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References

1. Negro R, Gobessi S, Longo PG, et al. Overexpression of the autoimmunity-associated phosphatase PTPN22 promotes survival of antigen-stimulated chronic lymphocytic leukemia cells by selectively activating the AKT pathway. *Blood.* 2012;119(26):6278-6287.

2. Bottini N, Musumeci L, Alonso A, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet.* 2004;36(4):337-338.

3. Chew GYJ, Sinha U, Gatenby PA, et al. Autoimmunity in primary antibody deficiency is associated with protein tyrosine phosphatase nonreceptor type 22 (PTPN22) [published online ahead of print July 31, 2012]. *J Allergy Clin Immunol.* doi:10.1016/j.jaci.2012.06.023.

4. Stanford SM, Mustelin TM, Bottini N. Lymphoid tyrosine phosphatase and autoimmunity: human genetics rediscovers phosphatases. *Semin Immunopath.* 2010;32(2):127-136.

5. Arechiga AF, Habib T, He Y, et al. Cutting edge: the PTPN22 alleleic variant associated with autoimmunity impairs B cell signaling. *J Immunol.* 2009;182:3343-3347.

6. Fiorillo E, Orru V, Stanford SM, et al. Autoimmune-associated PTPN22 R620W variation reduces phosphorylation of lymphoid phosphatase on an inhibitory tyrosine residue. *J Biol Chem.* 2010;285(34):26506-26518.

7. Zhang J, Zahir N, Jiang Q, et al. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degredation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nat Genet.* 2011;43(9):902-907.

8. Menard L, Saadoun D, Isnardi I, et al. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J Clin Invest.* 2011;121(9):3635-3644.

9. McCarty CA, Wilke RA, Giampietro PF, Wesbrook SD, Caldwell MD. Marshfield Clinic Personalized Medicine Research Project (PMRP): design, methods and recruitment for a large population-based biobank. *Per Med.* 2005;2:49-79.

10. Slager SL, Rabe KG, Achenbach SJ, et al. Genome-wide association study identifies a novel susceptibility locus at 6p21.3 among familial CLL. *Blood.* 2011;117(6):1911-1916.

Response

Increased expression or activity of PTPN22 may compromise negative selection of autoreactive CLL cells

The finding of Hebbring et al that the PTPN22 R620W autoimmunity risk allele is present at a higher frequency in chronic lymphocytic leukemia (CLL) patients originating from northern/western Europe than in matched controls[1] is very interesting and provides further support to the recent findings of Negro et al suggesting an important role for PTPN22 in the pathogenesis of CLL.[2]

The study by Negro et al investigated the expression of PTPN22 in a large series of CLL patients from Italy, where the PTPN22 R620W autoimmunity risk allele is relatively infrequent, and showed that PTPN22 is markedly overexpressed in the majority of investigated CLL samples.[2] Additional experiments showed that overexpression of PTPN22 attenuates BCR signals that can potentially induce leukemic cell apoptosis while simultaneously increasing the activity of the AKT kinase, a key survival signaling molecule in CLL cells. This selective uncoupling of AKT from downstream proapoptotic BCR pathways was shown to protect CLL cells from activation-induced cell death. Overall, these data suggested that the purpose of PTPN22 overexpression in CLL could be to protect the malignant B cells, which frequently express autoreactive BCRs, from immunologic tolerance mechanisms that eliminate autoreactive lymphocytes.

A similar mechanism is believed to be responsible for the increased risk of autoimmune disease in subjects carrying the PTPN22 R620W allele.[3] The risk allele has been shown to inhibit antigen-receptor signaling more strongly than the wild-type protein,[4] suggesting that it could compromise autoantigen-induced negative selection of autoreactive lymphocytes. Therefore, the finding by Hebbring et al that the PTPN22 R620W autoimmunity risk allele is present at a higher frequency in northern/western European CLL patients than in matched controls suggests that both

increased expression and increased activity of PTPN22 could play a role in the pathogenesis of CLL. It will be interesting to investigate in future studies whether these 2 mechanisms operate in the same or different patients. Given the possibility of targeting PTPN22 or PTPN22-regulated pathways, this issue could be of considerable importance.

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References

1. Hebbring SJ, Slager SL, Epperla N, et al. Genetic evidence of PTPN22 effects on chronic lymphocytic leukemia. *Blood.* 2013;121(1):235-236.
2. Negro R, Gobessi S, Longo PG, et al. Overexpression of the autoimmunity-associated phosphatase PTPN22 promotes survival of antigen-stimulated CLL cells by selectively activating AKT. *Blood.* 2012;119(26):6278-6287.
3. Rhee I, Veillette A. Protein tyrosine phosphatases in lymphocyte activation and autoimmunity. *Nat Immunol.* 2012;13(5):439-447.
4. Vang T, Liu WH, Delacroix L, et al. LYP inhibits T-cell activation when dissociated from CSK. *Nat Chem Biol.* 2012;8(5):437-446.

To the editor:

CLL B-cell receptors can recognize themselves: alternative epitopes and structural clues for autostimulatory mechanisms in CLL

Chronic lymphocytic leukemia (CLL) may be driven by antigen recognition through the B-cell receptor (BCR).[1-7] A recent paper in *Nature*[8] suggested a new mechanism for such antigenic drive by functionally characterizing an epitope previously identified by our group as being recognized by virtually all CLL BCRs within a large cohort of CLL patients.[9] This epitope has now been shown to be part of the CLL BCR itself, thus conferring apparently autonomous signaling of BCRs within the cell membrane that may promote growth and survival of the leukemic cell. This provides an entirely new view on CLL pathobiology and its mechanism of disease that appears to apply to the majority of CLL patients.[8]

We describe herein an additional epitope involved in self-recognition of CLL BCRs. We screened random phage display peptide libraries for peptides specifically binding to the CLL BCR (expressed as a Fab fragment) of a randomly chosen patient (Fab007). We identified peptides mimicking the epitope recognized by Fab007 and validated their strong binding to this BCR (data not shown). The consensus amino acid sequence of this epitope (YYC) is homologous to Ig heavy and light chains of different gene families used by Igs of the IgG, IgM, IgA, IgE, and IgD isotype, including the respective CLL Fab007 itself (data not shown). Binding of the CLL Fab007 to the Fab part of Igs was verified in a protein array (Figure 1A) and in ELISAs (Figure 1B). Further binding assays indicated that CLL Fab007 recognizes itself and other CLL BCRs even in patients (eg, CLL Fab005) in whom the recently described epitope VRQ[8] is not present (Figure 1C). A large binding study on primary CLL samples indicated that approximately 50% of CLL cases interact with the YYC motif, most of them showing also considerable reactivity with the VRQ motif,[9] suggesting redundant recognition of alternative epitopes. Interestingly, our new epitope, which is located in framework region 3 of the variable part of Igs, is sterically adjacent and builds a structural continuum with the VRQ epitope located in framework region 2 that was recently suggested as driving autonomous BCR signaling in most CLL patients[8] (Figure 1D bottom right panel). This remarkable colocalization of different epitopes mediating self-recognition of CLL BCRs may explain how autonomous signaling can occur even at a

single-cell level[8] (Figure 1D blue circles) and not just in the context of 1 CLL cell recognizing receptors in adjacent cells (Figure 1D red circles). This is because the region containing both described epitopes is (1) structurally exposed on the surface of the protein and (2) approximately the same distance from the cell surface as the CDR3 region mediating this autorecognition (Figure 1D). This allows interaction of adjacent BCRs within the membrane of the same CLL cell. Our data support the recently established theory of autostimulatory mechanisms in CLL pathobiology and point out redundant recognition profiles and structural explanations that provide the basis for self-recognition of the BCR and self-antigen binding despite the low-affinity typically attributed to CLL BCRs.

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