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To the editor:

Regulation of human dendritic cells by B cells depends on the signals they receive

B cells are classically known for producing antibodies. However, several reports also indicate that B cells are potent regulators of immune responses including those mediated by dendritic cells (DCs), although direct effect of B cells on DC was relatively unexplored.[1-4] Recently, Morva et al demonstrated that CD40 + TLR9 (CpG)–stimulated human B cells restrain the differentiation of monocyte-derived DCs and their maturation.[5] They further found that stimulation of B cells is necessary to trigger potent regulatory activities on DCs.

Under physiological conditions, there is a constant cross-talk between DCs and B cells. Although suppression of DCs may play a role in preventing the autoimmunity, normal functioning of DCs without suppression is also critical for immune homeostasis and immune response to tumor cells and foreign antigens. These views indicate that B cells are not continually inhibitory on DCs. As B cells can receive activation signals via diverse receptors including B-cell receptor (BCR), CD40 and TLR, we surmised that the effect of stimulated B cells on DCs depends on the type of signals they receive. In view of the importance of BCR signaling in B-cell activation and in generation of regulatory B cells,[1,2] we explored the role of BCR-stimulated B cells either alone or in combination with TLR9 stimulation on the differentiation and maturation of DC.

We found that when monocytes were differentiated into DCs in the presence of cytokines GM-CSF and IL-4 and B cells that were activated by combination of BCR + TLR9 (CpG) stimuli, there was a significant reduction in the expression of DC markers such as DC-SIGN, CD83, HLA-DR, CD40, CD80, CD86, and CD58 (Figure 1A). Thus, in accordance with Morva et al,[5] our results revealed a regulatory role of BCR + CpG-stimulated B cells on differentiation of DCs. However, B cells that received signals only via BCR were not inhibitory (Figure 1A), indicating that in the absence of TLR stimuli, activated B cells do not block differentiation of DCs. In addition, we report a novel mechanism of modulation of DCs by regulatory B cells. We found that BCR + CpG-stimulated B cells induce a high percentage of apoptosis in differentiating DCs (Figure 1B). Thus, regulatory B cells can modulate DC-mediated immune responses by controlling the number of DCs. Of note, Fas-FasL–mediated apoptosis of target cells is proposed to be one of the mechanisms of immune regulation by regulatory B cells.[1]

Furthermore, compared with profound inhibition of LPS-mediated maturation of DCs by CD40 + CpG-activated B cells,[5] we found that B cells that received BCR signaling alone were only partially inhibitory on DCs (Figure 1C). Together, our results indicate that regulation of DC differentiation and maturation by B cells depends on the type of stimuli they receive. B cells receiving BCR stimulation alone are not inhibitory on DCs while under inflammatory conditions as in TLR9 stimulation; these TLR-9–stimulated B cells can act as inflammation-limiting factors in part via inhibition of DC activation. These functional differences of B-cell stimuli were also reflected in their ability to induce the expression of key molecules CD62L and CD80/CD86 on B cells (Figure 1D) that are proposed to be important in the regulation of DC and T-cell functions, respectively, by regulatory B cells.[5,6]

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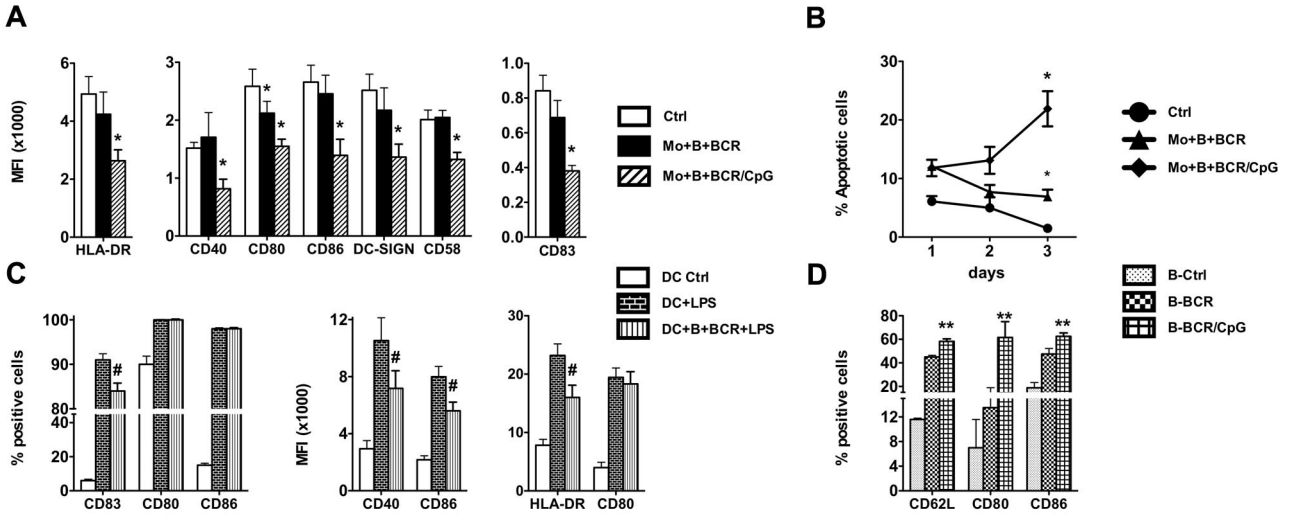

Figure 1. Regulation of human dendritic cells by B cells depends on the signals they receive. (A-B) Peripheral blood CD14$^+$ monocytes from healthy donors were cultured in the presence of GM-CSF (1000 IU/10^6 cells) and IL-4 (500 IU/10^6 cells) alone (Ctrl) or cytokines and BCR-activated (10 μg/mL of F(ab')$_2$ anti–human IgM) CD19$^+$ B cells (at ratio of 1:4; Mo + B + BCR) or cytokines and BCR + CpG-ODN-2006 (0.25μM; Mo + B + BCR/CpG) for 6 days. (A) Expression of DC surface markers (mean fluorescence intensities [MFI]) as analyzed by flow cytometry on CD20-negative cells (n = 5 experiments). (B) Percentage of annexin V$^+$ apoptotic DCs from days 1 to 3 after coculture with B cells (n = 3 experiments). (C) Five-day-old monocyte-derived immature DCs were cultured in the presence of GM-CSF and IL-4 alone (DC Ctrl) or stimulated with LPS (100 ng/mL; DC + LPS) or cocultured at 1:4 ratio with BCR-activated CD19$^+$ B cells in the presence of LPS (DC + B + BCR + LPS) for 48 hours to analyze the expression of surface markers (% positive cells and MFI) on CD20-negative cells. (n = 5 experiments). (D) CD19$^+$ B cells were either nonstimulated (B-Ctrl) or stimulated with BCR (B-BCR) or BCR + CpG (B-BCR/CpG) for 3 days and expression of surface markers (% positive cells) was analyzed (n = 3 experiments). The statistical significance as determined by 2-tailed paired Student *t* test is indicated (*$P < .05$ v/s Ctrl; #$P < .05$ v/s DC + LPS, and **$P < .05$ vs B-BCR).

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To the editor:

Rearrangement of *NOTCH1* or *BCL3* can independently trigger progression of CLL

Recent data indicate that *NOTCH1* mutations significantly increase the risk of CLL progression toward Richter syndrome (RS) and chemoresistance,[1,2] and that activation of *NOTCH1* at time of CLL diagnosis is an independent prognostic factor of poor survival.[1,3] We report here a case of CLL with a novel rearrangement of *NOTCH1* identified at the time of RS. The patient, a 58-year-old male, was diagnosed with CLL (unmutated *VH*) in RS in June 2003. Cytogenetic analysis and FISH on peripheral blood (PBL), bone marrow (BM), and lymph node (LN) cells showed 2 related clones: one with an isolated +12 and a second with +12 and dic(9;14)(q34;q32) (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). FISH analysis of dic(9;14)(q34;q32) indicated that this aberration resulted in juxtaposition of 3′*IGH* and 5′*NOTCH1,* as evidenced by the loss of sequences telomeric to the breakpoints (Figure 1A-D). These imbalances were confirmed by array CGH (data not shown). The targeting of *NOTCH1* by dic(9;14) was evidenced by qRT-PCR analysis, which showed a 10-fold up-regulation of *NOTCH1* mRNA (Figure 1E) and a low expression of the neighboring genes (*GPSM11, CARD9, DNL2*). Immunoblotting of a cell lysate from LN with a NOTCH1 antibody recognizing active, cleaved NOTCH1 (Val1744) identified a band corresponding to activated intracellular NOTCH1 (Figure 1F), suggesting an additional truncating mutation. Indeed, sequence analysis identified a 2 basepair deletion, ΔCT7544–7545/P2515fs, in the nucleotide sequence encoding the PEST domain (Figure 1G). This mutation resulting in expression of a truncated intracellular *NOTCH1* allele is recurrent in T-ALL[4] and CLL.[1-3,5]

The patient was treated and achieved complete remission (supplemental Table 1). In 2007, however, CLL relapsed and an examination of BM identified a clone with a sole +12 negative for ΔCT7544–7545/P2515fs. Two years later, CLL progressed and BM revealed an evolved clone with complex aberrations including +12 and t(14;19)(q32;q13)/*IGH-BCL3* but lacking dic(9;14)(q34;q32). Of note, BM was again positive for ΔCT7544–7545/P2515fs. Despite treatment, 2 clones harboring +12, one with t(14;19) and a second with new additional karyotypic changes, were seen in the analyzed BM (06/2011) positive for ΔCT7544–7545/P2515fs. *TP53* disruption frequently associated with RS[6] was not observed in the analyzed