of accumulating data now supporting a link between oxidative enzymopathies and vascular disease.[5] In G6PD-deficient endothelial cells, there is increased oxidative stress and decreased NO levels.[6] This "enzymatic vasculopathy" hypothesis could be particularly relevant in SCA, a monogenic disease with highly variable clinical profile, probably due to polymorphism in other factors, including those influencing vascular biology.

**Françoise Bernaudin**
*Centre Hospitalier Intercommunal Créteil*
*Créteil, France*

**Suzanne Verlhac**
*Centre Hospitalier Intercommunal Créteil*
*Créteil, France*

**Martine Torres**
*University of Southern California*
*Los Angeles*

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

**Correspondence:** Françoise Bernaudin, Centre Hospitalier Intercommunal Créteil, 40 avenue de Verdun, Créteil, France 94010; e-mail: francoise.bernaudin@chicreteil.fr.

## References

1. Rees DC, Dick MC, Height SE, et al. A simple index using age, hemoglobin, and aspartate transaminase predicts increased intracerebral blood velocity as measured by transcranial Doppler scanning in children with sickle cell anemia. *Pediatrics.* 2008;121(6):e1628-e1632.
2. Bernaudin F, Verlhac S, Arnaud C, et al. Cerebral vasculopathy outcome in a sickle cell anemia (SCA) newborn cohort screened early with transcranial Doppler. *Blood.* 2008;112(11):Abstract 2497.
3. Neish AS, Blews DE, Simms CA, Merritt RK, Spinks AJ. Screening for stroke in sickle cell anemia: comparison of transcranial Doppler imaging and nonimaging US techniques. *Radiology.* 2002;222(3):709-714.
4. Bernaudin F, Verlhac S, Kamdem A, et al. Risk factors for silent infarcts in a pediatric sickle cell anemia cohort. *Blood.* 2008;112(11):Abstract 2487.
5. Leopold JA, Loscalzo J. Oxidative enzymopathies and vascular disease. *Arterioscler Thromb Vasc Biol.* 2005 Jul;25(7):1332-1340.
6. Leopold JA, Dam A, Maron BA, et al. Aldosterone impairs vascular reactivity by decreasing glucose-6-phosphate dehydrogenase activity. *Nat Med.* 2007;13(2):189-197.

# To the editor:

## Proportions of immature CD19$^+$CD21$^-$ B lymphocytes predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease

B lymphocytes are increasingly assigned an important role in the pathogenesis of auto-and alloimmune diseases including chronic graft-versus-host disease (cGVHD)[1-4] where abnormalities of B-cell homeostasis in patients with active cGVHD have been observed.[1,4] Extracorporeal photopheresis (ECP) achieves high response rates in steroid-refractory cGVHD.[5] At present, no reliable biomarkers are available for prediction of response to ECP and monitoring of patient outcome. We thus investigated the distribution of immature CD19$^+$CD21$^-$ B cells and memory CD19$^+$CD27$^+$ B cells in peripheral blood (PB)[1] before ECP (n = 19), 6 (n = 28), 12 (n = 34), and 21 months (n = 33) after start of ECP in 49 patients (28 male, 21 female) with moderate (n = 25) or severe (n = 24) cGVHD[6] and correlated results with clinical response.[7] Informed consent was obtained in accordance with the Declaration of Helsinki. Eighteen patients were included in a prior report.[1]

Complete (CR) and partial response (PR) to ECP was observed in 25 of 34 patients (74%) after 12 months. ECP nonresponders at 6 months had a significantly higher ($P = .02$) percentage of immature CD19$^+$CD21$^-$ B lymphocytes (mean 22%, range 4%-52%) in PB before start of ECP compared with CR (mean 8%; range 1.3%-29%) and PR (mean 16%; range 1.6%-55%) patients (Figure 1A). Percentages of memory CD19$^+$CD27$^+$ B cells before first ECP were not significantly different ($P = .12$) between the groups. The CD21$^-$/CD27$^+$ ratio was significantly higher ($P = .03$) in ECP nonresponders (mean 17.4; range 0.5-50) compared with CR patients (mean 1.6; range 0.3-5).

Percentages of immature CD19$^+$CD21$^-$ B lymphocytes were significantly ($P < .001$) lower in CR patients compared with ECP nonresponders 6 (mean 5% vs 25%; range 1%-15% vs 14%-43%), 12 (mean 6% vs 24%, range 1.2%-16% vs 1.5%-58%), and 21 (mean 6% vs 26%; range 1%-21% vs 2%-60%) months after start of ECP (Figure 1B). In addition, in ECP responders compared with ECP nonresponders the CD21$^-$/CD27$^+$ ratio was significantly lower 6 months (mean 2.2 vs 9.6; range 0.1-6.5 vs 1.2-20; $P = .001$), 12 (mean 2.7 vs 6; range 0.2-15 vs 0.6-16; $P = .006$), and 21 months (mean 2 vs 17; range 0.2-9 vs 0.6-76; $P = .001$) after start of ECP.

To our knowledge, relative amounts of immature CD19$^+$CD21$^-$ B lymphocytes represent the first reported cellular biomarker able to predict response to ECP. Whether our findings are ECP-specific has to remain speculative because no other treatment cohorts were analyzed and the mechanisms of action of ECP are still subject to further research.[5] CD19$^+$CD21$^-$ B lymphocytes could also serve as a novel biomarker for measuring disease activity of cGVHD quantitatively and objectively, which would be highly desirable.[8] Of note, CD21$^-$ B lymphocytes are increased in proportion in autoimmune diseases such as systemic lupus erythematosus and active cGVHD.[1,10] Increased proportions of CD21$^-$ B lymphocytes could be part of the autoimmune pathogenesis compatible with inefficient censoring of autoreactive B cells in cGVHD.[9] Disrupted B-cell homeostasis and relative rather than absolute preponderance of an activated B-cell pool has recently been reported in cGVHD.[4] Our findings warrant larger prospective studies analyzing the predictive value of B-cell subsets for successful ECP treatment of cGVHD.

**Zoya Kuzmina**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

**Hildegard T. Greinix**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

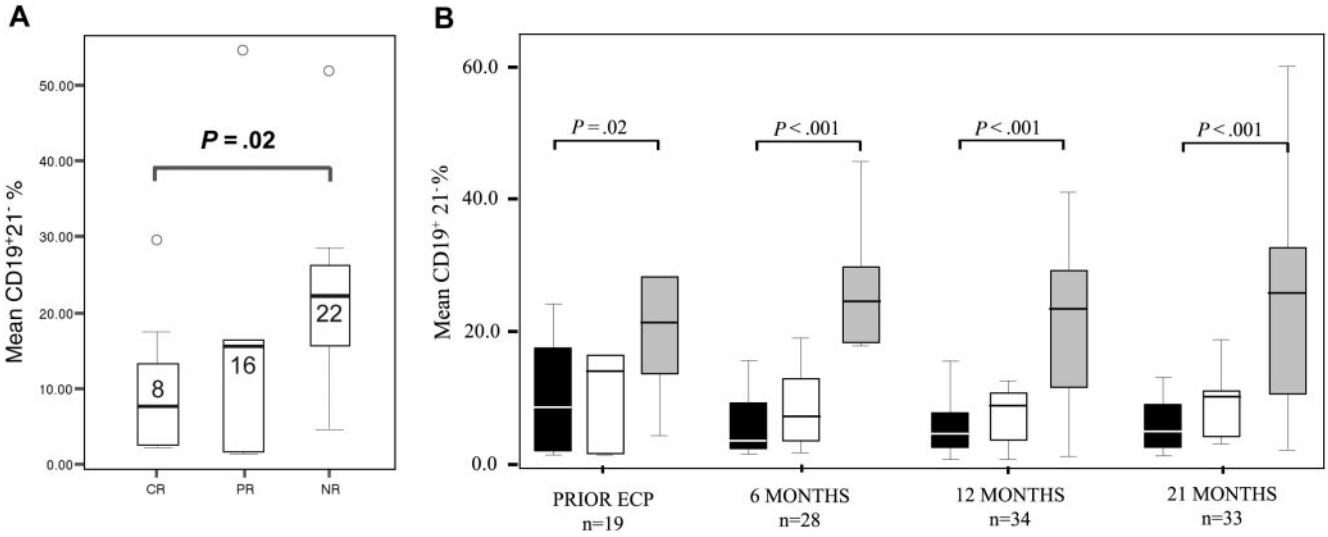


**Figure 1. Comparison of relative amounts of immature CD19$^+$CD21$^-$ B lymphocytes between ECP responders and ECP nonresponders.** (A) Low percentages of immature CD19$^+$CD21$^-$ B cells before therapy correlate significantly with complete resolution of chronic graft-versus-host disease to extracorporeal photopheresis (ECP). Patients were divided into 3 groups, complete responders (CR, n = 6), partial responders (PR, n = 6), and nonresponders (NR, n = 7) 6 months after start of ECP. Immature CD19$^+$CD21$^-$ B cells assessed before start of ECP are shown in box plot format. Numbers indicate mean percentages (bold horizontal lines). Comparison of groups was performed using unpaired Student *t* test. (B) Complete ECP responders have significantly lower percentages of immature CD19$^+$CD21$^-$ B cells 6, 12, and 21 months after start of ECP therapy compared with ECP nonresponders. Patients with complete response (CR; black bars), partial response (PR; white bars) and no response (NR; gray bars) to ECP are shown. Results are shown in box plot format. The bold horizontal line indicates the mean percentages. Comparison of groups was performed using unpaired Student *t* test.

**Robert Knobler**
*Department of Dermatology*
*Division of Special Dermatology*
*University of Vienna*
*Vienna, Austria*

**Nina Worel**
*Department of Blood Group Serology and Transfusion Medicine*
*University of Vienna*
*Vienna, Austria*

**Michal Kouba**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

**Roman Weigl**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

**Ulrike Körmöczi**
*Institute of Immunology*
*University of Vienna*
*Vienna, Austria*

**Arno Rottal**
*Institute of Immunology*
*University of Vienna*
*Vienna, Austria*

**David Pohlreich**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

**Christoph Zielinski**
*Department of Internal Medicine I, Bone Marrow Transplantation*
*University of Vienna*
*Vienna, Austria*

**Winfried F. Pickl**
*Institute of Immunology*
*University of Vienna*
*Vienna, Austria*

**Contribution:** H.T.G. and W.F.P. designed the research study, analyzed and interpreted the data, and coauthored the manuscript; Z.K., D.P., and M.K. performed the clinical research, collected and analyzed data; R.W., U.K., and A.R. performed the flow cytometric analyses; R.K. and N.W. performed the ECP treatments; and C.Z. interpreted the data and contributed to the manuscript.

**Conflict-of-interest disclosure:** H.T.G. and R.K. have served as consultants to Therakos, Inc and participated as lecturers in the speakers bureau for Therakos. The remaining authors declare no competing financial interests.

**Acknowledgments:** This work was supported by European Commission Grant MCRTN-CT-2004-512253 TRANSNET, by 037703 STEMDIAGNOSTICS and a research grant of the Austrian Society for Transplantation Austrotransplant.

**Correspondence:** Hildegard T. Greinix, MD, Medizinische Universitaet Wien, Klinik fuer Innere Medizin I, Knochenmarktransplantation, Waehringer Guertel 18-20, A-1090 Vienna, Austria; e-mail: hildegard.greinix@meduniwien.ac.at; or Winfried F. Pickl, MD, Institut fuer Immunologie, Medizinische Universitaet Wien, Borschkegasse 8, A-1090 Vienna, Austria; e-mail: winfried.pickl@meduniwien.ac.at.

## References

1. Greinix HT, Pohlreich D, Kouba M, et al. Elevated numbers of immature/transitional CD21$^-$ B lymphocytes and deficiency of memory CD27$^+$ B cells identify patients with active chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2008;14:208-219.
2. Miklos DB, Kim HT, Miller KH, et al. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. *Blood.* 2005;105:2973-2978.
3. Fujii H, Cuvelier G, She K, et al. Biomarkers in newly diagnosed pediatric extensive chronic graft-versus-host disease: a report from the Children's Oncology Group. *Blood.* 2008;111:3276-3285.
4. Sarantopoulos S, Stevenson KE, Kim HT, et al. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. *Blood.* 2009;113:3865-3874.
5. Greinix HT, Socie G, Bacigalupo A, et al. Assessing the potential role of photopheresis in hematopoietic stem cell transplant. *Bone Marrow Transplant.* 2006;38:265-273.
6. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease. I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2005;11:945-956.

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.

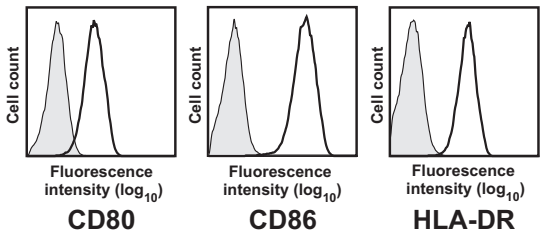


**Figure 1. Phenotype of CD40-activated B cells.** Surface expression of CD80, CD86, and human leukocyte antigen (HLA)–DR of $CD19^+$ CD40-activated B cells. Results are representative of more than 50 experiments.

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.