To the editor:

The V_{H}1-69–expressing marginal zone B cells expanded in HCV-associated mixed cryoglobulinemia display proliferative anergy irrespective of CD21^{low} phenotype

We read with great interest the article by Charles et al describing anergy of V_{H}1-69^{+} CD21^{low} B cells from patients with hepatitis C virus (HCV)–associated mixed cryoglobulinemia (MC). Anergy of these cells was evident by decreased calcium mobilization and low production of IgM. These authors reported that the V_{H}1-69^{+} B cells with a CD21^{high} phenotype were not anergic, but data from our laboratory challenge this conclusion. It is worth noting that in Charles et al’s study, calcium mobilization in different subsets of B cells was very variable, and that the analyses could be biased by contaminating VH1-69^{+} CD21^{high} B cells because G6^{+} cells were not gated in those experiments. Furthermore, IgM production does not appear to be a reliable marker of anergy in CD21^{low} B cells, because the CD21^{low} B cells of patients with common variable immunodeficiency or with HIV infection have defects of proliferative responses although they produce significant amounts of IgM. Unfortunately, proliferation studies were not reported in Charles et al’s article.

We investigated the functional properties of V_{H}1-69^{+} B cells from several patients with HCV-associated MC. As with Charles’s study, we exploited the G6 antibody (provided by R. Jefferis, University of Birmingham, Birmingham, United Kingdom) to identify these cells. Phenotypic characteristics suggest that the V_{H}1-69^{+} CD21^{high} (IgM^{+} CD27^{+}) marginal zone (MZ)–like B cells are the precursors of their CD21^{low} (CD11c^{+}) counterparts, considering that some of them express low-level CD11c (Figure 1A,C). The proliferative responses of V_{H}1-69^{+} B cells were analyzed by the CFSE dilution flow cytometric method; the strategy of analysis is illustrated in Figure 1B and D. We found that both the CD21^{low} (Figure 1B) and the CD21^{high} (Figure 1D) V_{H}1-69^{+} B cells failed to proliferate in response to the stimulation of Toll-like receptor (TLR) 9 with CpG; similar findings were obtained with the TLR7 ligand R848 (not shown). Furthermore, V_{H}1-69^{+} B cells failed to differentiate to CD20^{low/neg} plasmablasts (Figure 1B,D). The data shown in Figure 1D are representative of the findings in 3 HCV^{+} MC patients with highly enriched (>75% of total B cells) CD21^{high} V_{H}1-69^{+} B cells.

Clonal B cells of HCV^{+} MC patients provide a unique model for investigating the pathophysiology of human MZ B cells. Murine MZ B cells expand massively on stimulation by blood-borne microbial antigens and similarly, the antigenic pressure of HCV appears to result in the robust clonal expansion of V_{H}1-69^{+} MZ B cells in MC. We provide evidence that these cells undergo premature proliferative anergy, which takes place when the V_{H}1-69^{+} B cells still retain a CD21^{high} MZ-like phenotype and have not yet shifted to a fully “exhausted” CD21^{low} phenotype. Interestingly, microarray gene expression profiling studies showed that murine MZ B cells stimulated in vivo by Streptococcus pneumoniae rapidly up-regulate Stra13, a negative regulator of B-cell proliferation. Thus, proliferative anergy may serve to constrain the excessive expansion of MZ B cells activated by microbial antigens, and to attenuate the risk of autoimmune disease and of malignant transformation.

References

Figure 1. Phenotype and proliferative responses of CD21<sub>low</sub> and CD21<sub>high</sub> VH1-69<sup>+</sup> B cells. (A-B) FACS plots from a representative (n = 8) MC patient with predominance of CD21<sub>low</sub> VH1-69<sup>+</sup> B cells. (A) Expression of surface markers by electronically gated VH1-69<sup>+</sup> B cells stained with the G6 antibody.<sup>4</sup> (B) Gating strategy for the analysis of proliferative responses of VH1-69<sup>+</sup> and VH1-69<sup>−</sup> B cells to CpG (2.5 μg/mL, 5-day culture). Cells were permeabilized before staining. The regions of analysis encompass CD20<sup>+</sup> B cells and CD20<sup>low/neg</sup> plasmablasts, which were also characterized as CD38<sup>+</sup> cytoplasmic-IgM<sup>+</sup> cells (not shown). Plasmablasts are present only in the VH1-69<sup>−</sup> gating region. Percent values in the histograms denote the number of cells entering division (precursor cohort)<sup>5</sup>. (C-D) FACS plots from a representative (n = 3) MC patient with highly enriched CD21<sub>high</sub> VH1-69<sup>+</sup> B cells. Analyses of surface marker expression (C) and of proliferative responses to CpG (D) were done as in panels A and B, respectively.
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