A conditional form of Bruton's tyrosine kinase is sufficient to activate multiple downstream signaling pathways via PLC Gamma 2 in B cells

ABSTRACT

These data suggest that Btk:ER regulates downstream signaling pathways primarily via PLCγ2 in B cells. While it is not known whether activated Btk:ER precisely mimics activated Btk, this conditional system will likely facilitate the dissection of the role of Btk and its family members in a variety of biological processes in many different cell types.

INTRODUCTION

Background Mutations in Bruton's tyrosine kinase (Btk) are responsible for the human disease termed X-linked agammaglobulinemia (XLA) (reviewed in reference). The B cell antigen receptor (BCR) signaling defect is very severe such that XLA patients have a block in the pro-B to pre-B cell transition and consequently have no mature B cells. The xid mouse, in which Btk is mutated, and the Btk knockout mouse display similar, although somewhat less severe, phenotypes. Btk is the prototypical member of the Tec family of non-receptor protein tyrosine kinases (PTKs) that includes Bmx, Itk, Tec and Txk. In addition to a COOH-terminal PTK domain, Btk has an NH2- terminal pleckstrin homology (PH) domain, a proline-rich Tec homology domain, a Src-homology 3 (SH3) domain and a Src-homology 2 (SH2) domain. Btk was originally identified in B cells but is now known to be expressed in most leukocytes with the exception of T cells and NK cells. Btk is thought to be activated upon BCR cross-linking by a two-step mechanism involving phosphatidylinositol (PI) 3-kinase and the Src family PTK Lyn (reviewed in references). PI 3-kinase induces Btk membrane targeting by generating phosphatidylinositol 3,4,5-trisphosphate (PIP3) to which the PH domain of Btk binds. Lyn transphosphorylates Btk within the activation loop of the kinase domain, allowing subsequent autophosphorylation of the Btk SH3 domain and full Btk activation. The SH2 domain-containing inositol phosphatase (SHIP) downregulates Btk by dephosphorylating PIP3. Biochemical studies of Btk-deficient B cells have shown Btk to be necessary for BCR-induced phospholipase C-γ2 (PLCγ2) phosphorylation, calcium mobilization, extracellular signal-regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) activation, NF-κB activation and apoptosis. These signaling events are generally not entirely abrogated and kinetic analyses have revealed that the sustained phases are particularly impaired in the absence of Btk. In contrast, these pathways are completely inhibited in the absence of the Syk/ZAP-70 family tyrosine kinase Syk. These data have led to the hypothesis that Btk co-operates with Syk in PLCγ2-dependent BCR signaling and that Btk functions as a signal duration modulator (reviewed in references). However, it is not clear if Btk alone is sufficient to mimic aspects of BCR signaling, nor is it known whether all downstream signals mediated by Btk are PLCγ2-dependent. Indeed, additional functions for Btk have recently been proposed, namely in PI 3-kinase activation, cytoskeletal reorganization and DNA transcription. In this study we have generated a conditional form of Btk by fusing the full length Btk protein with the hormone-binding domain of the estrogen receptor (Btk:ER). We show that Btk:ER activation alone is sufficient to activate multiple downstream signaling pathways in B cells, including calcium mobilization, ERK and JNK MAPK, and apoptosis. Moreover, by analysing Btk:ER function in PLCγ2-deficient cells, we show that PLCγ2 is necessary for Btk:ER to transmit these signals.

CONCLUSION

Conclusions The Btk mutations found in XLA patients and the xid mouse show that Btk is required for normal B cell development, but the important pathways activated by Btk have yet to be fully identified. In this paper we show that a conditional form of Btk (Btk:ER) is sufficient to activate many signaling pathways downstream of the antigen receptor, including PLCγ2 phosphorylation, calcium mobilization, apoptosis and ERK and JNK activation. Our findings clearly indicate that all of these Btk:ER-mediated responses require PLCγ2. In future studies, Btk:ER may be a useful tool for the identification of Btk phosphorylation sites on PLCγ2, and for the discovery of novel substrates.