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To the editor:

Complex interactions between B cells and dendritic cells

In the last decade, we have gained increasing insights into the antibody-independent functions of B cells. It has now become clear that apart from producing antibodies, B cells also contribute to immunity by antigen presentation, cytokine production, and a wealth of cellular interactions with other components of the immune system. In their 2012 paper, Morva et al[1] report that human B cells that were activated via CD40 and Toll-like receptor 9 (TLR9) are able to regulate dendritic cells (DCs) in a cell contact–dependent fashion. In response to this article, Maddur et al[2] furthermore show that the nature of the regulatory activity of B cells on DC function depends on the activation stimulus used to activate the B lymphocytes. These two papers provide important insights into the intricate mutual interactions between B lymphocytes and DCs.

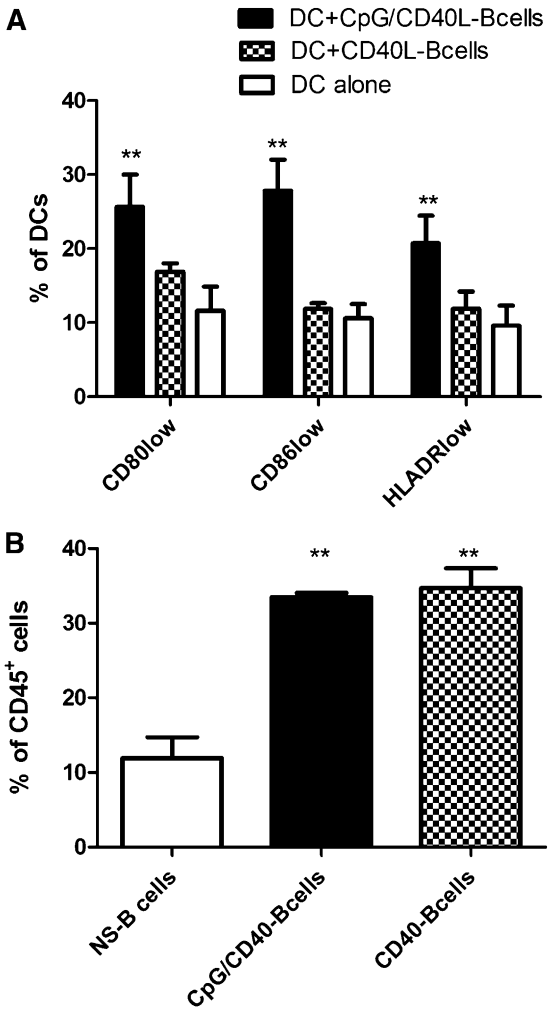

Figure 1. Interaction between B cells and DCs. (A) Human monocyte-derived DCs were cultured in triplicates alone or were cocultured with human $CD19^+$ B cells, which were activated via CD40L or CD40L+CpG, at a DC/B-cell ratio of 1:4 for 48 hours. Subsequently, the cell surface expression of CD80, CD86, and HLA-DR was determined using a Gallios flow cytometer (Beckman Coulter, Krefeld, Germany). The bar charts represent the mean percentage of positive DCs ± SD. (B) To determine the effect of the initial B-cell stimulation on their survival in the coculture, we determined the percentage of remaining B cells among the total $CD45^+$ cells after 48 hours in the coculture with DCs at a DC/B-cell ratio of 1:4. Shown are the mean percentages of $CD19^+$ B cells ± SD. $^{**}P < .01$.

The effect of CD40 on B-cell function has been controversial. While signaling through CD40 is generally believed to have pro-inflammatory effects on B lymphocytes, leading to enhanced antigen presentation and cytokine production, some groups have found that CD40-activation can induce regulatory features in B cells.[3-5] As exemplified by the results of Maddur et al,[2] the type, strength, and duration of the activation stimulus has a crucial impact on the function of human B lymphocytes.[2,6] Similarly, we found striking functional differences between B cells that were stimulated by CD40L compared with B cells that were stimulated by CD40L and cytosine guanine dinucleotide (CpG). The $CD80^{low}CD86^{low}HLA\text{-}DR^{low}$ DC population described by Morva et al[1] was increased in the cocultures with CD40L/CpG-stimulated B lymphocytes but not with CD40L-stimulated B lymphocytes (Figure 1A). Collectively, the experiments suggest that TLR9-mediated signals seem to induce a regulatory capacity in B cells and counter the pro-inflammatory effects of CD40-stimulation on B cells.

Importantly, even though we could confirm the findings by Morva et al[1] and Maddur et al,[2] there is one major caveat in the interpretation of these data. As far as one can judge from the descriptions of the methods, both research groups cocultured DCs with B cells and subsequently performed a mixed lymphocyte reaction without prior purification of the DCs. Since signaling through CD40 has anti-apoptotic effects, it could favor the survival of the CD40-activated B cells in the cocultures. Using an identical experimental setup, we found significant differences in the composition of the cell mixture after the end of the 48-hour coculture period. B cells activated by CD40L or CD40L and CpG seemed to survive longer, and they made up a higher percentage of the cells after coculture (Figure 1B). Thus, the DC/B-cell ratio in the cell mixture was 2:1 and 7:1 in the cocultures with CD40L/CpG-stimulated and unstimulated B cells, respectively. As a consequence, the mixed lymphocyte reactions with DCs exposed to CD40L/CpG-activated B cells contained a relatively higher number of B cells and a lower number of DCs. It should therefore be taken into consideration that the observed effects on T-cell activation

and proliferation could be, at least in part, attributable to the "contaminating" B cells.

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To the editor:

FcμR (Toso/Faim3) is not an inhibitor of Fas-mediated cell death in mouse T and B cells

Since the publication by Nguyen et al[1] showing that Toso/FcμR is an inhibitor of Fas-mediated apoptosis, there has been debate regarding whether FcμR regulates apoptosis.[2-4] An obvious problem is that these original studies used cell lines instead of normal primary T and B cells. To clarify the physiological role of FcμR in Fas-mediated apoptosis, we have now used FcμR-

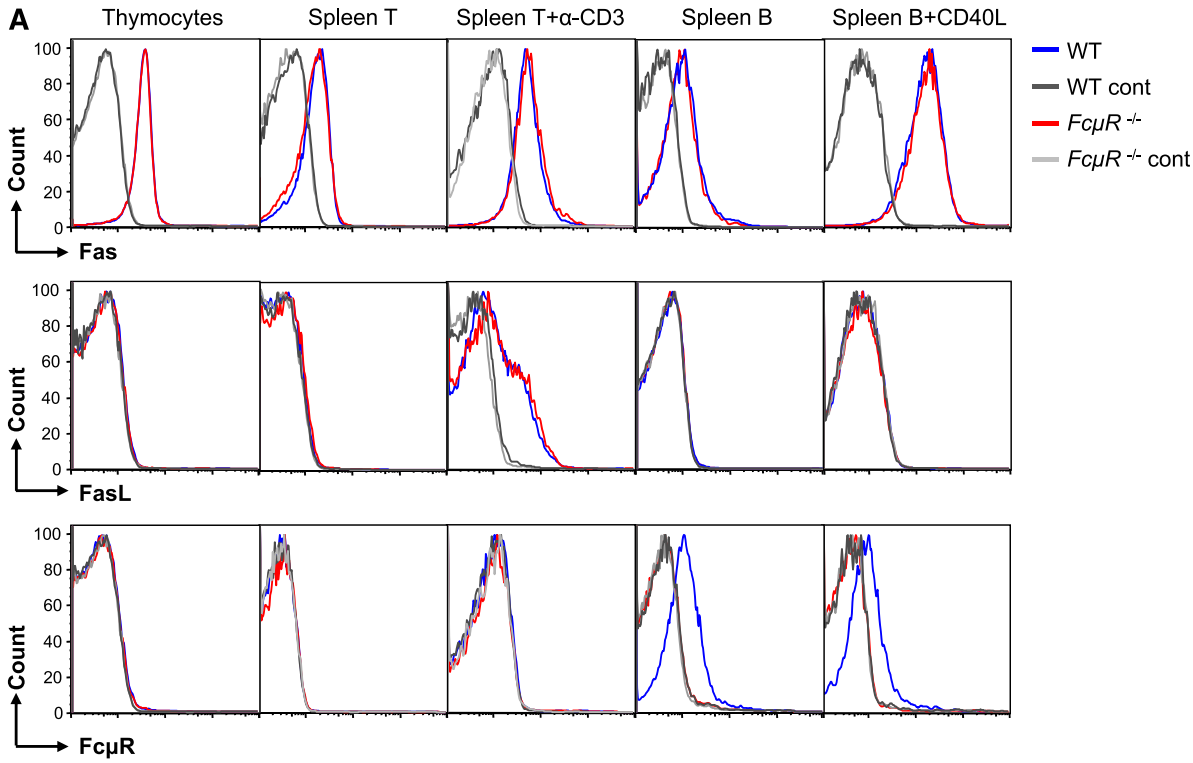

Figure 1. FcμR is not involved in Fas-mediated apoptosis in mouse T and B cells. (A) Expression of Fas, FasL, and FcμR by WT and *FcμR*$^{-/-}$ thymocytes and T and B cells before and after activation. To analyze Fas, FasL, and FcμR expression, cells were first incubated with a rat IgG_{2b} anti-mouse CD16/CD32 antibody (clone 2.4G2; BD Biosciences) to block FcγR. For Fas expression, cells were stained with PE/Cy7 anti-CD95 (clone Jo2; BD Biosciences) or an isotype control (PE/Cy7 hamster IgG2, clone Ha4/8; BD Biosciences). For FasL expression, cells were stained with PE anti-CD178 (clone MFL3; Biolegend) or an isotype control (PE hamster IgG, clone HTK888; Biolegend). For FcμR expression, cells were first stained with the rat anti-mouse FcμR mAb (clone 4B5) or an isotype control (rat IgG2a; BD Biosciences), and then stained with PE anti-rat IgG2a (clone RG7/1.30; BD Biosciences). Purified spleen T and B cells were stimulated with plate-bound anti-CD3 (clone 2C11, 10 μg/mL) and CD40L for 44 hours, respectively. CD40L, CD40 ligand; Ig, immunoglobulin; mAb, monoclonal antibody; PE, phycoerythrin.