IMMUNOLOGY

A switch-variant model integrates the functions of an autoimmune variant of the phosphatase PTPN22

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The R620W polymorphism in protein tyrosine phosphatase nonreceptor type 22 (PTPN22) predisposes carriers to several autoimmune diseases. Two papers in *Science Immunology* and *Science Signaling* on this human disease–associated variant lead us to propose a new "switch-of-function" model.

Protein tyrosine phosphatase nonreceptor type 22 (PTPN22; also known as LYP in humans and PEP in mice) is a protein tyrosine phosphatase that is expressed in hematopoietic cells. PTPN22 acts as a negative regulator of signaling in T and B cells by dephosphorylating immunoreceptor-proximal proteins, Src and Syk families of kinases, and VAV (*1*). PTPN22 consists of an N-terminal catalytic domain, an interdomain, and a C-terminal binding domain that contains four polyproline-binding motifs (named P1 to P4). The 1858C>T single-nucleotide polymorphism in *PTPN22* results in an R620W missense mutation that maps to the P1 motif. This mutation disrupts the interaction of PTPN22 with its only ligand, c-Src kinase (CSK) (*1*). The R620W variant is a known risk factor for autoimmune diseases, although the mechanism by which this variant confers susceptibility remains unclear (*1*). Previous studies have noted the effects of PTPN22 R620W in B cells, T cells, and innate immune cells.

Carriers of the R620W variant are predisposed to autoimmune disorders characterized by the production of autoantibodies (*1*). Consistent with this, the R620W variant is associated with the development of greater frequencies of polyreactive and autoreactive B cell receptors (BCRs) (*2*). Because PTPN22 R620W is a stronger inhibitor of BCR signaling than is the wild-type (WT) phosphatase, expression of the former may prevent the deletion of self-reactive populations during B cell development (*3*, *4*). Mature naïve B cells from healthy R620W carriers also display altered expression of various signaling proteins, including increased amounts of components associated with signaling downstream of the BCR, CD40, and Toll-like receptors. In addition, these cells are also more responsive to CD40 stimulation.

To determine whether B cell–intrinsic expression of the R620W variant was sufficient to break tolerance, Schickel *et al.* transplanted human hematopoietic stem cells carrying WT *PTPN22* or the disease-associated *1858T* allele into NOD/SCID (nonobese diabetic/severe combined immunodeficient)–common γ chain knockout mice. They found that the presence of *PTPN22 1858T* in these humanized mice substantially increased the frequencies of developing polyreactive and autoreactive B cells (*5*). These observations recapitulate findings made with *PTPN22* genotyped human subjects and indicate that the *PTPN22 1858T* allele is associated with defective central B cell tolerance. Furthermore, 20-fold overexpression of the R620W variant, but not WT PTPN22, in B cells directly correlated with increased autoreactivity. Knockdown of PTPN22 R620W or treatment with the PTPN22 inhibitor LTV-1 restored central B cell tolerance in these mice, suggesting that the inhibition of PTPN22 R620W by LTV-1 could reset central B cell tolerance. Together, these data demonstrate the rationale for targeting the R620W variant and emphasize the clinical potential of a previously described PTPN22 inhibitor (*6*).

The role of PTPN22 is best described in T cells where it inhibits proximal T cell receptor (TCR) signaling (*1*). Originally described as a gain-of-function polymorphism, the R620W mutant exhibits approximately 50% more phosphatase activity than that of WT PTPN22 and is a more potent inhibitor of TCR signaling in humans (*3*, *7*, *8*). This appears to contrast with findings in transgenic mice, where expression of the corresponding mutation (Ptpn22-R619W) increased TCR-dependent signaling and the accumulation of activated effector T cells (*9*). However, caution should be exercised when extrapolating data from one species to another. Although the catalytic domains of human PTPN22 and mouse Ptpn22 share 89% identity, outside this region, these proteins share only 61% identity. Additionally, alternative splice isoforms of PTPN22 that lack the P2 to P4 motifs have been described. Analyses of human $CD4^+$ T cells from R620W donors show reduced CD3ζ phosphorylation and Ca^{2+} flux after TCR stimulation, whereas CD28-mediated AKT activation is enhanced (*3*, *8*). Similar alterations in TCR- and CD28-mediated signals are seen in activated regulatory T cells (defined as $CD4^+CD45RA^-FoxP3^{high}$). These cells from R620W donors do not control interferon-γ production from conventional $CD4^+$ T cells, which leads to exaggerated T helper 1 cell responses (*8*). At the mechanistic level, reduced TCR signaling in T cells from R620W carriers could result from the enhanced phosphatase activity of the R620W variant, although it remains unclear how the catalytic domain can be affected by a polymorphism located more than 300 amino acids away from the catalytic domain. Reduction in proximal TCR signaling in clinical samples may also result from the R620W variant partitioning into lipid rafts more efficiently than does WT PTPN22 (*6*), suggesting that localization may control the functional activity of mutant PTPN22. These two models are not mutually exclusive. Together, the data indicate that the R620W variant–mediated changes in T cell sensitivity may not be as simplistic as direct augmentation of phosphatase activity.

Indeed, work by Burn *et al.* using super-resolution microscopy demonstrated that T cell adhesion is also affected by the PTPN22 R620W polymorphism (*10*). In T cells expressing WT PTPN22, stimulation of the integrin LFA-1 causes PTPN22 recruitment to LFA-1 signaling modules at the plasma membrane, where PTPN22 inhibits signaling. T cells expressing the R620W variant do not similarly

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cluster PTPN22 at the membrane under stimulating conditions. As a consequence, VAV guanine nucleotide exchange factor signaling and cell adhesion are augmented. These data indicate that the polymorphism affects not only antigen receptor and co-receptor signaling but also the nature of the interactions between T cells and other cells. These results further emphasize that the R620W mutation has effects on multiple pathways that feed into TCR signal strength.

From the aforementioned studies, it is clear that PTPN22 R620W can function as both a gain-of-function and a loss-of-function variant. Therefore, we propose that the R620W polymorphism is a switch-of-function polymorphism, where both the catalytic activity and spatiotemporal regulation of the R620W variant determine how a given signaling pathway is affected (Fig. 1). This integrated model also predicts that the R620W mutation may affect immune cells in many ways, and further studies are certainly warranted.

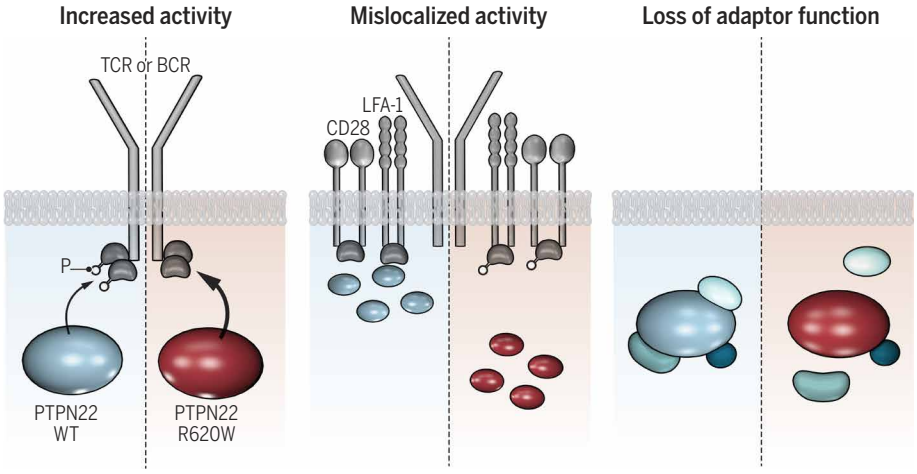

Fig. 1. The PTPN22 R620W mutation is a switch-of-function polymorphism. (**Left**) The R620W variant is more catalytically active than WT PTPN22. This could increase dephosphorylation of proximal TCR and BCR signaling proteins, including CD3, Igαβ, and Src and Syk family kinases, and reduce downstream signaling. (**Middle**) The R620W variant localizes improperly. Mislocalization could prevent dephosphorylation of CD28- or LFA-1–associated kinases and VAV, thus augmenting costimulation or adhesion. (**Right**) Independent of its phosphatase activity, WT PTPN22 can affect signaling by acting as a scaffold for CSK and other signaling molecules. However, the exact composition and stoichiometry of WT PTPN22–containing complexes are not known. Many of the known binding partner interactions are disrupted in the R620W variant.

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