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The role of Bruton's tyrosine kinase in autoimmunity and implications for therapy

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Summary

Bruton's tyrosine kinase (BTK) mediates B cell signaling and is also present in innate immune cells but not T cells. BTK propagates B cell receptor (BCR) responses to antigen-engagement as well as to stimulation via CD40, toll-like receptors (TLRs), Fc receptors (FCRs) and chemokine receptors. Importantly, BTK can modulate signaling, acting as a "rheostat" rather than an "on-off" switch; thus, overexpression leads to autoimmunity while decreased levels improve autoimmune disease outcomes. Autoreactive B cells depend upon BTK for survival to a greater degree than normal B cells, reflected as loss of autoantibodies with maintenance of total antibody levels when BTK is absent. This review describes contributions of BTK to immune tolerance, including studies testing BTK-inhibitors for treatment of autoimmune diseases.

Keywords

Bruton's tyrosine kinase; autoimmunity; B lymphocyte signaling; autoimmune inflammatory arthritis; type 1 diabetes; systemic lupus erythematosus; BTK inhibitors

Introduction

The primary contribution of Bruton's tyrosine kinase (BTK) to human health is to support humoral immunity, as demonstrated by its first mention in the literature, a description of a boy who was highly susceptible to infection with encapsulated bacteria (1). This report, by Colonel Ogden C. Bruton, described a disease that came to be known as X-linked

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agammaglobulinemia (XLA). Patients with XLA lack most antibodies, yet they can do relatively well with treatment, consisting of regular administration of replacement immunoglobulins from pooled human serum, and antibiotics (2, 3). The protein defective in XLA was discovered in 1993, both in humans and in the similar X-linked immunodeficiency (*xid*) mouse model, and is now known as Bruton's tyrosine kinase (4–8). That same year, *xid* mice were reported to be protected against collagen-induced arthritis (CIA), the first report to specifically link Btk with autoimmune inflammatory arthritis (9). Since that time, BTK's structure and function have been painstakingly delineated and a profusion of small molecular BTK-inhibitors has been developed for use in lymphoma and autoimmune disease(10–22). There is evidence from mouse models that Btk has a special role in governing immune tolerance in B cells (23–26). Thus, unlike other methods of targeting B lymphocytes, BTK inhibitors hold promise for improving B cell-related autoimmunity without inducing the degree of immunodeficiency seen in XLA. This review describes the known features of BTK pertinent to immune tolerance and its potential as a therapeutic target in autoimmunity.

B cell contributions to autoimmunity

B cell signaling is critical to B cell tolerance, and BTK plays a central role. Autoantibodies are often considered to be a readout of autoreactive T cell help, but this approach ignores contributions of B cell intrinsic tolerance mechanisms, which begin in the bone marrow, prior to T cell interactions. Approximately 70–80% of developing B cells are autoreactive, but most are culled at the immature stage via a process known as receptor editing or by apoptosis (27). In genetic backgrounds that favor autoimmunity this selection process is flawed, and there are increased numbers of naïve autoreactive B cells available to interact with T cells (28–30). These B cells act as antigen-presenting cells (APCs), specialized to concentrate autoantigen, and can be the exclusive APC that drives T cell mediated autoimmunity (31–33). B cells also produce cytokines and have regulatory functions (34). B cells in inflamed tissue may have specialized roles, as their removal can prevent autoimmune disease, even when T cells remain (35, 36). B cell responses in germinal centers that form in these inflamed tissues may lead to autoantibodies and autoreactive memory B cells. Therefore, understanding how B cell signaling mediates B cell tolerance is a key to preventing and treating autoimmune disease (23–26, 37, 38).

BTK-mediated signaling

The B cell receptor (BCR) is the primary sensor that initiates signaling (Figure 1). There are two primary components of the BCR: Membrane-bound antibody, and Igα/Igβ heterodimers that provide the cytosolic signaling function. Each B cell expresses 2×10^5 identical BCRs, and antigen-engagement triggers the signaling cascade, prompting phosphorylation by SRC-family kinases of immunoreceptor tyrosine-based activation motifs (ITAMs) on Igα and Igβ (39). Dual phosphorylation of these ITAMs allows spleen tyrosine kinase SYK to dock and become activated(40). These proximal, or initiating, signaling components are critical to the survival of B cells, and loss of any of these components results in severe depletion of the B cell compartment. Initiation of the signaling cascade affects multiple components that interact to propagate the signal. BTK is a 659 amino acid protein, arranged in five domains that enable multiple functions (Figure 2 and (41)). The N-terminal pleckstrin homology (PH)

domain binds phosphatidylinositol 3,4,5-triphosphate (PIP3) generated by phosphoinositide 3-kinase (PI3K) in response to BCR signaling, resulting in recruitment of BTK from the cytosol to the cell membrane. The PH domain of Btk is critical, as *xid* mice that have a mutation (R28C) in this component have a phenotype that is almost identical to that of *Btk*-deficient mice in which the protein is absent (6–8, 10, 42, 43). BTK's SRC homology 2 (SH2) domain binds phosphorylated tyrosines, which facilitates docking to the adaptor protein BLNK, a signalosome hub that anchors multiple proteins in close proximity for signaling interactions. BTK is activated by tyrosine phosphorylation at Y551, classically accomplished by LYN, as well as by SYK, which requires BLNK docking to facilitate this interaction (17, 44, 45). BTK's SRC homology 3 (SH3) binds various other proteins, in some cases dependent on its own autoregulated phosphorylation status (17, 41, 46). Thus, BTK has docking functions, as well as enzymatic activity, and there is evidence that it may make important contributions as an adaptor molecule, independent of its catalytic function (11, 12, 22). This has potentially important implications for predicting and understanding the effects of small molecular inhibitors that exclusively target the kinase function. The primary action of BTK's kinase domain is to phosphorylate phospholipase C gamma 2 (PLCγ2), which then cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate two second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (41, 47). IP_3 binds its receptor on the endoplasmic reticulum to initiate calcium flux, leading to nuclear transport of the transcription factor nuclear factor of activated T cells (NFAT). DAG activates Protein Kinase C β (PKCβ) with downstream activation of the NFκB pathway, as well as several mitogen-activated protein kinase (MAPK) pathways(15, 48–50). Of note, BTK also mediates signaling via CD40, Fc Receptors, chemokine receptors and TLRs, in myeloid cells as well as in B cells, although many of those pathways are less well-defined (10, 14, 15, 37, 49–53).

BTK regulation of B cell signaling responses relevant to autoimmunity

B cells are highly sensitive to the loss of proximal signaling proteins such as IgM or Syk, which cause B cell immunodeficiency(54–56). However, proteins involved in signal propagation can modulate signal responsiveness, rather than turning it on or off. Because tolerance induction and maintenance require nuanced responses to signaling, these proteins are good targets for fine-tuning and improving tolerance, and Btk regulation in mouse models has been shown to be important for B cell tolerance. Transgenic Btk overexpression causes a systemic lupus erythematosus-like disease, propagated by spontaneous germinal centers and autoantibody formation (25), and a model featuring constitutively activated Btk results in autoreactive IgM plasma cells (57). Conversely, Btk expression at 25% normal levels abrogates autoantibody production and an autoimmune syndrome produced in lyn-deficient mice (23). Autoreactive-prone B1a cells and anergic (An1) B cells both rely on Btk, and our lab has shown that Btk-deficiency eliminates 95% of anti-insulin B cells and protects against Type 1 diabetes (T1D) in nonobese diabetic (NOD) mice (10, 26, 37). Btk may also affect cytokine contributions to autoimmune disease, by supporting IL10 production by B cells(38). We showed that this B cell cytokine alteration may have downstream effects on T cells from $Btk^{-/-}$NOD mice, which also produce lower levels of IL10 in response to stimulation with anti-CD3/anti-CD28. This was specific to IL10, as IFNγ and IL17 production were normal (37). Importantly, IL10 has been shown to drive

autoantibody production by human B cells from patients with systemic lupus erythematosus (SLE, lupus) and IL10 blockade has provided protection against lupus in the NZB x NZW model (58, 59).

Functional outcomes of BTK deficiency in humans

XLA is caused by more than 600 different mutations in the *BTK* gene, and is characterized by near-complete absence of B cells in humans, due to developmental arrest at the pre-B cell stage. These patients have fewer than 1% normal B cell levels, undetectable plasma cells, and very low serum immunoglobulin levels (1, 60–62). They are highly susceptible to infections with encapsulated bacteria that cause pneumonia, otitis media and sinusitis, requiring lifelong immunoglobulin replacement purified from donor pools. This treatment generally allows patients to live otherwise healthy lives, implying that BTK's primary importance in humans resides in humoral immunity(2, 3). Patients with XLA are not generally thought to develop autoimmune disease, despite the fact that their few remaining B cells have an immature, high-IgM, phenotype, and are enriched for polyreactive, autoreactive-prone specificities (62, 63). There has been only one report of a patient developing T1D and a few reports of juvenile arthritis (64–66). Of note, however, a recent survey of XLA patients showed a majority had some self-reported symptoms consistent with inflammation or autoimmunity, although few had been formally diagnosed with autoimmune disease(67). The authors of that study noted that so little antibody is present that it is unlikely to cause these findings, but hypothesized that myeloid cell defects might contribute. Indeed, macrophages, neutrophils, dendritic cells and mast cells also express BTK (51, 68–71), although its role is not well-defined in those cell types. Some TLR responses are aberrant in the absence of BTK, which could contribute to susceptibility to infectious diseases (72, 73). In addition, overproduction of inflammatory cytokines in response to TLR signaling has been reported, which could contribute to inflammation in XLA patients (74, 75). In mouse models, *Btk*-deficient bone marrow-derived dendritic cells (BMDCs) exhibited increased levels of CD86 in response to lipopolysaccharide (LPS), and became antigen-presenting MHC class-II^{high} cells at a higher rate than their *Btk*-sufficient counterparts. This inflammatory phenotype was linked to a decreased ability of these BMDCs to produce IL-10, impairing the regulation of these cells (71).

BTK-deficiency as models for BTK-inhibitors

It is important to recognize that BTK-deficiency in human patients does not predict BTK inhibitor effects, for at least two reasons. First, BTK is a complex molecule with multiple functions, as discussed above. (Fig 2 and (11)). BTK-inhibitors target only the kinase domain, leaving other BTK activities intact, and in fact, ibrutinib does not recapitulate the XLA phenotype, as it does not reduce total IgG levels(19). Second, patients suffering from autoimmunity have a full repertoire of mature B cells, including those with normal and autoreactive specificities, cell populations not available for study in XLA patients. Thus there is no "natural experiment" in humans that allows *in vivo* evaluation of BTK function in mature B cells, or to differentiate their effects on autoreactive versus normal B cells. Btk-deficient murine B cells offer an advantage for these studies. Although they show slight delay in developmental progression at the pre-B cell stage, in addition to decreased Vκ transcription, this is offset by increased IL-7-driven proliferation that allows development of

mature peripheral B cells for study of BTK function (76, 77) Btk-deficient murine B cells in the periphery have a developmental block in maturation at the transitional stage, but have 50–80% of normal B cell numbers, and contain some B cells in all subsets except for peritoneal B1a cells (8, 10, 37). These Btk-deficient B cells are suboptimally activated in response to stimulation of BCR, CD40, and TLRs (10, 37). While deficient in T-independent humoral responses, they are nevertheless able to respond to T-dependent immunizations. This suggests that B cells that have matured beyond the bone marrow stage may not rely heavily on Btk to respond to T cell-dependent vaccines (41). Therefore using small molecular inhibitors to target BTK in humans with mature B cells may not cause major humoral immunodeficiency, in contrast to XLA, in which B cells lack BTK from birth.

BTK contributions to pre-clinical models of autoimmune disease

Studies identifying a role for Btk in autoimmunity began using the *xid* mouse model more than a decade before the protein itself was discovered (Table I). Most of these early studies tested this x-linked antibody defect in a variety of murine models that depend upon autoantibodies, such as SLE and hemolytic anemia. The first of these used F1 males of New Zealand Black (NZB) mice crossed with CBA/N mice that carried the *xid* mutation during studies to understand gender differences in autoimmunity and showed data suggesting reduced autoantibody (anti-erythrocyte) production (78). The same group next found that NZB mice with the *xid* mutation did not develop anti-DNA antibodies, while aged (16 month old) MRL/1 mice with *xid* still did (79). The authors noted that NZB autoantibodies are mostly IgM and likely to arise from the T-independent B1 subset that is absent in *xid* mice, while MRL/1 autoantibodies were mostly IgG, associated with T cell driven lymphoproliferation. These findings implied that different B cell subsets and immune processes were responsible for the autoantibodies in the different models. Of note, a second group found that anti-erythrocyte and anti-DNA autoantibodies did occur in some NZB mice with *xid*, implying heterogeneity in this model (80). Next, congenic NZB mice with only the *xid* component of the CBA/N strain were developed (81). These mice were protected against autoantibody production, splenomegaly, hemolytic anemia, and early death, despite retaining the T cell abnormalities associated with NZB mice. Further work with this model showed that aging, or polyclonal stimulation with TLR ligands, could overcome disease protection(82, 83). Additional murine models of SLE, including NZBxNZW, C3H.gld/gld, and MRL.lpr/lpr have also been crossed with *xid* mice with similar results (Table I and references (84–86)). The first published work to use Btk-deficiency in a model of autoimmune arthritis was performed as part of a study of x-linked genes in 1993, just prior to identification of the protein responsible for the defect. The *xid* locus from CBA/N mice was crossed onto DBA/1 mice. DBA/1-*xid* offspring proved resistant to induction of collagen-induced arthritis, and failed to develop autoantibodies to type II collagen (9). Fourteen mice were used in this experiment, and the mechanism of disease protection was not elucidated at that time, although the findings were considered surprising, since CIA depends on T cells, and T cell-dependent B cell responses, not thought to be affected by *xid*. Our lab recently used the K/BxN spontaneous and serum transfer models to further investigate the role of Btk in arthritis, and discovered that its primary contribution is in the B cell compartment, especially germinal center development and function. Interestingly, autoantibodies were severely reduced while total IgG remained at near normal levels in Btk-

deficient K/BxN mice, and spontaneous autoimmune arthritis was strikingly reduced (87). However, Btk-deficient recipients of K/BxN serum transfer developed arthritis at the same rate as Btk-sufficient littermates, indicating that innate contributions to arthritis are not affected by loss of Btk. This differs from a number of studies using BTK inhibitors that have shown efficacy in both innate and adaptively driven forms of autoimmune arthritis (88–90). These findings suggest that off-target effects of the inhibitors may contribute to their disease outcomes, and highlight the importance of including genetic approaches to define functional effects of Btk, rather than relying exclusively on inhibitors. We have also shown that Type 1 diabetes (T1D), considered to be T cell-mediated, is prevented by Btk-deficiency in the nonobese diabetic (NOD) mouse model of this disease, despite the absence of this protein in T cells, reinforcing the evidence that the primary function of B cells in this disease is to present autoantigens to T cells (31–33, 37). Anti-insulin B cells and autoantibodies were severely reduced, while total B cell numbers and total IgG levels were not (26, 37). We also showed that Btk supports naturally occurring autoreactive-prone anergic (An1) and autoreactive marginal zone (MZ) NOD B cells (26, 37, 91), which may also have implications for arthritis, as this subset was recently shown to be a primary source of anti-collagen B cells in collagen-induced arthritis (92).

BTK inhibitors

The recognition of BTK as an important B cell target has generated a race to produce BTK-specific kinase inhibitors. The first inhibitor was described in 1999, and now dozens of recent publications report the outcomes of pre-clinical and clinical trials for these drugs, including ibrutinib, CC-292, RN486 and CGI1746 (Table II and (18, 21, 53, 88–90, 93–101). Many BTK-inhibitors are dosed orally and appear to be fairly well tolerated (18). Ibrutinib was recently the first-in-class to be FDA approved for treatment of mantle cell lymphoma and Waldenstrom macroglobulinemia (101, 102). This orally dosed daily drug is an irreversible inhibitor that binds BTK Cys481 (103). The irreversibility allows it to bind BTK in cells for 24 hours, even though the serum half-life is 2–3 hours, a feature which is thought to reduce side effects. Known off-target binding includes EGFR, HER2, HER4, BMX, JAK3, TEC and BLK (21). Ibrutinib also binds interleukin-2-inducible T cell kinase (ITK), shifting T cells away from T_H2 toward T_H1 cytokine profiles (103). Ibrutinib was shown to prevent both collagen induced arthritis (CIA) and collagen autoantibody induced arthritis (CAIA), and to reduce inflammatory cytokine release by macrophages and monocytes (89). CGI1746 stabilizes BTK in an inactive nonphosphorylated enzyme conformation, and was found to decrease FcγR mediated inflammation induced by immune complexes, and to prevent autoantibody production and arthritis in the CIA, CAIA, and K/BxN serum transfer models (88). CC-292 (formerly AVL-292) also binds Cys481 irreversibly and prevented CIA as well as improving arthritis outcomes when used therapeutically after disease onset in that model (96). Of note, a search of ClinicalTrials.gov reveals an on-going study of CC-292 versus placebo as co-therapy with methotrexate in active rheumatoid arthritis (NCT01975610). RN486 is a reversible inhibitor shown to prevent and reverse autoimmune and inflammatory arthritis in CIA, CAIA, and rat adjuvant induced arthritis (AIA) (90, 104). An early, non-covalent, reversible, BTK-inhibitor, LFM-A13 was shown to affect BTK responses and to effectively prevent graft-versus-host disease in murine allogenic bone marrow transplant studies (93, 105). However, it also has off-target

effects as well as a high IC 50 (17.2 μM), requiring high doses for effect, and use of this drug in pre-clinical trials has become less common recent years (99). This explosion in the number of therapeutic trials of BTK-inhibitors highlights the importance of fully understanding their mechanisms of action. Our data suggest that low, intermittent dosing might be used to eliminate autoreactive B cells without inducing global B cell immunodeficiency. Lower dosing may be particularly useful for improving specificity of drug action, as specific kinase inhibition is difficult due to similarities in the kinase domains of different proteins. These drugs are designed very carefully for exclusive binding of BTK, yet even the best are known to have off-target effects, which may affect outcomes (103). Of note, the fact that other kinase inhibitors have had difficulty translating to clinical use for autoimmune disease has raised some skepticism, leading some experts to express only guarded optimism about this class of drugs (106).

Early studies of B cell-related outcomes in patients on BTK-inhibitors

The effect of BTK inhibitors on normal or autoreactive human B cells is not yet known. Some recent studies of patients with B cell malignancies treated with ibrutinib show that immunoglobulin levels are not reduced, indicating that targeting BTK with inhibitors need not replicate the typical immunodeficient phenotype associated with XLA(19, 107, 108). For example, patients with chronic lymphocytic leukemia (CLL) treated with ibrutinub showed no reduction in IgG for the first 6 months, followed by a decrease of 23% at 24 months, then stabilizing after that. IgM increased transiently and IgA levels showed substantial and sustained improvement, doubling at 24 months. Patients with the best IgA recovery also had fewer infections and infection rate correlated with IgA, not IgG levels. Polyreactive IgM and IgA, but not IgG, antibodies also increased, thought to be concurrent with renewed B cell populations, but also possibly similar to polyreactive antibodies seen in XLA patients (63). The number of normal B cells increased but did not recover entirely, remaining abnormally low even at 24 months, even in patients with otherwise normal lymphocyte counts. B cell precursors were present in about half of bone marrow samples (109). This evidence of improvement from an impaired baseline after treatment needs to be replicated in patients who do not start treatment in a state of humoral immunodeficiency however. There is also early evidence from patients undergoing treatment for leukemia that BTK inhibitors may have a beneficial effect on autoimmunity. One retrospective study that assessed autoimmune cytopenias in CLL showed a much lower than expected incidence in patients treated with ibrutinib (110). This was not a randomized controlled trial, and also does not address autoimmunity in other disease processes, but is early evidence suggestive that BTK-inhibitors may be useful for targeting autoreactive B cells in humans.

Conclusions

BTK structure, and function in murine B cells, has been well-defined since its discovery more than two decades ago. BTK contributions to human immunity are best-known by the defects conferred when it is absent, including loss of B lymphocytes and antibodies, while its role in shaping mature and autoreactive human B cell repertoires is not clear. BTK-inhibitors target only the kinase domain and do not necessarily impair the linker function. Their use in humans so far does not appear to recapitulate the XLA phenotype, although

published studies at this time are limited to studies of patients with B cell related lymphomas. Furthermore, off-target effects of these drugs limit the interpretation of the role of BTK. Nevertheless, these drugs appear to be well-tolerated, and work in preclinical models suggests that they may prove useful for targeting autoreactive B cells in patients with autoimmune disease.

Expert Commentary

After more than two decades of careful work to understand BTK contributions to immunity and autoimmunity, we now stand at the verge of applying this knowledge to human health. Small molecular inhibitors are in the pipeline and the first is now in use in patients with B cell related lymphoma. Pre-clinical trials for autoimmunity are underway. Questions that must be answered include how these inhibitors affect patients, including normal and autoreactive B cells and antibodies as well as autoimmune disease outcomes, and potential for immunodeficiency. Given the fact that XLA patients have B cells that tend to be autoreactive it is also important to maintain vigilance for a possible increase in autoimmunity. B cell repertoire studies currently applied to patients with autoimmune disease would be valuable in understanding the impact of BTK inhibitors on patient repertoire (28, 63, 111). Also, BTK has been reported to have a role in B cell migration and adhesion (112), and in clinical trials with Ibrutinib-treated mantle cell lymphoma (MCL), patients exhibited egress of malignant cells from lymphatic tissue into peripheral blood (113). The impact of this mechanism of action may vary by autoimmune disease, as disruption of lymphoid structures by CXCL13 blockade has been shown in mouse models to ameliorate CIA and EAE (114, 115), but not T1D (116). Increased study of how lymphocytosis may affect autoimmune pathogenesis may be important in the future. In addition, the role of BTK in inflammatory responses mediated by innate cell populations is very poorly understood. Detailed studies of innate signaling responses such as those governed by TLRs have mostly been performed in B cells and there is evidence that BTK-mediated myeloid cell responses to the same TLRs may differ. BTK inhibitors that recently became commercially available for research are currently commonly used in these investigations and results of those studies are often cited as evidence of BTK's role in various signaling pathways. However, their off-target effects may confound some of those outcomes and other approaches using specific genetic targeting are also needed. Understanding which drug effects can be attributed to BTK signaling, and which may be due to off-target kinase binding, is important for these studies, as well as for best practices in drug design and dosing. Finally, some chronic lymphocytic leukemia (CLL) patients have been reported to develop resistance to Ibrutinib, through mutations affecting binding to BTK or by hyper induction of PLCγ2 (117–120). Though autoreactive B cells lack the oncogenes that drive B cell lymphomas to continuously proliferate, the possibility of acquired resistance should not be ignored when considering treatment with BTK inhibitors.

Five-year view

The rapid advances in the development of BTK-inhibitors, including their current use in cancer and clinical trials for autoimmunity, suggests that these drugs will be in clinical use for autoimmune disease within five years. It may be hoped that careful dosing would blunt autoimmunity without inducing immunodeficiency, which would represent an advance over

other anti-B cell drugs, such as rituximab, which eliminates all B cells. Caveats, however, are the unknown outcomes cited above, including the possible paradoxical increases in autoreactive B cells due to altered selection during development or in germinal centers, as well as the potential for immunodeficiency. Careful analysis of these outcomes during clinical trials will help refine the transition of this promising class of drugs into useful treatments for patients with autoimmune disease.

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Reference annotations

* Of interest

** Of considerable interest

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Key Issues

- Autoreactive B cells depend more upon B cell signaling than normal B cells do, as indicated by loss of autoreactive prone B cells including B1a, An1, transgenic anti-insulin B cells, marginal zone B cells in the nonobese-diabetic mouse model of autoimmune diabetes.
- Total IgG is normal or near-normal while autoantibodies are significantly reduced in Btk-deficient mouse models.
- Btk-deficient mice can still make T-dependent immune responses, suggesting that precise BTK targeting may have an advantage over global B cell elimination for treatment of autoimmunity without induction of humoral immunodeficiency.
- X-linked aggamaglobulinemia cannot predict the potential effect of BTK-inhibitors on mature human B cells, as cells do not develop beyond the pre-B cell stage.
- BTK is a multi-component signaling protein with adaptor function as well as kinase function, and most kinase inhibitors target only the kinase component.
- Several BTK-inhibitors have proven effective in multiple pre-clinical models of autoimmune and inflammatory arthritis.
- Comparison of BTK-deficiency with effects of BTK-inhibition suggest that some proportion of their disease protective effects may be due to off-target binding.
- BTK-signaling in innate cells requires further investigation.#

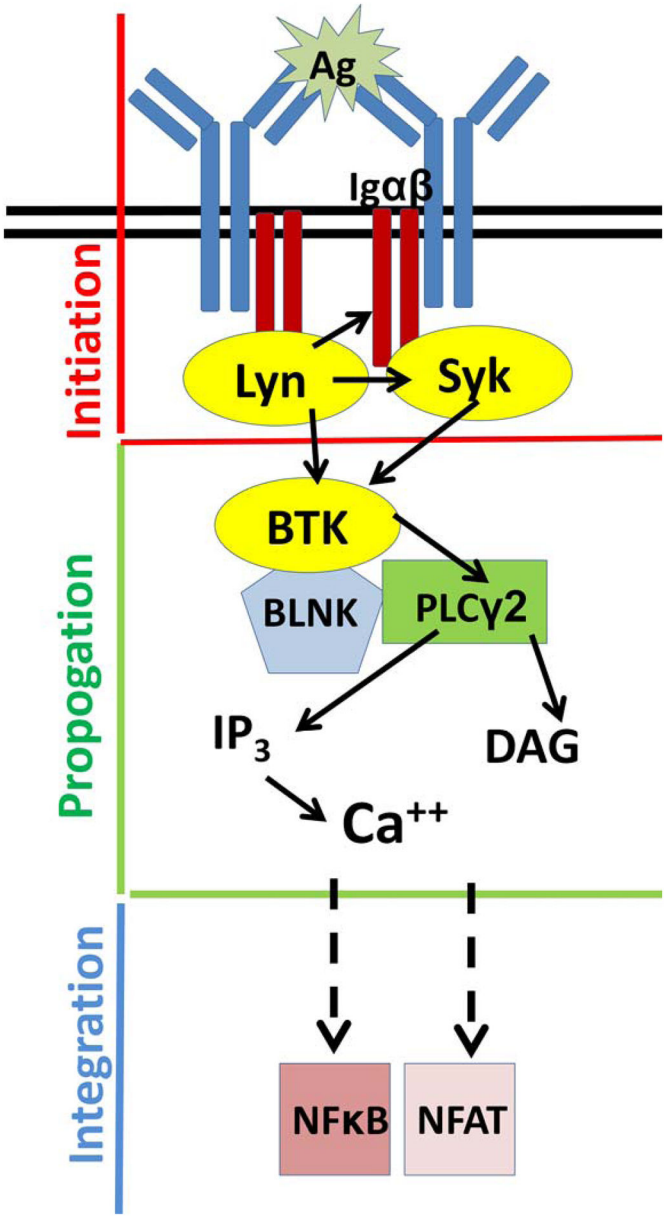

Figure 1. Simplified schematic of BTK's position in the signaling cascade

Antigen-BCR binding triggers a signaling pathway in which BTK is recruited to the cell membrane from the cytosol, docks with the linker protein BLNK and phosphorylates PLCγ2, with downstream calcium flux and cellular activation via nuclear translocation of transcription factors NFκB and NFAT. Yellow=kinase, blue=adaptor, pink=transcription factor. Dashed arrows=multiple proteins involved. Inspired by Dal Porto and Cambier (129).

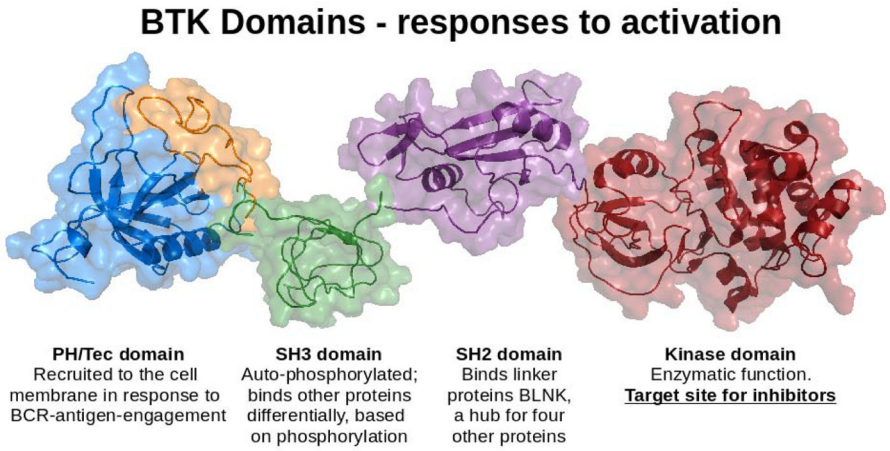

Figure 2. Structural rendering of subunits of BTK

PH/Tec domain, blue and orange (130) (residues 2–170, 1BTK.pdb). SH3 domain, green (131)(residues 212–275, 1AWW.pdb). SH2 domain, purple (132) (residues 270–386, 2GE9.pdb). Kinase domain, red (133) (residues 397–659, 1K2P.pdb). The arrangement of the structures is for context, and is not intended to imply relative position or lack of inter-domain motion. PH=pleckstrin homology; SH=SRC homology. Image made with the PyMOL Molecular Graphics System, Version 1.6.0.0 (Schrödinger, LLC).

Table I

BTK and autoimmunity in murine models

Autoimmune disease studies	Model used	Immune outcomes	Disease outcomes
Hemolytic anemia Ref: (78) 1979	CBA/N (*xid*) x NZB	Reduced anti-erythrocyte antibodies	N/D
Lupus Ref: (79) 1980	CBA/N (*xid*) x NZB and CBA/N (*xid*) x MRL/1	Reduced anti-DNA antibodies in NZB (mostly IgM) but not aged MRL/1 (mostly IgG).	N/D
Hemolytic anemia and Lupus Ref: (80) 1980	CBA/N (*xid*) x NZB	Found that some offspring did have anti-erythrocyte and anti-DNA antibodies, suggesting heterogeneity in the model.	N/D
Lupus Refs: (81–83) 1981, 1982, 1987	NZB.*xid*	Loss of anti-DNA autoantibodies. Loss of splenomegaly. T cell abnormalities of NZB were not reversed.	Protection from fatal renal disease. Disease could be restored by age or chronic stimulation with TLR ligands.
Lupus Ref: (84) 1982	NZBxNZW.*xid/xid*	Loss of anti-DNA autoantibodies, but not normal antibodies. Even immunization to DNA could not induce autoantibody formation.	Protection from fatal renal disease.
Lupus Refs:(85, 86) 1983, 1987	MRL.lpr/lpr *xid* and C3H.gld/gld *xid*	Decreased autoantibody production, despite persistence of T cell abnormalities and lymphadenopathy.	Reduced renal damage and death from autoimmunity.
Collagen-induced arthritis (CIA) Ref: (9) (1993)	*Xid*	Lack of antibodies against type II collagen	Protection against arthritis.
Lupus-like autoimmune disease induced by Lyn deficiency Ref:(121, 122) 1998	Lyn-deficient *xid* and $Btk^{-/-}/Lyn^{-/-}$	Severe reduction of B cell numbers and impaired B cell function; Protection against autoantibodies seen in $Lyn^{-/-}$	Protection against glomerulonephritis and/or splenomegaly seen in $Lyn^{-/-}$
Lupus-like autoimmune disease induced by Lyn deficiency Ref: (23) 2003	$Btk^{lo}/Lyn^{-/-}$	Restoration of B cell numbers and function compared with $Btk^{-/-}/Lyn^{-/-}$; Protection against autoantibodies seen in $Lyn^{-/-}$	Protection against splenomegaly seen in $Lyn^{-/-}$
Experimental autoimmune encephalomyelitis (EAE) Ref: (123) 2002	Myelin oligodendrocyte glycoprotein-induced (MOG)-EAE DBA/1-*xid*	Reduced anti-MOG antibodies; increased MOG-specific T cell responses without change in IFNγ	Reduced severity of EAE
EAE, Dextran sulfate sodium (DSS)-induced colitis, carrageenan-induced edema Ref: (124) 2004	*Xid* mice	Poor macrophage survival and production of reactive oxygen intermediates (ROI) in response to lipopolysaccharide (LPS); normal phagocytosis, normal CD80/CD86 regulation, and near-normal motility of macrophages.	Reduced EAE, colitis and foot-pad edema
Lupus Ref: (38) 2008	56R.$Btk^{-/-}$ and Btk^{lo}/56R.$Btk^{-/-}$	Loss of anti-DNA antibodies seen in 56R model; Btk^{lo} restored anti-DNA IgM but not IgG	ND
Lupus-like autoimmune disease Ref: (25) 2012	B cell-specific BTK overexpression	Increased B cell activation and survival, spontaneous germinal centers, increased plasma cells and autoantibodies	Lupus-like autoimmune disease in lungs, kidneys and salivary glands
Type 1 diabetes Refs: (26, 37, 91)	$Btk^{-/-}$/NOD	Loss of most anti-insulin Tg B cells, An1 B cells, a subset of MZ B cells,	Protection against T1D in NOD mice. Disease

Autoimmune disease studies	Model used	Immune outcomes	Disease outcomes
2014, 2009, 2015		and anti-insulin antibody. Preservation of total IgG levels.	restored by presence of residual *Btk*$^{-/-}$ anti-insulin Tg B cells

Table II

Pre-clinical studies of BTK-inhibitors for autoimmunity.

Autoimmune disease studies	Models	Drug	Immune outcomes	Disease outcomes
Murine autoimmune arthritis Ref: (100) 2007	Collagen induced arthritis (CIA)	"Compound 4" by Celera	ND	Improved arthritis scores
Murine Lupus Ref: (125) 2010	MRL/Fas	Ibrutinib	Decreased autoantibodies	Improved renal impairment
Murine autoimmune arthritis Ref:(88) 2011	CIA, serum transfer models: collagen autoantibody induced arthritis (CAIA) and K/BxN	CGI1746	Decreased FcγR mediated inflammation induced by immune complexes; prevented autoantibody production	Prevented autoimmune (CIA) and inflammatory arthritis (CAIA and K/BxN)
Rat autoimmune inflammatory arthritis Ref: (126) 2011	Rat Collagen induced arthritis	GDC-0834	ND	Reduced ankle swelling
Murine autoimmune and inflammatory arthritis Ref: (89) 2011	CIA and CAIA	Ibrutinib	Reduced inflammatory cytokine release by macrophages and monocytes	Prevented arthritis in both CIA and CAIA.
Rodent arthritis Refs: (90, 104) 2012, 2015	mouse CIA, CAIA, and rat adjuvant-induced arthritis (AIA)	RN486	Reduced tumor necrosis factor α (TNFα) production by monocytes stimulated with IgG-coated beads, reduced B cell activation, reduced autoantibodies and inflammatory markers in serum	Prevented arthritis in CIA and CAIA models and reduced arthritis when used therapeutically in CIA; reduced splenomegaly and AIA in rats
Glomerular nephritis (Lupus) Ref: (127) 2012	B6.sle1 and B6.Sle1.Sle3	PCI-32675 (Ibrutinib), given for 56 days	Reduced autoantibodies, B cells, DCs, macrophages, neutrophils and activated T cells; normal numbers of naïve T cells	Reduced splenomegaly and glomerular nephritis, Reduced B cells in the kidneys despite no decrease in cellularity overall
Murine autoimmune arthritis Ref: (96) 2013	CIA	CC-292	ND	Prevented and treated CIA
Glomerular nephritis (Lupus) Ref: (20) 2013	NZB x NZW	RN486 given for 8 weeks beginning at age 32 weeks	Reduced IgG autoantibodies, autoreactive B cells, B cell activation and splenic plasma cells, with preserved total IgG	Reduced proteinuria and glomerulosclerosis, IgG, IgM and C3 deposition, reduced macrophage infiltrations
Glomerular nephritis (Lupus) Ref: (128) 2013	NZBxW_F1 and Anti-glomerular basement membrane (GBM) antibody induced nephritis	PF-06250112, given for 12 weeks beginning at age 26 weeks	Reduced autoantibodies compared with vehicle-treated, reduced naïve B cells at high doses, reduced germinal center (GC) B cells and splenic plasma cells at all doses, reduced activated T cells with normal numbers of naïve T cells, preserved total IgG and IgA levels.	Reduced proteinuria, glomerular injury, cellular inflammatory infiltrates, IgG and C3 in the spontaneous model and prevention of proteinuria in the antibody-induced model.
Type 1 diabetes Ref: (26) 2014	Non-obese diabetic mice	Ibrutinib	Eliminated transgenic anti-insulin B cells in vitro	ND
Human rheumatoid arthritis and psoriatic arthritis Ref: (53) 2014	In vitro assays using synovial explants and human macrophages	RN486	BTK was present in B cells, macrophages, monocytes and neutrophils in tissues. RN486 significantly	ND

Autoimmune disease studies	Models	Drug	Immune outcomes	Disease outcomes
			inhibited IL6 production by stimulated macrophages, but only trended toward TNFα reduction.	