7. Pavletic SZ, Martin P, Lee SJ, et al. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: IV. Response criteria working group report. *Biol Blood Marrow Transplant.* 2006;12:252-266.
8. Schultz KR, Miklos DB, Fowler D, et al. Toward biomarkers for chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: III. Biomarker working group report. *Biol Blood Marrow Transplant.* 2006;12:126-137.
9. Keenan RA, De Riva A, Corleis B, et al. Censoring of autoreactive B cell development by the pre-B cell receptor. *Science.* 2008;321:696-699.
10. Sims GP, Ettinger R, Shirota Y, et al. Identification and characterization of circulating human transitional B cells. *Blood.* 2005;105:4390-4398.

To the editor:

Activated human B cells: stimulatory or tolerogenic antigen-presenting cells?

In recent years the antibody-independent functions of B cells have gained increasing attention. B cells can become potent antigen-presenting cells (APCs) after activation. Contrary to activated B cells, resting B cells can act as immunoregulatory cells. Two interesting recent papers in *Blood* now show that activated human B lymphocytes can also obtain regulatory functions. The fact that activated B cells can inhibit T-cell responses is somewhat surprising, as we and others have shown that B cells stimulated via CD40 induce $CD4^+$ and $CD8^+$ T-cell responses in vitro and in vivo.[1-3] How can these discrepancies be explained?

Tretter et al show that activation by *Staphylococcus aureus* Cowan I (SAC) or CpG-containing oligonucleotides induces B cells which down-regulate T-cell responses by inducing anergy and apoptosis of $CD4^+$ T cells in an IL-2–dependent fashion.[4] The suppressive effect was restricted to the activated large B-cell subpopulation expressing the high-affinity interleukin-2 (IL-2) receptor CD25. CD25 expression is not a marker for B cells with regulatory function, though, because many other stimuli, including CD40 activation and IL-4, also induce CD25 expression. Like the regulatory B-cell population described by Tretter et al, CD40-activated B cells express CD25 as well as high levels of costimulatory molecules (Figure 1). Despite these similar features, they do activate T cells even in the presence of 50 U/mL IL-2, a concentration at which Tretter et al observed an inhibition of T-cell proliferation.[1,3] Therefore, the functional consequences of CD25 expression on activated B cells appear to be dependent on the activation stimulus. Bacterial activation by stimuli such as CpG, SAC, and lipopolysaccharide[5] seem to confer regulatory functions whereas activation via CD40 induces stimulatory functions in B cells exposed to IL-2.[1,5] In addition, because SAC preferentially activates; immunoglobulin variable heavy chain gene 3 (VH3)–expressing B cells, it could be that suppressive function is characteristic for this subpopulation of B cells.[6]

It has previously been shown that murine and human resting B cells can expand regulatory T cells in vitro.[7,8] Tu et al demonstrate that alloantigen-specific human regulatory T cells can be generated in vitro using autologous CD40-activated (CD40-B) cells.[9] The CD40-B cells seem to be more heterogeneous than typical CD40-B cells, though (Figure 1). Based on the expression of major histocompatibility complex class II, 2 distinct populations represented by 2 separate peaks can be identified in Figure 1C of their article. Because Tu et al used cryopreserved CD40-B cells, the process of cryopreservation and thawing might have affected the function of the CD40-B cells.

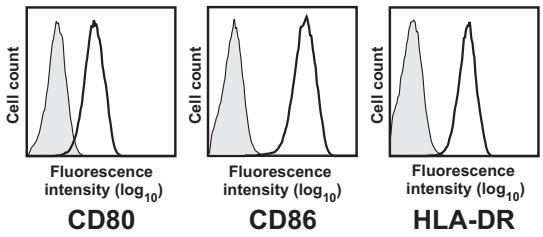

Figure 1. Phenotype of CD40-activated B cells. Surface expression of CD80, CD86, and human leukocyte antigen (HLA)–DR of $CD19^+$ CD40-activated B cells. Results are representative of more than 50 experiments.

In conclusion, these 2 studies exemplify the activation state–dependent plasticity of B-cell function. Several factors such as the type, duration, and strength of the activation stimulus, the B-cell subset, and microenvironmental setting seem to determine the final outcome. The role of different modes of B-cell activation in determining B-cell function therefore requires further clarification. One should thus be cautious before drawing general conclusions about the function of activated B cells from studies that use only a limited set of activation stimuli.

Alexander Shimabukuro-Vornhagen
Max Eder Junior Research Group and Stem Cell Transplantation Program
Department I of Internal Medicine
University Hospital of Cologne, Cologne, Germany

Eisei Kondo
Max Eder Junior Research Group, University Hospital of Cologne
Cologne, Germany

Tanja Liebig
Max Eder Junior Research Group, University Hospital of Cologne
Cologne, Germany

Michael von Bergwelt-Baildon
Max Eder Junior Research Group and Stem Cell Transplantation Program
Department I of Internal Medicine
University Hospital of Cologne, Cologne, Germany

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Alexander Shimabukuro-Vornhagen, University Hospital of Cologne, Kerpener Strasse 62, Cologne, Germany 50924; e-mail: alexander.shimabukuro-vornhagen@uk-koeln.de.

References

1. von Bergwelt-Baildon MS, Vonderheide RH, Maecker B, et al. Human primary and memory cytotoxic T lymphocyte responses are efficiently induced by means of CD40-activated B cells as antigen-presenting cells: potential for clinical application. *Blood.* 2002;99:3319-3325.
2. Ahmadi T, Flies A, Efebera Y, Sherr DH. CD40 Ligand-activated, antigen-specific B cells are comparable to mature dendritic cells in presenting protein antigens and major histocompatibility complex class I- and class II-binding peptides. *Immunology.* 2008;124:129-140.

3. Fujiwara H, Melenhorst JJ, El Ouriaghli F, et al. In vitro induction of myeloid leukemia-specific CD4 and CD8 T cells by CD40 ligand-activated B cells gene modified to express primary granule proteins. *Clin Cancer Res.* 2005;11:4495-4503.
4. Tretter T, Venigalla RK, Eckstein V, et al. Induction of CD4+ T cell anergy and apoptosis by activated human B cells. *Blood.* 2008;112:4555-4564.
5. Parekh VV, Prasad DV, Banerjee PP, Joshi BN, Kumar A, Mishra GC. B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J Immunol.* 2003;170:5897-5911.
6. Kristiansen SV, Pascual V, Lipsky PE. Staphylococcal protein A induces biased production of Ig by VH3-expressing B lymphocytes. *J Immunol.* 1994;153:2974-2982.
7. Chen X, Jensen PE. Cutting edge: primary B lymphocytes preferentially expand allogeneic FoxP3+ CD4 T cells. *J Immunol.* 2007;179:2046-2050.
8. Reichardt P, Dornbach B, Rong S, et al. Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse. *Blood.* 2007;110:1519-1529.
9. Tu W, Lau YL, Zheng J, et al. Efficient generation of human alloantigen-specific CD4+ regulatory T cells from naive precursors by CD40-activated B cells. *Blood.* 2008;112:2554-2562.

Response

Stimulatory or tolerogenic role of CD40-activated B cells depends on the strength of the activation to T cells

We thank Shimabukuro-Vornhagen et al for their comments on our recent study where we developed a simple and low-cost protocol using allogeneic CD40-activated B cells to induce and expand highly efficient human alloantigen-specific $CD4^{high}CD25^{+}Foxp3^{+}$ regulatory T cells (Tregs) from naive $CD4^{+}CD25^{-}$ T cells in large scale.[1] First, we have to clarify that we did not use autologous CD40-activated B cells but we used allogeneic cells to induce and expand Tregs instead. Second, the CD40-activated B cells used in our system are live cells but not the irradiated peptide-pulsed cells as others used.[2,3] Third, the ratio of B to T cells for induction and expansion of $CD4^{high}CD25^{+}$ Tregs is 1:10 in our system but not 1:4 as others used to induce antigen-specific T and cytotoxic T cells.[2,3] Indeed, several reports have demonstrated that weekly stimulation with antigen-presenting cells (APCs) such as dendritic cells (DCs) is an effective way to induce and expand Tregs in vitro and in vivo.[4-9] Similar to our report,[1] Jonuleit et al demonstrated that allogeneic immature DCs induced Tregs from naive CD4 T cells at a 1:10 ratio of DC:T cells.[6] Therefore, it is not surprising that CD40-activated B cells, as one of the APCs,[2,3] can induce and expand Tregs, in particularly under weekly stimulation.

Although the process of cryopreservation and thawing would undoubtedly affect the absolute number of live CD40-activated B cells (the recovery rate of live B cells preserved in liquid nitrogen for 6 months is approximately 80% in our system), it does not affect their function. As shown in Table 1, there are no significant differences in the induction, expansion, and suppressive ability of $CD4^{high}CD25^{+}$ Tregs induced by frozen versus fresh CD40-activated B cells.

Another question raised by von Bergwelt-Baildon et al was the phenotype of CD40-activated B cells. We think the difference of the major histocompatibility complex (MHC-II) expression in B cells resulted from the different antibodies used. In our study, we determined MHC–II expression in these B cells with fluorescein isothiocyanate (FITC)–anti-human MHC-II antibody, which reacts with all MHC class II molecular HLA-DR, DP, and most DQ antigens (BD Biosciences, San Jose, CA). In contrast, von Bergwelt-Baildon et al only examined the HLA-DR expression in these B cells. We further checked the MHC-II and HLA-DR expressions in CD40-activated B cells (Figure1). Consistent with our previous report,[1] more than 1 peak of MHC-II expressions were observed in these B cells, whereas only 1 peak of HLA-DR expression in the B cells was found (Figure 1).

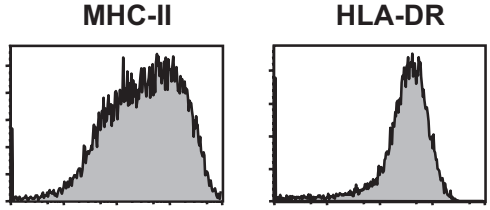

Figure 1. MHC-II and HLR-DR expressions in human CD40-activated B cells. Expression of MHC-II and HLR-DR on the CD40-activated B cells cultured for 8 days. Data shown here are representatives of B cells from 4 different healthy adult donors.

More evidence of CD40-activated B cells as the tolerogenic cells was also found in our recent study (Zheng J. and Tu W. manuscript submitted). In this study, we demonstrated that allogeneic CD40-activated B cells induced novel $CD8^{high}CD25^{+}$ cells from naive $CD8^{+}$ T cells. These $CD8^{high}CD25^{+}$ T cells were alloantigen-specific Tregs with relatively poor alloantigen-specific cytotoxicity.

Taken together, the process of cryopreservation and thawing does not affect the function of CD40-activated B cells to induce and expand alloantigen-specific Tregs, and the strength of the activation to T cells by CD40-activated B cells is critical for determining whether B cells act as the stimulatory or tolerogenic cells.

Wenwei Tu
Department of Paediatrics and Adolescent Medicine
LKS Faculty of Medicine
The University of Hong Kong
Hong Kong SAR, PR China

Table 1. Comparisons of the functions of frozen versus fresh CD40-activated B cells

	Frozen CD40-activated B cells (n = 10)	Fresh CD40-activated B cells (n = 6)	*P*
Percent of induced $CD4^{high}CD25^{+}$ Tregs after 6 days of coculture	65.30 ± 3.04	62.83 ± 3.25	.6066
Absolute number ($\times 10^6$) of induced $CD4^{high}CD25^{+}$ Tregs from 10^6 precursors after 6 days of coculture	0.84 ± 0.06	0.82 ± 0.09	.8475
Percent of inhibition at a Treg/responder cell ratio of 1:64	51.75 ± 1.25	50.00 ± 1.07	.3664