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The Structural Wedge Domain of the Receptor-like Tyrosine Phosphatase CD45 Enforces B Cell Tolerance by Regulating Substrate Specificity

Julie Zikherman,* Ramya Parameswaran,* Michelle Hermiston,† and Arthur Weiss*‡

CD45 is a receptor-like tyrosine phosphatase that positively regulates BCR signaling by dephosphorylating the inhibitory tyrosine of the Src family kinases. We showed previously that a single point mutation, E613R, introduced into the cytoplasmic membrane-proximal “wedge” domain of CD45 is sufficient to drive a lupus-like autoimmune disease on a susceptible genetic background. To clarify the molecular mechanism of this disease, we took advantage of a unique allelic series of mice in which the expression of CD45 is varied across a broad range. Although both E613R B cells and those with supraphysiologic CD45 expression exhibited hyperresponsive BCR signaling, they did so by opposite regulation of the Src family kinase Lyn. We demonstrated that the E613R allele of CD45 does not function as a hyper- or hypomorphic allele but rather alters the substrate specificity of CD45 for Lyn. Despite similarly enhancing BCR signaling, only B cells with supraphysiologic CD45 expression became anergic, whereas only mice harboring the E613R mutation developed frank autoimmunity on a susceptible genetic background. We showed that selective impairment of a Lyn-dependent negative-regulatory circuit in E613R B cells drove autoimmunity in E613R mice. This demonstrates that relaxing negative regulation of BCR signaling, rather than enhancing positive regulation, is critical for driving autoimmunity in this system.  The Journal of Immunology, 2013, 190: 2527–2535.

Hyperresponsive BCR signaling has been implicated in the pathogenesis of lupus-like autoimmunity in mice and humans (1, 2). Dysregulated BCR signaling in mice can produce systemic autoimmunity, whereas human genome-wide association studies identified a role for BCR-signaling genes in lupus pathogenesis (1–3). However, the molecular and cellular mechanisms by which abnormal BCR signaling breaks tolerance and drives autoimmune disease remain uncertain.

AgR signaling relies upon the sequential activation of the Src and Syk family kinases (4, 5). In response to receptor ligation, the Src family kinases (SFKs) phosphorylate tyrosines within cytoplasmic ITAMs of the BCR or the TCR (TCR). Doubly phosphorylated ITAMs in B cells and T cells recruit the tandem-SH2 domain–containing Syk family kinases, Syk and Zap70, respectively (4, 6). Once activated, Src and Syk family kinases phosphorylate critical downstream targets, generating extensive signal diversification, and driving diverse cellular outcomes.

Numerous inhibitory coreceptors containing ITIMs are expressed on B cells and serve as critical negative regulators of BCR signaling (6). The B cell SFK Lyn phosphorylates ITIMs, which, in turn, recruit the inhibitory effector protein and lipid phosphatases SHP-1 and SHIP1, respectively (7, 8). Unlike the SFKs Fyn and Blk, which play exclusively positive-regulatory roles during BCR signaling, Lyn plays a nonredundant negative-regulatory role in addition to its positive-regulatory role (7, 8). Importantly, loss or impairment of SHP-1, SHIP1, or Lyn function were each shown to produce autoimmunity in mice, suggesting that this negative-regulatory circuit is essential to maintain tolerance (2, 9–14).

Situated at the top of the AgR-signaling cascades, the SFKs are themselves tightly regulated. Phosphorylation of the C-terminal tyrosine of the SFKs stabilizes an autoinhibitory conformation, whereas phosphorylation of the activation loop tyrosine of the SFKs is required for full enzymatic activity (15). The receptor-like tyrosine phosphatase CD45 serves as a critical positive regulator of AgR signaling by dephosphorylating the inhibitory tyrosine of the SFKs (15, 16). The inhibitory tyrosine of the T cell–specific SFK Lck (Y505) is hyperphosphorylated in CD45-deficient primary thymocytes (15, 17). Consequently, TCR signaling and T cell development are severely disrupted in mice deficient for CD45 (17–20). BCR signaling and B cell development are also impaired in CD45-deficient mice, but this phenotype is much milder than that of T lineage cells because of the expression of a partially redundant phosphatase, CD148 (21).

Although CD45 is the most abundantly expressed phosphatase on the surface of nucleated hematopoietic cells (15), the function of its large, heavily glycosylated extracellular domain remains unknown. Alternate splicing of this extracellular domain generates multiple CD45 isoforms whose expression are tightly regulated in a cell type–, activation–, and developmental stage–specific manner (15, 22). A genetic polymorphism that alters such splicing but not amino acid sequence, is associated with human autoimmune disease (23). These data imply that the extracellular domain of CD45 must have an important function. However, a bona fide ligand has
yet to be identified, raising the possibility that the extracellular domain of CD45 may regulate its activity by other means. Postulated functions for this domain have included association with the CD4 coreceptor or dimerization (24, 25).

Previously, our laboratory explored the importance of a cytoplasmic juxtamembrane wedge domain in CD45 that may function to mediate dimerization-induced inhibition of phosphatase activity. Introduction of a point mutation (E613R) at the tip of this structural wedge domain relieves its inhibitory function in cell line studies of forced dimerization (26, 27). In vivo, this mutation produces a lupus-like phenotype on susceptible genetic backgrounds and renders B cells hyperresponsive to BCR signaling (28, 29). Although this mutation was predicted to generate a hypermorphic allele of CD45, E613R T and B cells exhibit hyperphosphorylation of the inhibitory tyrosine of the SFKs, suggesting that an alternative mechanism might account for functional and cellular phenotypes in these mice (29, 30). The normal function of the wedge domain and the precise biochemical mechanism by which E613R alters CD45 function remain unclear.

We recently generated an allelic series of mice in which CD45 splicing is intact, but expression is varied across a broad range (0–180% in B cells) (31, 32). In T and B cells, increasing CD45 expression across this series reduces phosphorylation of the inhibitory tyrosines of Lck and Lyn, respectively (31, 32). We (32) and other investigators (33) demonstrated, through studies of this and a similar series, that CD45 plays a dual positive- and negative-regulatory role in response to its transduction and BCR signaling, such that even a very low CD45 expression is sufficient to reconstitute TCR signaling, but supraphysiologic CD45 expression inhibits TCR signaling. The unexpected negative-regulatory role of CD45 in T cells is thought to be mediated by dephosphorylation of the activation loop tyrosine of the SFKs. In contrast, in B cells, CD45 plays a purely positive-regulatory role (31). Because the mechanism by which the E613R allele of CD45 produces dysregulated AgR signaling and autoimmunity remains unclear, we decided to compare the CD45 E613R allele with our allelic series. In particular, we focused attention on so-called “HE” (H−/− or H/H) mice in which one or two copies, respectively, of normally spliced CD45 are overexpressed on top of endogenous CD45. This represents a bona fide CD45 “hypermorphic” and serves as a critical contrast to E613R mice.

We find that the CD45 E613R allele is neither a pure hypomorphic nor a hypermorphic variant of CD45; instead, it alters specificity for individual SFK substrates, suggesting that the wedge domain of CD45 normally plays a role in substrate selectivity. We show that both E613R and H/H mice exhibit hyperresponsive proximal BCR signaling but do so through opposing regulation of Lyn. We further show that these differences have important functional consequences, such that H/H, but not E613R, B cells are partially anergic. On a susceptible genetic background, E613R, but not H/H, mice exhibit frank autoimmunity. Finally, we show that impairment of a Lyn-dependent negative-regulatory circuit in E613R, but not H/H, B cells accounts for these phenotypes. This demonstrates that relieving negative regulation of BCR signaling, rather than enhancing positive regulation, is critical for driving systemic autoimmunity in this model.

Materials and Methods

Mice

Lightning mice were generated directly on the C57BL/6 genetic background during N-ethyl-N-nitrosourea mutagenesis screen conducted at Australian National University (32). Lightning mice were backcrossed to C57BL/6 mice for at least six generations. H/H (HE) mice and CD45+/− mice were described previously (19, 34), as were CD45 E613R and Lyn−/− mice (9, 28). All knockout and transgenic strains were fully backcrossed to the C57BL/6 genetic background. Mice were used at 5–9 wk of age for all functional and biochemical experiments. All mice were housed in a specific pathogen-free facility at the University of California, San Francisco, according to the University Animal Care Committee and National Institute of Health guidelines.

Abs and reagents

Murine CD3, CD4, CD8, CD19, CD21, CD22, CD23, CD44, panCD45, B220, CD45-1, AA4.1, IgM, and IgD Abs conjugated to FITC, PE, PerCP-Cy5.5, PE-Cy5.5, PE-Cy7, Pacific Blue, allophycocyanin, or Alexa Fluor 647 were obtained from eBiosciences or BD Biosciences. p-Erk, with the exception that phospho-S6 Ab is directly conjugated to the fluorophore. A phospho-Erk assay was also carried out after 15 min of preincubation with U0126. Mek1/2 inhibitor (Cell Signaling) at 10 nM concentration to confirm the specificity of Abs.

Calcium measurements

Assays were performed as previously described (35), with the exception that Indo-1 dye (Invitrogen) was used to load cells, and a UV laser on the BD Fortessa was used for detection. Prior to stimulation and analysis, lymphocytes were surface stained for expression of CD45 and AA4.1 (GraphPad Software). Densitometry measurements were performed using Kodak Image Station/Molecular Imaging software.

Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were performed as previously described (21). Purified cell populations of splenic B cells and CD4+ T cells were obtained by sorting with MACS kits, as per the manufacturer’s instructions (Miltenyi Biotech). Blot densitometry was performed using Kodak Image Station software.

BAFF ELISA

The BAFF ELISA kit was obtained from R&D Systems and used per the manufacturer’s instructions.

dsDNA ELISA, proteinuria, and histopathology

dsDNA ELISA, proteinuria, and histopathology were assessed as previously described (35).

Results

Differential regulation of SFKs Lck and Fyn by the CD45 E613R allele in T cells

SFK phosphorylation and AgR signaling are tightly regulated by CD45 in T cells. We sought to identify a biochemical “signature” of hypermorphic and hypomorphic CD45 function in T cells against which to compare the E613R allele of CD45. To do so, we took advantage of an allelic series of mice in which CD45 expression is varied across a broad range. The series includes mice harboring...
one or two copies of the Lightning (L) allele in which surface levels of CD45 expression are reduced relative to wild-type mice (L−/− = 7%; L/L = 15%) (32). In addition, the series includes mice with either one or two copies of the “H” CD45 transgene (H−/− and H/H mice, respectively) that express supraphysiologic levels (140 and 180%, respectively) of the protein (32, 34).

T cells express two SFKs, Lck and Fyn, whose inhibitory and activating sites of tyrosine phosphorylation serve as CD45 substrates (32, 33, 36, 37). We assessed tyrosine phosphorylation of Lck and Fyn in thymocytes and peripheral T cells from E613R and allelic series mice (Fig. 1A, 1B, Supplemental Fig. 1A). As reported previously, E613R thymocytes and peripheral T cells exhibit hyperphosphorylation at both of these tyrosines in Lck (Fig. 1A, 1B, Supplemental Fig. 1A) (30). Interestingly, this biochemical phenotype most closely resembles that of L−/− and L/L mice with low levels of CD45 expression, suggesting that CD45 E613R might be a hypomorphic allele (31, 32). In contrast, T cells with high CD45 expression exhibit reduced phosphorylation of both Lck regulatory tyrosines (Fig. 1A, 1B) (31, 32). However, regulation of Fyn in these mice differed markedly from that of Lck (Fig. 1A–C). In contrast to Lck, phosphorylation of the activation loop tyrosine of Fyn was not appreciably altered in E613R T cells relative to wild-type cells (Fig. 1A–C). This suggests that access to Lck tyrosine phosphorylation sites but not of Fyn’s are specifically impaired in E613R T cells. It was suggested that dual phosphorylation of the activation loop and inhibitory tyrosines of Lck can enhance its enzymatic activity (38) and that low CD45 expression in thymocytes results in TCR hyperresponsiveness, unmasking an additional negative-regulatory role for CD45 in TCR signaling that is most likely mediated by dephosphorylation of the activation loop tyrosine of Lck (Fig. 1E). E613R thymocytes are also hyperresponsive to TCR stimulation (Fig. 1E) (30). In this study, we show that E613R thymocytes appear to functionally resemble L+/− thymocytes most closely (Fig. 1E). However, in response to CD3/CD4 coligation, E613R thymocytes uniquely hyperresponsive, in contrast to low CD45−expressing mice. This suggests that E613R in T cells is not a simple hypomorphic allele of CD45. Rather, the CD4−associated pool of Lck in E613R thymocytes appears to be hypocoactive, suggesting that the E613R mutation at the wedge domain may alter access to this particular substrate pool.

FIGURE 1. Selective dysregulation of Lck phosphorylation in E613R T cells. (A) Whole-cell lysates of resting lymph node CD4 T cells from +/+ H/H, and E613R mice were blotted with Ab to the inhibitory and activating tyrosines of Lck (Lck pY505/Src pY416). Src416 Ab binds activating tyrosines of all SFKs. The lower band represents p56 Lck; the upper band represents p59 Fyn. Densitometry was performed on the lower Lck band. Total Lck is detected as a loading control. (B) Whole-cell lysates of resting thymocytes from +/+ H/H, and E613R mice were blotted with Ab to the inhibitory and activating tyrosines of Lck (Lck pY505/Src pY416). The single band detected by Src416 in thymocytes represents Lck. Total Lck is detected as a loading control. (C) Fyn was immunoprecipitated from whole-cell lysates of resting thymocytes from +/+ H/H, and E613R mice. Immunoprecipitates were subsequently blotted with Ab to the inhibitory and activating tyrosines of Fyn (pSrc527/pSrc416). Total Fyn is detected as loading control. (D) TCR-associated ζ-chain was immunoprecipitated from whole-cell lysates of resting thymocytes from CD45 allelic series and E613R mice. Immunoprecipitates were subsequently blotted for total ζ and total phosphotyrosine (pY) levels. Data in (A)–(D) are representative of at least two independent experiments. (E) TCR-stimulated, fixed, and permeabilized thymocytes were stained for phospho-Erk and costained for CD4 and CD8 so that double-positive subsets could be identified. Data were collected by flow cytometry. Graphs depict intracellular phospho-Erk in double-positive subsets from allelic series and E613R thymi. Data are representative of at least three independent experiments. Densitometry measurements in this and all subsequent figures were normalized to wild-type data.
Differential regulation of the SFKs Lyn and Fyn by the CD45 E613R allele in B cells

SFK phosphorylation and AgR signaling in B cells are also tightly regulated by CD45. It was shown that E613R B cells are markedly hyperresponsive to BCR stimulation (29). Studies of the CD45 allelic series showed that increasing CD45 expression produces increasing BCR signaling, suggesting a similarity between E613R and high CD45–expressing H/H B cells (31). To determine whether E613R might function as a hypermorphic allele of CD45 in B cells, we compared SFK phosphorylation in B cells from E613R and allelic series mice. B cells express three predominant SFKs: Lyn, Fyn, and Blk (6). We observed increased phosphorylation of the inhibitory tyrosine of Lyn (Y507) and reduced phosphorylation of the activation loop tyrosine of the SFKs (detected with mAb against Src pY416) in E613R B cells, suggesting reduced Lyn kinase activity (Fig. 2A, Supplemental Fig. 1B) (29). In contrast, increasing CD45 expression in allelic series B cells results in progressive dephosphorylation of the inhibitory tyrosine of Lyn (Fig. 2A, Supplemental Fig. 1B) (31). In contrast to B cells expressing either low or high CD45, E613R B cells appear to exhibit a unique “biochemical signature.”

Interestingly, we observed a consistent increase in the amount of Lyn, but not Fyn, protein expressed in E613R B cells, comparable to that observed in CD45

2/2 B cells (Fig. 2A, Supplemental Fig. 1B). It was reported that constitutively active Lyn kinase results in protein degradation and reduced Lyn protein expression (41). Consistent with the phosphorylation pattern of Lyn in E613R B cells, this suggested to us that Lyn kinase was less enzymatically active in E613R B cells. Importantly, immunoprecipitation of individual SFKs revealed that the altered SFK phosphorylation and expression patterns observed through analysis of whole-cell lysates corresponded primarily to Lyn, rather than Fyn, kinase (Fig. 2B, 2C). We conclude that the E613R allele has disparate effects on Lyn and Fyn in B cells.

Because both phosphorylation and expression of Lyn appeared to be altered in E613R B cells, we assessed Lyn-specific substrates to clarify the activity level of Lyn. To do so, we next evaluated phosphorylation of the Lyn-specific substrates, SHP-1 and SHIP1 phosphatases, which, in turn, serve as negative regulators of BCR signaling (Fig. 2D). We observed a reduction in basal phosphorylation of both phosphatases in E613R B cells relative to wild-type cells. In addition, inducible phosphorylation of these phos-
phosphatases in response to BCR stimulation was also reduced in E613R B cells. These data are consistent with partial inhibition of Lyn kinase activity in E613R B cells in both the basal and inducible states. Because SHP-1 and SHIP1 play negative-regulatory roles in the BCR-signaling pathway, these results suggest selective impairment of the Lyn-dependent negative-regulatory circuit in E613R B cells.

We next probed more downstream BCR-signaling events in E613R and allelic series B cells. We observed enhanced and prolonged Erk phosphorylation, S6 ribosomal protein phosphorylation downstream of P13K, and an increase in intracellular calcium both in E613R B cells and in H/H B cells with supraphysiologic CD45 expression (Fig. 2E–G). Importantly, Erk phosphorylation in all genotypes is lost upon pretreatment with the Mek1/2 inhibitor U0126, confirming the specificity of Ab staining (Supplemental Fig. 2A). These data confirm that BCR signaling in both E613R and H/H B cells is hyperresponsive, as shown previously (29, 31).

However, our studies of SFK phosphorylation suggest that the mechanism for hyperresponsiveness is biochemically distinct in these two lines. Low CD45 expression is associated with impaired BCR signaling, further confirming that E613R does not appear to function as a simple hypomorphic allele of CD45 (31). This is consistent with the selective dysregulation of Lyn, but not Fyn, phosphorylation observed in E613R B cells (Fig. 2B, 2C).

Opposing functional effects of supraphysiologic CD45 expression and the E613R allele on B cell development and activation

Because BCR signaling is perturbed in both E613R and allelic series mice, we assessed B cell development, a BCR-dependent function as a simple hypomorphic allele of CD45 (31). This is consistent with selective dysregulation of Lyn, but not Fyn, phosphorylation observed in E613R B cells (Fig. 2B, 2C).

We next determined whether differences in AgR signaling and activation of E613R and H/H B cells had functional consequences in vivo. On susceptible genetic backgrounds, E613R mice develop lupus-like autoimmune disease (28, 29) (M. Hermiston and A. Weiss, unpublished observations). We compared the disease phenotype of E613R mice to that of H/H mice with supra-physiologic CD45 expression on the susceptible B6/129 F1 genetic background (Fig. 5A–D). Although E613R mice develop a robust autoimmune disease with high-titer dsDNA Ab, proteinuria, renal inflammation, and premature mortality, H/H mice do not differ significantly from those bearing wild-type CD45 (Fig. 5A–D). These results reinforce biochemical and genetic data suggesting that the E613R allele of CD45 does not represent a CD45 gain-of-function variant.

Like E613R animals, Lyn−/− B cells develop a lupus-like autoimmune disease, albeit on the B6 genetic background (9). It was recently reported that, although Lyn−/− B cells are hyperresponsive, lupus-like autoimmune in these animals is driven partly by the myeloid compartment and BAFF hyperproduction (43). To test whether disease pathogenesis in E613R mice indeed resembles
that in Lyn<sup>−/−</sup> mice, we assessed serum BAFF levels in these animals. We found elevated BAFF levels in E613R, but not in H/−, animals along with splenomegaly and myeloid expansion (Fig. 5E, 5F, data not shown). These results suggest that E613R mice share common disease-pathogenesis features with the well-studied Lyn<sup>−/−</sup> model of lupus-like disease, whereas mice with supra-physiologic CD45 expression were protected from disease, despite hyperresponsive proximal BCR signaling.
Discussion

Introduction of the E613R mutation into the membrane-proximal wedge domain of CD45 drives systemic autoimmune disease in mice, but the molecular mechanism by which it does so has been unclear. In this study, we took advantage of the CD45 allelic series mice genetic sufficient or deficient for Lyn and H/H mice genetically sufficient or deficient for Lyn. Values are mean ± SEM of three biological replicates. Data in (B) and (C) are representative of at least three biological replicates. (E and F) Graphs representing mean fluorescence intensity (MFI) of CD69 expression on lymph node B cells from E613R and allelic series mice genetically sufficient or deficient for Lyn stimulated for 16 h with varying doses of anti-IgM. Values are mean ± SEM of three biological replicates. Data in (E) and (F) are representative of at least three independent experiments.

Although H/H B cells with supraphysiologic CD45 expression and E613R B cells both exhibit enhanced Erk phosphorylation, PI3K signaling, and calcium signaling in response to BCR ligation, the biochemical mechanisms underlying this hyperresponsiveness are distinct. Lyn inhibitory tyrosine (Y507) phosphorylation is increased in E613R B cells but reduced in H/H B cells, suggesting that Lyn is inactivated in the former but overactive in the latter. Importantly, E613R B cells do not phenocopy CD45+/− B cells nor do they resemble B cells with low CD45 expression. Consistent with biochemical evidence in T cells, these results support the notion that the E613R mutation does not simply enhance or impair phosphate activity of CD45.

We establish through both biochemical and genetic epistasis experiments that Lyn, but not other SFKs, is uniquely inactivated in E613R mice. B cells from Lyn−/− mice and B cells harboring constitutively active Lyn (Y507F; so-called “Lynup/up mice”) were described previously, and both exhibit hyperresponsive BCR signaling, reminiscent of E613R and H/H mice (41). These phenotypes have been attributed to dual positive- and negative-regulatory roles for Lyn via its phosphorylation of ITAM- and ITIM-containing receptors, respectively. Interestingly, like H/H B cells, Lynup/up mice are characterized by downregulation of multiple cell surface coreceptors and paradoxically impaired activation of B cells, suggestive of anergy (41). In contrast, neither Lyn−/− nor E613R B cells exhibit such functional unresponsiveness. We conclude that E613R B cells resemble Lyn−/− B cells, whereas H/H B cells phenocopy Lynup/up B cells.

Lyn regulates signaling by ITIM-containing inhibitory receptors both by phosphorylation of the ITIM tyrosine, which leads to recruitment of the inhibitory effectors SHP-1 and SHIP1, and by direct phosphorylation of these effector phosphatases (8). We demonstrated that both SHP-1 and SHIP1 phosphorylation are...
impaired in E613R B cells, consistent with selective Lyn inhibition. Importantly, both of these phosphatases have been implicated in enforcing B cell anergy (51). We show through genetic epistasis that the functional unresponsiveness of H/H B cells is mediated by Lyn. We speculate that H/H B cells recruit SHP-1 and SHIP1 to phosphorylated ITIM receptors more efficiently than do wild-type cells, thereby impairing downstream-signaling events despite enhanced proximal BCR signaling. We propose that impaired activation of SHP-1/SHIP1 phosphatases via Lyn contributes to autoimmunity in E613R mice, whereas the converse maintains anergy in mice with supraphysiologic CD45 expression. Consistent with this model, several mouse mutants characterized by enhanced BCR signaling have selective defects in this negative-regulatory circuit and develop systemic autoimmunity (e.g., CD22^{+/--}, FcγRIIβ^{+/--}, Shp-1^{Mev/Mev}, and Lyn^{--}) (1, 2). It is incompletely understood how Lyn, SHP-1, and SHIP1 enforce anergy. One possible mechanism is a reduction in phosphatidylinositol 3,4,5-phosphate levels and selective dampening of the PI3K-signaling pathway (14). It remains to be determined which ITIM-bearing surface receptors are most critical in this process and whether additional inhibitory effectors are recruited via Lyn. Finally, it is uncertain whether the anergic state is enforced by transcriptional or by posttranslational changes (14, 52).

What role does this pathway play in spontaneous, as opposed to genetically engineered, autoimmune disease? B cells from both the polygenic NZB/W lupus model and from patients with lupus exhibit hyperresponsive BCR signaling (1). It is uncertain whether selective impairment of ITIM pathways is responsible for these signaling phenotypes and for subversion of anergy. Altered expression of Lyn and genetic polymorphisms in Lyn, as well as the ITIM-containing receptor FcγRIIβ, are associated with human lupus, suggesting that this may be the case (3, 53–57). Indeed, it was shown recently that haploinsufficiency for Lyn in mice is sufficient to produce lupus-like disease and that this phenotype cooperates genetically with CD22, SHP-1, and SHIP-1 haploinsufficiency, suggesting that disease is a quantitative trait, and partial dysregulation of this inhibitory circuit is sufficient to produce disease (58, 59). In the current study, we identified a molecular mechanism for the CD45 E613R autoimmune disease that converges on this pathway and, thereby, highlights the importance of the Lyn-mediated negative-regulatory circuit in maintaining B cell tolerance.

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Disclosures

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References
