Response

Extended analysis of microarray data does not contradict preplasmablast phenotype of human CD20⁺CD27⁺CD43⁺ cells

Li et al¹ used additional approaches to analyze the microarray data presented in our article describing a preplasmablast phenotype for the proposed human B1 cells.² Their analysis positions “B1” cells closer to memory B cells (CD19⁺CD20⁺CD27⁺CD43⁺) than to plasmablasts (CD19⁻CD20⁻CD27⁻CD43⁻), which, according to them, contradicts our conclusions.³ On the contrary, we feel that the analysis by Li et al¹ supports and extends our findings. Both our analysis and theirs are compatible with the notion that the putative B1 cells have a phenotype intermediate between memory B cells and plasmablasts. In addition, the observation that surface expression of CD43 and CD27 is intermediate between that of memory B cells³,⁴ and plasmablasts supports our conclusions. Importantly, an independent analysis of our data demonstrates that transcriptional features of mouse B1 cells are clearly distinct from those of the proposed human B1 cells, whereas mouse and human germinal center B cells share features.⁵

Other concerns were raised by Li et al.¹ We agree that B1 cells can switch to (mostly) immunoglobulin A (IgA). However, in mice, the majority of B-1 is known to secrete IgM.⁶ In our study, we found that IgA was the predominant isotype produced and even found similar frequencies of cells producing IgM and IgG. Rather, our data demonstrate a parallel between plasmablasts and the proposed B-1 cells.

Li et al¹ suggested that tetanus toxin–specific cells in our sorted “B1” population result from a small inadvertent contamination. However, our enzyme-linked immunospot data demonstrate that if production of anti-tetanus toxin antibodies by B1 cells was due to containing plasmablasts, the contamination of B1 cells by plasmablasts would have accounted for up to 50%. Moreover, such contamination would have aligned “B1” cells much closer to plasmablasts in the gene expression analysis, which is inconsistent with Li et al’s interpretation of the gene expression analysis.¹ The absence of antibody production in memory B cells (both spontaneous and vaccination-induced) further underscores the purity of our sorted samples.²,⁷

Li et al¹ argued that differentiation of cells to plasmablasts and plasma cells does not discriminate between B-cell lineages, as we actually discussed previously.⁸ However, this assertion contradicts the Rothstein group’s previous publications in which the inability of human “B1” cells to differentiate to plasmablasts was twice claimed to distinguish B-1 cells from plasmablasts.⁹,¹⁰

Last, Li et al¹ raised technical concerns about the absence of CD69 and CD70 expression in our stimulated B-cell cultures, pointing toward the lack of positive controls and that the 5-day timespan might have led to decreased expression. Although not mentioned in our publication, we performed parallel control experiments in which peripheral blood mononuclear cells were stimulated with R-848 and interleukin-2 and consistently observed expression of CD69 and CD70 in CD19⁻ lymphocytes. Before choosing day 5, we performed kinetic studies and observed a gradual increase of CD20⁺CD27⁻CD43⁻ cells from days 3 to 5 without expression of CD69 or CD70.

In conclusion, the microarray analysis performed by Li et al¹ and by Mabbott and Gray⁵ support the concept that the putative B1 cells have a phenotype intermediate between memory B cells and plasmablasts.

Kris Covens
PharmAbs, The Katholieke Universiteit Leuven Antibody Centre, and Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, Katholieke Universiteit Leuven, Leuven, Belgium

Bert Verbinnen
PharmAbs, The Katholieke Universiteit Leuven Antibody Centre, and Experimental Laboratory Immunology, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium

Marc Jacquemin
PharmAbs, The Katholieke Universiteit Leuven Antibody Centre, and Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, Katholieke Universiteit Leuven, Leuven, Belgium

Xavier Bossuyt
Experimental Laboratory Immunology, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium

Acknowledgments: X.B. is a senior clinical investigator of the Fund for Scientific Research–Flanders.

Contribution: K.C. drafted the manuscript; and B.V., M.J., and X.B. critically revised the manuscript.

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

© 2013 by the American Society of Hematology
Correspondence: Xavier Bossuyt, Experimental Laboratory Immunology, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium; e-mail: xavier.bossuyt@uzleuven.be.

References


To the editor:

Complex subclone structure that responds differentially to therapy in a patient with essential thrombocythemia and chronic myeloid leukemia

BCR-ABL translocation and JAK2-V617F mutation can sometimes be found concomitantly in the same patient (reviewed in Hummel et al1). To date, 4 studies have examined the chronology and architecture of BCR-ABL and JAK2-V617F clones at the molecular level. In 3 studies, the 2 mutations were sequentially acquired by the same stem cell, with JAK2-V617F preceding the acquisition of BCR-ABL.2-4 Another report concluded that BCR-ABL and JAK2-V617F represented 2 distinct clones.5

Here, we studied a 56-year-old female patient diagnosed in May 2005 with JAK2-V617F-positive essential thrombocythemia (ET), normal cytogenetics, and absence of BCR-ABL (Figure 1A). With anagrelide (2 mg/day), platelet levels normalized. However, in October 2010, thrombocytosis, leukocytosis (white blood cell count = 30 x 10^9/L) and the presence of myelocytes and metamyelocytes in the peripheral blood was noted. Bone marrow showed typical features of chronic myeloid leukemia (CML) and cytogenetic analysis revealed the Philadelphia chromosome translocation t(9;22) (q34;q11). Molecular analysis confirmed expression of a BCR-ABL fusion (b3a2). Treatment with dasatinib induced remission of CML, but thrombocytosis persisted. Therefore, low-dose hydroxyurea (500 mg every second day) was added. A complete molecular remission with a 10^-4 to 10^-5 reduction of BCR-ABL transcripts (CMR^4,5) was reached within 12 months.

To allow detecting the translocation in DNA samples, we sequenced the BCR-ABL breakpoint and designed polymerase chain reaction (PCR) primers across the junction. The identity of the amplified fragment was confirmed by sequencing (Figure 1B). DNA derived from single colonies grown from the patient’s peripheral blood in methylcellulose was analyzed for the presence of JAK2-V617F by allele-specific PCR,6,7 and for BCR-ABL by PCR primers bridging the breakpoint. In October 2010, before dasatinib/hydroxyurea treatment, the majority of the colonies were either wild-type or carried both JAK2-V617F and BCR-ABL. However, 2 colonies harbored only BCR-ABL and 1 colony showed only JAK2-V617F (Figure 1C, left panel). Because the BCR-ABL breakpoint in this patient is unique, this pattern suggested that BCR-ABL was acquired before JAK2-V617F and implied that JAK2-V617F occurred twice. Genotyping of individual colonies with a microsatellite marker for chromosome 9p allowed excluding uniparental disomy as an alternative explanation for the single-mutant BCR-ABL colonies (Figure 1D). This biclonal pattern was confirmed in colonies derived from a second blood sample taken under dasatinib/hydroxyurea treatment 26 months later. Interestingly, the double-positive colonies completely disappeared, while several colonies positive for either JAK2-V617F or BCL-ABL persisted, suggesting that the double mutant may be more sensitive to treatment (Figure 1C, right panel). Despite the presence of BCR-ABL single-mutant colonies, BCR-ABL was undetectable in messenger RNA from bone marrow and peripheral blood and absent in DNA from granulocytes. We can only speculate that the BCR-ABL single-mutant progenitors can divide in vitro and form colonies but are unable to efficiently expand in vivo in the presence of dasatinib.

This patient displayed a novel pattern with 2 separate clones: 1 that first acquired BCR-ABL followed by a JAK2-V617F mutation within the same clone, and a second clone positive for JAK2-V617F only. Our data show that subclones can respond differentially to therapy. Knowledge of the clonal architecture in patients with MPN/CML could be useful to monitor remission and select therapy.

Jean Grisouard
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Mario Ojeda-Urube
Service d’Hématologie Clinique et Unité de Thérapie Cellulaire, Hôpital E. Müller, Mulhouse, France

Renate Looser
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Hui Hao-Shen
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Pontus Lundberg
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

© 2013 by The American Society of Hematology
Response: Extended analysis of microarray data does not contradict preplasmablast phenotype of human CD20⁺CD27⁺CD43⁺ cells

Kris Covens, Bert Verbinnen, Marc Jacquemin and Xavier Bossuyt