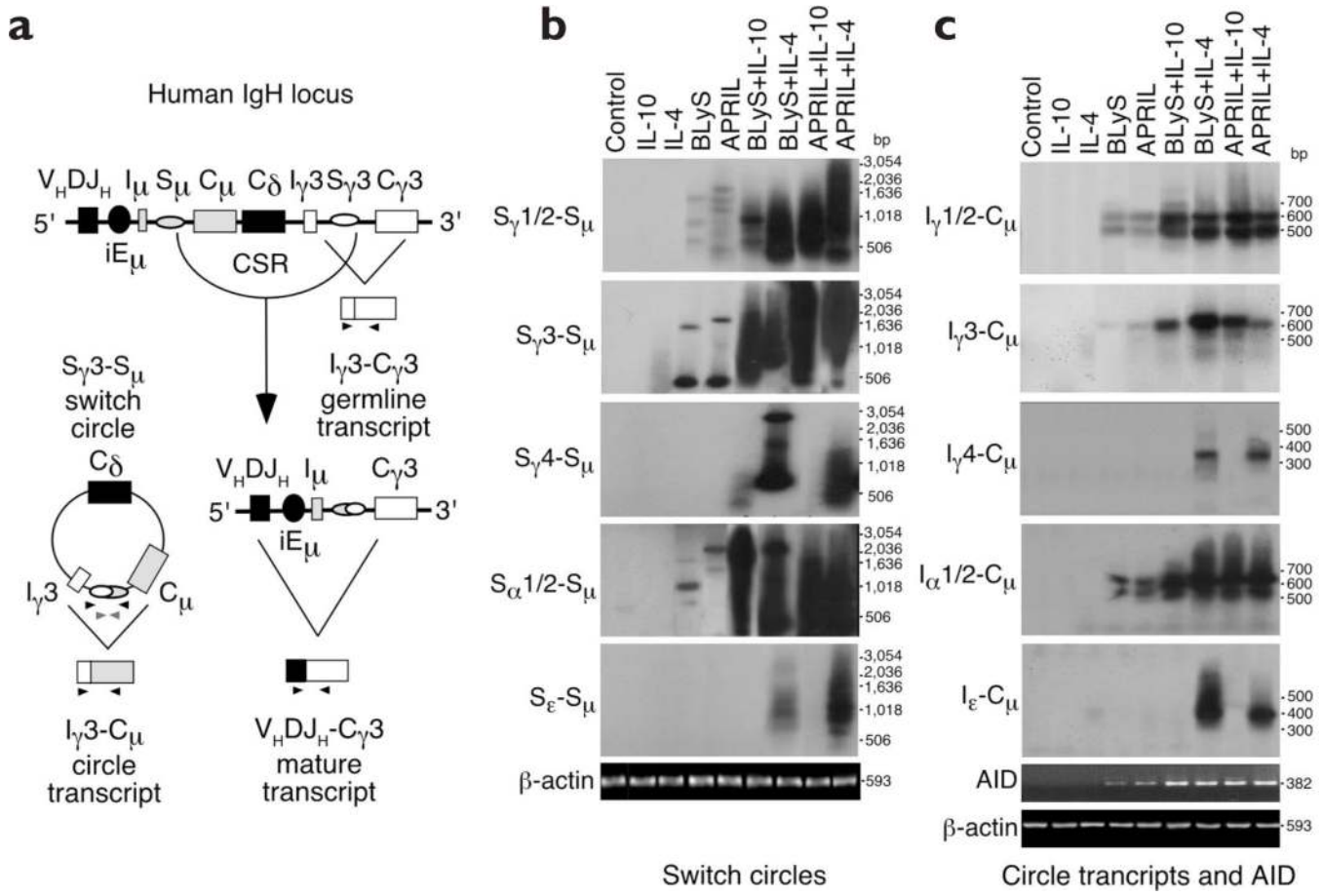

Figure 1. BLyS, APRIL, IL-4 and/or IL-10 induce CSR from C_μ to C_γ, C_α or C_ε

(**a**) Schematics of CSR from C_μ to $C_\gamma 3$. Ovals indicate S regions; rectangles are I_H exons and C_H gene exons; iE_μ is the IgH intronic enhancer; V-shaped lines indicate splicing; arrow-heads indicate the positions of the primers used to amplify SCs, CTs, germline I_H-C_H transcripts and mature V_HD_H-C_H transcripts, (**b**) Expression of SCs and β-actin (genomic DNA-loading control) in B cells incubated for 4 days with medium (control), IL-10, IL-4, BLyS or APRIL alone, BLyS + IL-10, BLyS + IL-4, APRIL + IL-10 or APRIL + IL-4 was assessed, (**c**) Expression of CTs, AID and β-actin transcripts; cells were treated as in **b**. One of three separate experiments that gave similar results is shown.

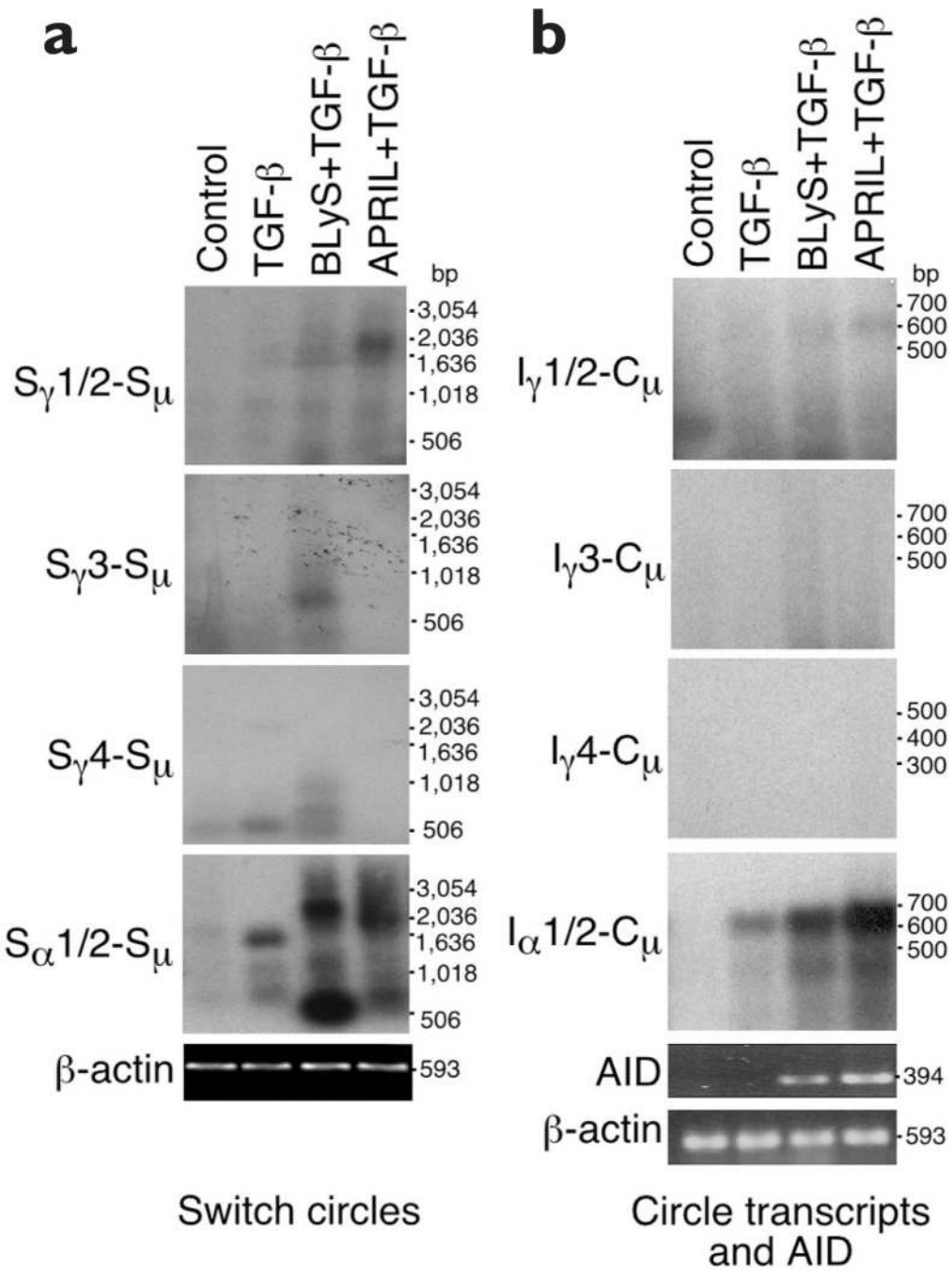

Figure 2. BLyS or APRIL and TGF-β induce CSR from C_μ to C_α

(**a**) IgD^+ B cells were incubated with medium (control), TGF-β1, BLyS + TGF-β1 or APRIL + TGF-β1. Expression of $S_\alpha 1/2S\mu$ and genomic β-actin after 4 days was assessed. (**b**) Expression of $I_\alpha 1/2\text{-}C_\mu$, AID and β-actin transcripts; cells were treated as in **a**. One of three separate experiments that gave similar results is shown.

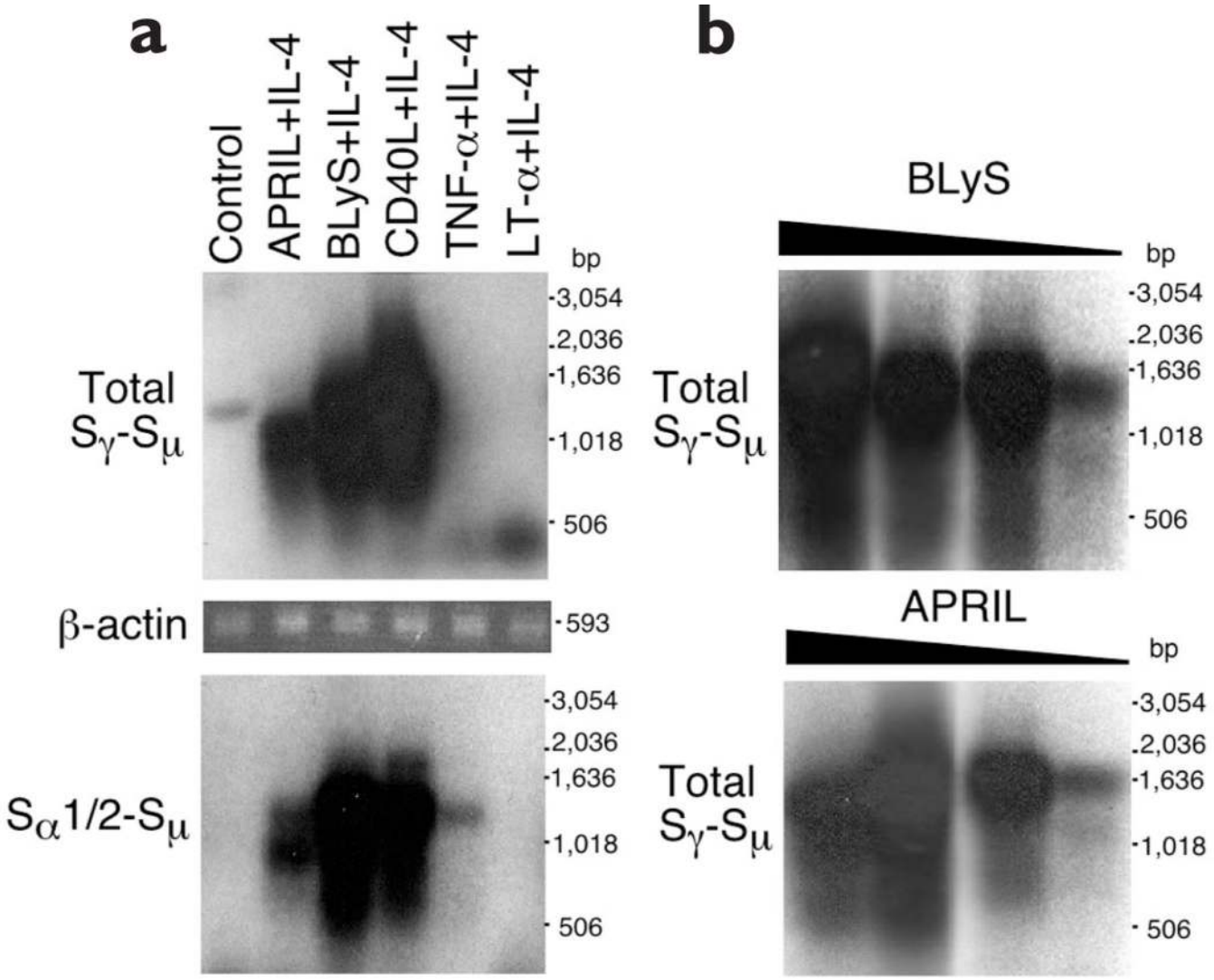

Figure 3. CSR is induced by BLyS, APRIL or CD40L but not by TNF-α or LT-α

(**a**) IgD$^+$ B cells were incubated with medium alone (control), APRIL + IL-4, BLyS + IL-4, CD40L + IL-4, TNF-α + IL-4 or LT-α + IL-4. Expression of total S_γ-S_μ SCs, S_α1/2-S_μ SCs and β-actin after 4 days was assessed, (**b**) IgD$^+$ B cells were incubated with IL-4 and 100, 10, 1 and 0.1 ng/ml of BLyS or APRIL. Expression of total S_γ-S_μ, after 4 days was assessed. One of three separate experiments that gave similar results is shown.

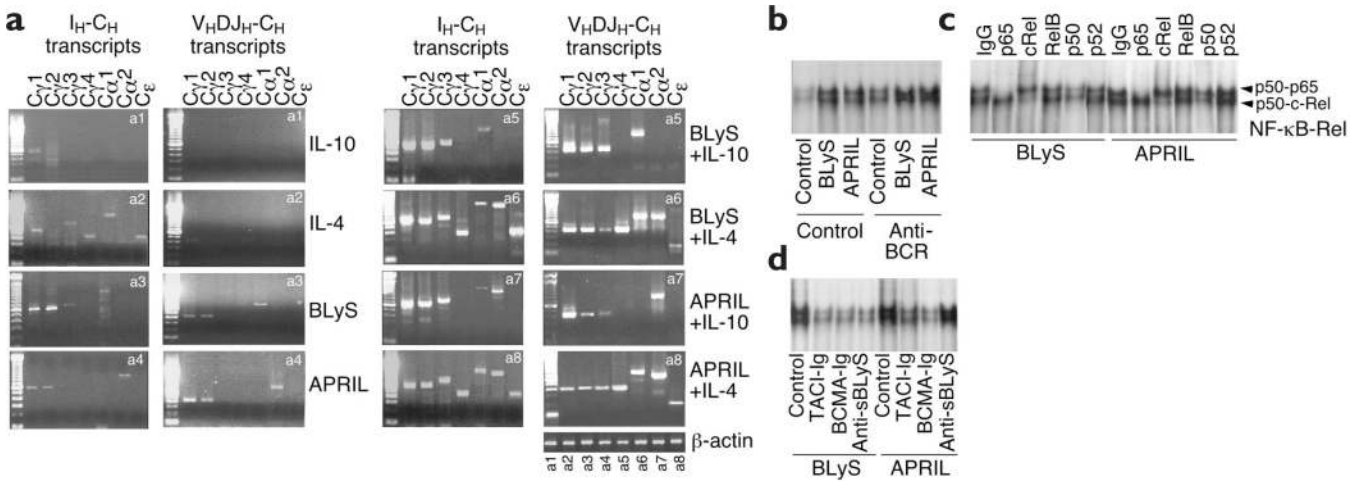

Figure 4. BLyS or APRIL up-regulate multiple germline I_H-C_H and mature V_HD_H-C_H transcripts

(**a**) Germline I_H-C_H, mature V_HD_H-C_H and β-actin transcripts from IgD^+ B cells incubated for 4 days with IL-10, IL-4, BLyS or APRIL alone, BLyS + IL-10, BLyS + IL-4, APRIL+ IL-10 or APRIL + IL-4 were analyzed. The first lane of each panel shows a 100-bp DNA ladder, (**b–d**) NF-κB binding to DNA after incubation of IgD^+ B cells with medium alone (control), BLyS or APRIL in the presence or absence of anti-BCR (**b**) and with or without a mouse control IgG1, TACI-Ig, BCMA-Ig or an antibody to soluble BLyS (sBLyS) (**d**). Arrows indicate p50–p65 and p50–c-Rel complexes, as identified by inhibition assays in which nuclear proteins from BLyS- or APRIL-activated B cells were first exposed to antibodies to p65, c-Rel, RelB and p50 or p52 and then incubated with the radiolabeled NF-κB–binding oligonucleotide (**c**). An irrelevant goat IgG was used as control. One of three separate experiments that gave similar results is shown.

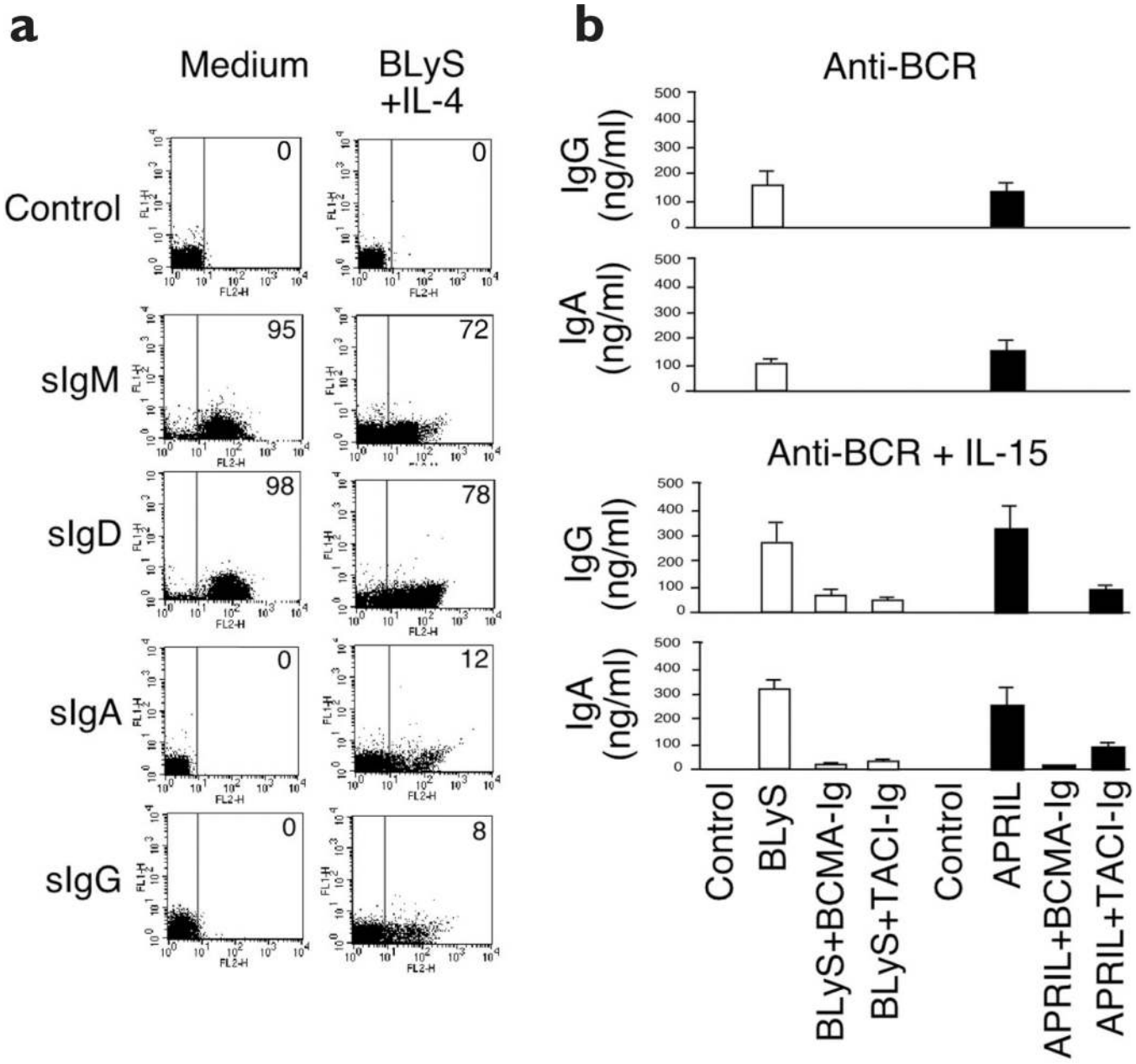

Figure 5. BLyS or APRIL up-regulate both membrane-bound and secreted IgG and IgA

(**a**) IgD^+ B cells were incubated with medium alone or BLyS and IL-4. Expression of surface IgM (sIgM), sIgD, sIgG and sIgA after 10 days was assessed. An isotype control is shown. Numbers indicate the percentages of class-switched B cells, (**b**) IgG and IgA secreted by IgD^+ B cells incubated for 10 days with BLyS or APRIL + anti-BCR or with BLyS or APRIL + anti-BCR and IL-15 and in the presence or absence of TACI-Ig or BCMA-Ig was assessed. Mean ± s.d. data of one of three separate experiments that gave similar results are shown.

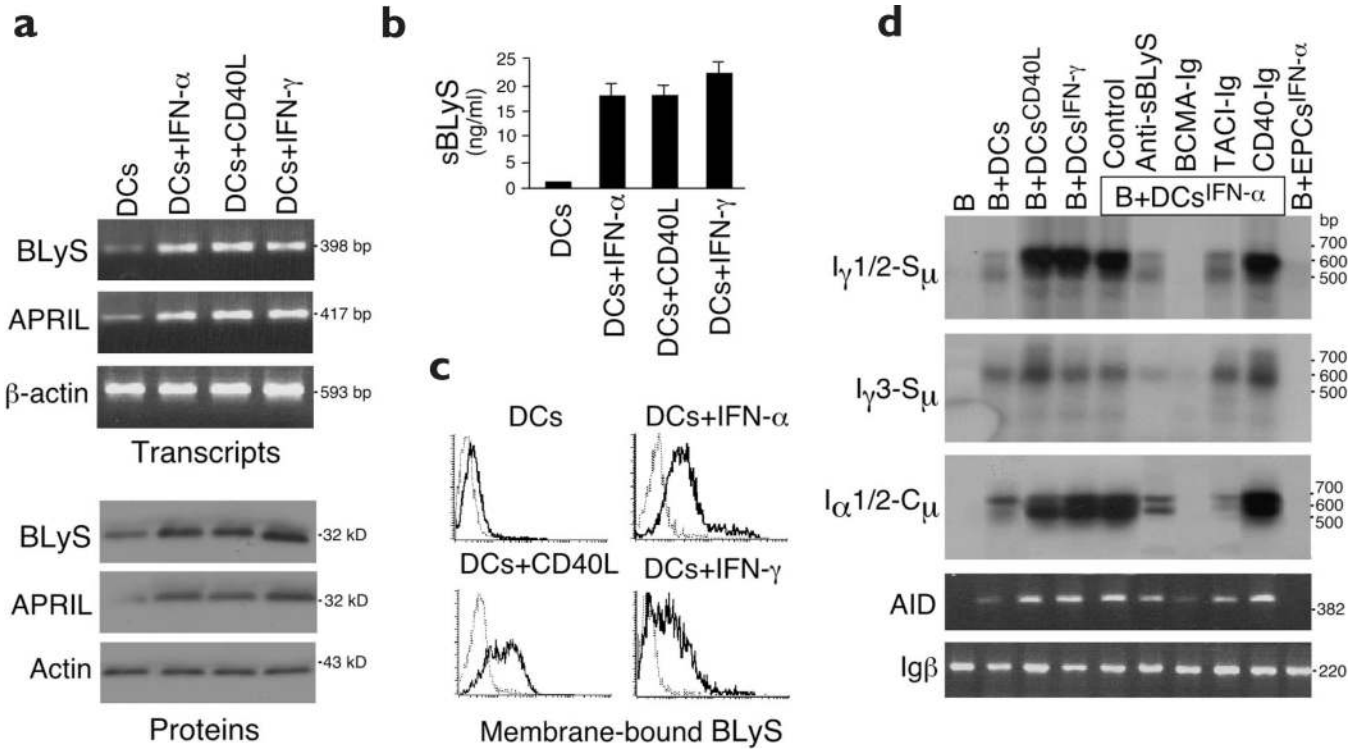

Figure 6. Activated DCs induce CSR through BLyS and APRIL

(**a**) DCs were incubated with medium alone, CD40L, IFN-α or IFN-γ. Expression of BLyS, APRIL and β-actin transcripts, and total BLyS, APRIL and actin proteins were assessed after 3 days. (**b**) Mean ± s.d. expression of sBLyS. (**c**) Expression of membrane-bound BLyS. Dotted and thick profiles depict control and BLyS antibodies, respectively, (**d**) IgD^+ B cells were cocultured with unstimulated or stimulated DCs or epithelial cells (EPCs) in the presence or absence of a control mouse antibody, TACI-Ig, BCMA-Ig, CD40-Ig or an antibody to sBLyS. $I_\gamma1/2\text{-}C_\mu$, $I_\gamma3\text{-}C_\mu$ and $I_\alpha1/2\text{-}C_\mu$ CTs as well as AID and Igβ (CD79b, B cell–specific loading control) transcripts were amplified after 4 days.

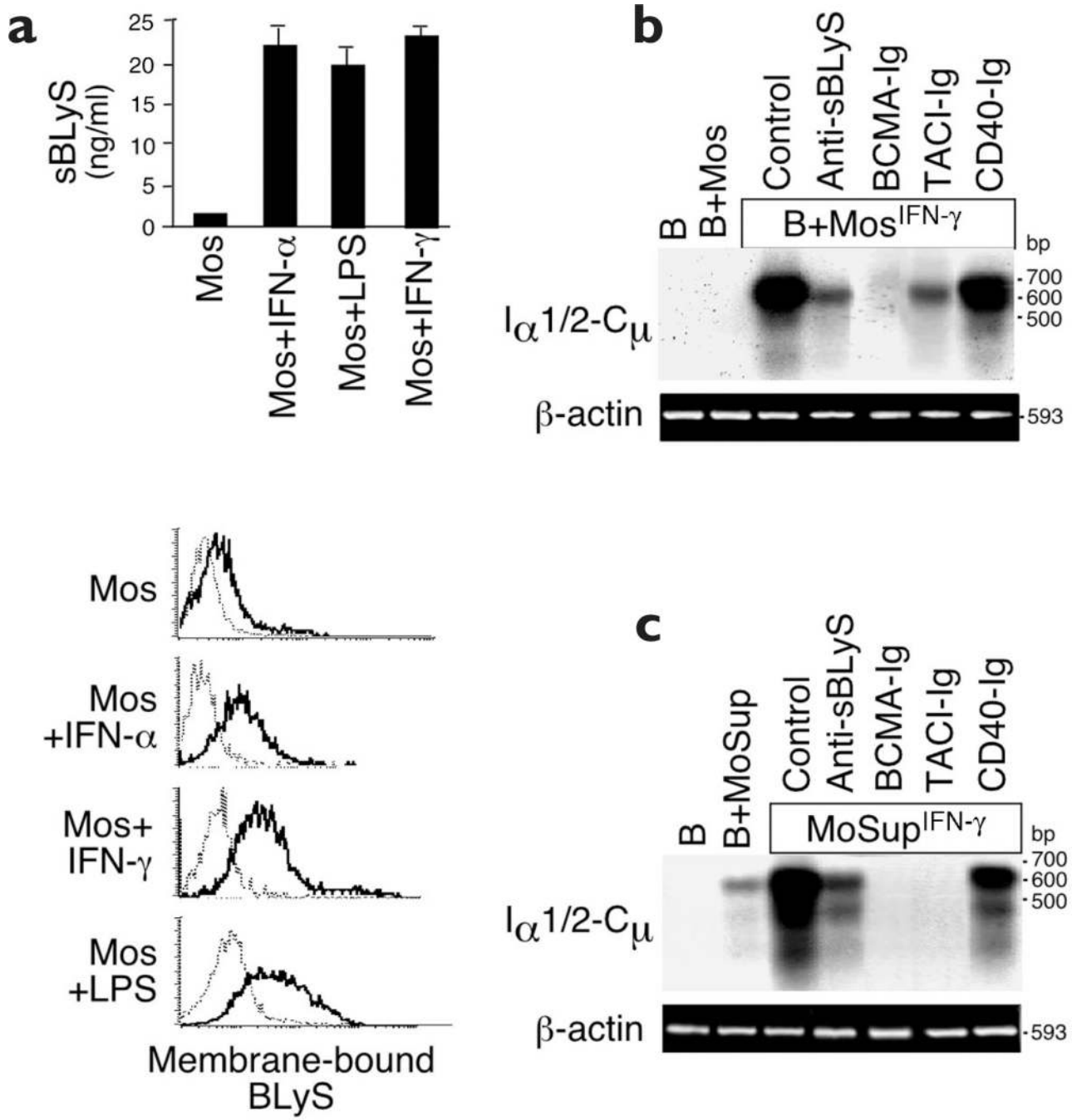

Figure 7. Activated monocytes induce CSR through BLyS and APRIL

(**a**) Monocytes (Mos) were incubated with medium alone, IFN-γ, IFN-α or LPS for 3 days. Soluble and membrane-bound BLyS were measured after 3 days, (**b**) IgD^+ B cells were incubated with resting or IFN-γ–activated monocytes in the presence or absence of a neutralizing antibodies to sBLyS, BCMA-Ig, TACI-Ig or CD40-Ig. Expression of $I_\alpha 1/2\text{-}C_\mu$ CTs and β-actin was assessed after 4 days, (**c**) IgD^+ B cells were incubated with supernatants from monocytes (MoSup) cultured for 3 days alone or with IFN-γ in the presence or absence of a control mouse antibody, BCMA-Ig, TACI-Ig, CD40-Ig or an antibody to sBLyS. $I_\alpha 1/2$-

C_μ CTs and β-actin were amplified after 4 days. One of three separate experiments that gave similar results is shown.

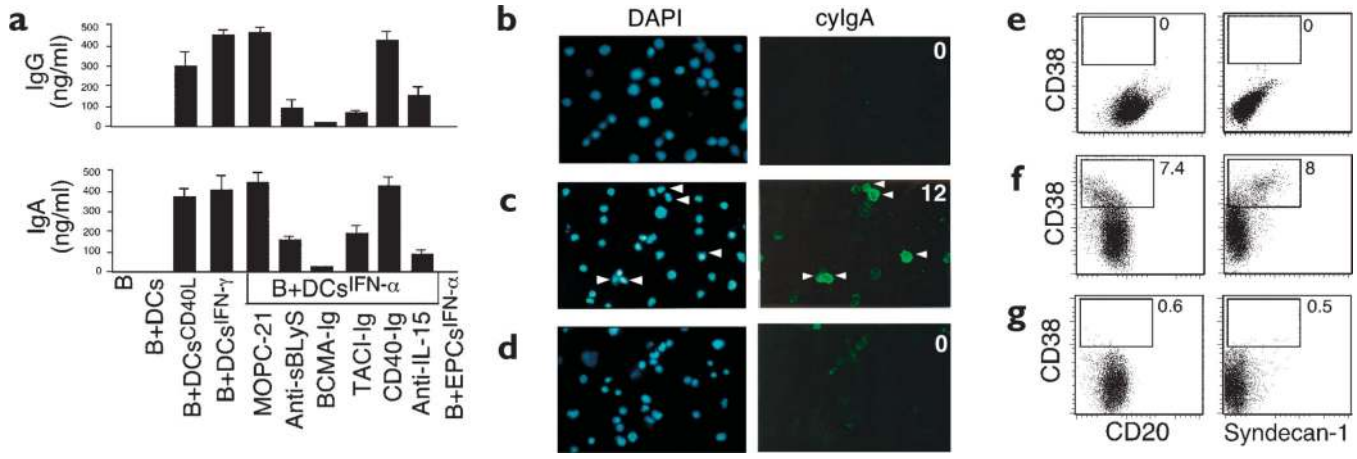

Figure 8. Activated DCs induce plasmacytoid differentiation through BLyS and APRIL

(**a**) BCR-activated IgD$^+$ B cells were incubated with resting or CD40L-, IFN-α–or IFN-γ–activated DCs or epithelial cells in the presence or absence of a control antibody, TACI-Ig, BCMA-Ig, CD40-Ig or a neutralizing antibody to sBLyS or IL-15. Secreted IgG and IgA were measured after 10 days, (**b–d**), BCR-activated IgD$^+$ B cells were incubated with IFN-α–activated epithelial cells, IL-15 and CD40-Ig (**b**), IFN-α–activated DCs, IL-15 and CD40-Ig (**c**), or IFN-α–activated DCs, IL-15 and BCMA-Ig (**d**). After 10 days, cells were stained with FITC-conjugated antibodies to IgA (green). Nuclear counterstaining was done with DAPI (blue, magnification:×1,000) and numbers indicate the percentages of cells expressing cytoplasmic IgA (**e–g**) IgD$^+$ B cells in **e–g** were cultured for 10 days as in **b–d**, respectively. Numbers indicate percentages of CD38^{hi}CD20^{lo} and CD38^{hi}CD 138$^+$ plasmacytoid cells. Data are mean ± s.d. of one of three separate experiments that gave similar results.