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Mini-Review

Bruton's tyrosine kinase—an integral protein of B cell development that also has an essential role in the innate immune system

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ABSTRACT

Btk is the protein affected in XLA, a disease identified as a B cell differentiation defect. Btk is crucial for B cell differentiation and activation, but its role in other cells is not fully understood. This review focuses on the function of Btk in monocytes, neutrophils, and platelets and the receptors and signaling cascades in such cells with which Btk is associated. *J. Leukoc. Biol.* 95: 243–250; 2014.

Introduction

In 1952, Ogden C. Bruton reported the first case of agammaglobulinemia. The patient was an 8-year-old boy who presented with recurrent severe respiratory infections and an absent γ-globulin fraction in his serum [1]. It was later demonstrated that patients with agammaglobulinemia lack B cells in the peripheral blood and that these patients do not generate plasma cells and B cells in peripheral tissues. Most of the patients with agammaglobulinemia display <2% B cells in peripheral blood [2]. Primary agammaglobulinemia can be an X-linked or an autosomal recessive disorder; the X-linked form is the most frequently diagnosed [3]. In 1993, the molecule responsible for the XLA was discovered by two independent groups [4, 5]. These groups reported that the gene involved in XLA is a tyrosine kinase of the Src family, named Btk, which is involved in B cell signaling and expressed through B cell differentiation [6]. Later, murine models for XLA were described, the Xid mice that carry a naturally occurring mutation in Btk (R28C), and a gene-targeted knockout mouse (Btk-deficient); both models show a decreased number of B cells (but not their absence) and an abnormal response to BCR signaling [7, 8]. For a long period, Btk was considered to be only essential for B cell development; however, growing evidence indicates that Btk also participates in other processes, such as TLR-mediated activation of myeloid cells, ROS production, and platelet activation. In this review, in addition to summarizing the relevance of Btk in B cells, we will describe its functions in nonlymphoid cells.

CLINICAL FINDINGS IN XLA

Some studies have shown that >50% of the cases of XLA are sporadic or lack a family history of this disease [9]. Most of the cases are diagnosed in early childhood (before 5 years of age) [9, 10]; however, there have been patients diagnosed at later ages, including during adulthood [11–13]. In XLA patients, B lymphocytes account for <2% of the total peripheral blood lymphocytes, and the levels of serum Igs (IgG, IgA, and IgM) are two SDs below the normal range for age. A few cases have shown normal IgM levels [14].

Affected boys present severe bacterial infections after 6 months of age (when passive maternal immunity has ceased). The type of infections that have been found in patients with agammaglobulinemia include (in order of frequency): pneumonia, acute otitis media, sinusitis, recurrent diarrhea, conjunctivitis, skin infections, meningitis/encephalitis, septic arthritis, hepatitis, and osteomyelitis [10, 15, 16]. The microorganisms most commonly isolated are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, Enterovirus, and EBV, whereas *Aspergillus fumigatus*, *Candida albicans*, and *Mycobacterium tuberculosis* have been isolated with

Abbreviations: BLNK=B cell linker protein, BM=bone marrow, BMDC=bone marrow-derived DC, Btk=Bruton's tyrosine kinase, c-MET=hepatocyte growth factor receptor, Flt3=FMS-related tyrosine kinase 3, GP=glycoprotein, Grb2=growth factor receptor-bound 2, IκBα=NF of κ light polypeptide gene enhancer in B cell inhibitor, α, IP_3-inositol triphosphate, mDC=monocytoid DC, p=phosphorylation, PH=pleckstrin homology, PIP2=phosphatidylinositol (4,5) bisphosphate, PIP3=phosphatidylinositol (3,4,5)-triphosphate, Rac=ras-related C3 botulinum toxin substrate, SH=Src-homology, SLP-76=Src-homology domain-containing leukocyte protein of 76 kDa, TH=Tec-homology, TIRAP=Toll/IL-1R-associated protein, XID=X-linked immunodeficient, XLA=X-linked agammaglobulinemia

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less frequency. Upon physical examination, tonsils were absent or hypoplastic in at least 79% of patients with XLA [10, 14, 15]. In addition, XLA manifests occasionally with diverse clinical spectra, such as autoimmunity, cancer, or severe neutropenia [15, 17]. In fact, neutropenia occurs in ~20% of XLA patients and is commonly associated with infectious episodes [17, 18]. Moreover, a small proportion of atypical cases of XLA has been reported. These patients are asymptomatic during childhood and do not present recurrent infections until reaching adulthood. These patients are commonly misdiagnosed with common variable immunodeficiency [13].

A late diagnosis contributes to the development of chronic lung disease, which is the most frequent complication [9, 14]. Other complications are failure to thrive and bronchiectasis [9, 19]. Some studies have reported an inverse association between the year of birth and age of diagnosis, which could reflect the importance of establishing a correct diagnosis early in life [9, 15, 19]. On average, 74% of patients are hospitalized because of an infectious process before the diagnosis [14].

MUTATIONS IN *BTK*

Mutation detection in XLA patients has been performed in many countries. Many of the mutations in *BTK* that have been reported in such publications are added into the international BTKbase (http://bioinf.uta.fi/BTKbase/). The most recent report that summarizes the data contained in this database was published by Väliaho et al. [20]. Briefly, of the 1111 entries in this report, most of the reported mutations are missense, resulting in an amino acid substitution (389; 35%) or a stop codon (161; 14.5%). These are followed by deletions (191; 17%), splice-site mutations (156; 14.04%), and insertions (68; 6.1%). Transitions are more frequent than transversions (69% vs. 31%, respectively), and CpG dinucleotides are the most common mutation events, frequently affecting R residues. In fact, 32.4% of the missense mutations caused a change, in order of frequency, of R to stop codons, W, Q, H, C, G, K, P, S, T, and L [20]. In addition, Lindvall et al. [21] reported, based on the data in the BTKbase, that SH1 is the most commonly mutated domain (44.7%), followed by PH (21.8%), SH2 (13.7%), TH (8.3%), and SH3 (7%). Interestingly, missense mutations in the SH3 domain have not been reported. The authors suggest that the stability of the SH3 domain might be very refractory to missense mutations [21].

In our experience, mutations in *BTK* in a cohort of 14 patients resembled very much the data summarized previously; however, we found two novel mutations (L111P and E605G) that affected the function and structure of Btk [22]. In addition, an important proportion (50%) of these cases has a splice site mutation [23]. The high frequency of these mutations in this cohort could be a result of the small group of XLA patients analyzed, but it is also possible that there is a relationship between splice-site mutations and the clinical progress in these patients. In fact, missense mutations are correlated with higher levels of IgG at diagnosis in these patients compared with those individuals with splice-site mutations [24]. These findings need to be verified in a larger patient population.

BTK IN EARLY B CELL DEVELOPMENT

The site of B cell generation is the BM. There are many factors that influence B cell development, e.g., the production of cytokines by stromal cells, the signaling through the BCR, and the expression of particular transcription factors [25]. Although it is not clear which cytokines drive B cell commitment in the human immune system, studies in mouse models indicate that IL-7 is crucial for this function [26]. Mice with IL-7R deficiency display an arrest in the early stages of B and T cell development [27]. Surprisingly, patients with mutations in the γ_c chain (a subunit of IL-7R) display normal levels of B cells in the peripheral blood, but these B cells are not functional [28].

Other factors that have been implied to be important in early B cell maturation are the stem cell factor and Flt3 ligand. Both proteins are soluble factors produced from stromal cells in the BM and interact with their respective receptors, cKit (a receptor with tyrosine kinase activity) and Flt3R, which are involved in the proliferation and differentiation of B cells [29, 30].

B cell development depends on the formation of the pre-BCR, which is formed by the λ-chain, VpreB, Igμ, Igα, and Igβ molecules. Signaling through this receptor will lead to B cell progression in the BM. Mutations in the genes encoding pre-BCR components lead to agammaglobulinemia in humans [31].

BTK AND BCR SIGNALING

Btk is a member of the Tec family of kinases [32, 33]. It is essential in the ontogeny of B cells and in signal transduction pathways that regulate B cell survival, activation, proliferation, and differentiation [34, 35]. Btk contains five structural domains: (1) PH, which is thought to mediate plasma membrane targeting, as mutations in this domain have profound effects on its activity [36–39]; (2) TH; (3) SH3; (4) SH2 [40, 41], and (5) the tyrosine kinase catalytic domain (SH1) [42] (**Fig. 1A**). The domains SH2 and SH3 are important in mediating intramolecular interactions or interactions with other proteins.

Btk is expressed continuously during B cell development from the late pro-B cell stage until the mature stage. The absence of functional Btk leads to the failure of many signal transduction pathways that regulate important physiological processes of the cell, e.g., apoptosis, growth, cell cycle, proliferation, and, in particular, antigen receptor-mediated signal transduction [43].

The primary event that occurs after BCR cross-linking is an increase in intracellular levels of PIP3, which as well as other lipids, binds efficiently to the PH domain of many proteins, including Btk [43]. This binding assists in the recruitment of Btk to the plasma membrane of the cell, where it subsequently becomes activated. In contrast, a naturally occurring PH domain Btk mutant [the arginine residue at Position 28 is substituted by a cysteine residue (R28C)], which does not interact with this lipid, fails to translocate to the cell surface. One of the initial events that occurs in response to the engagement of the BCR is the activation of nonreceptor protein tyrosine kinases, e.g., Syk, Lyn, and Fyn, and the phosphorylation of the intracellular sequences of the BCR, which are referred to as ITAMs [43]. Btk is phosphorylated by the kinases Lyn and/or

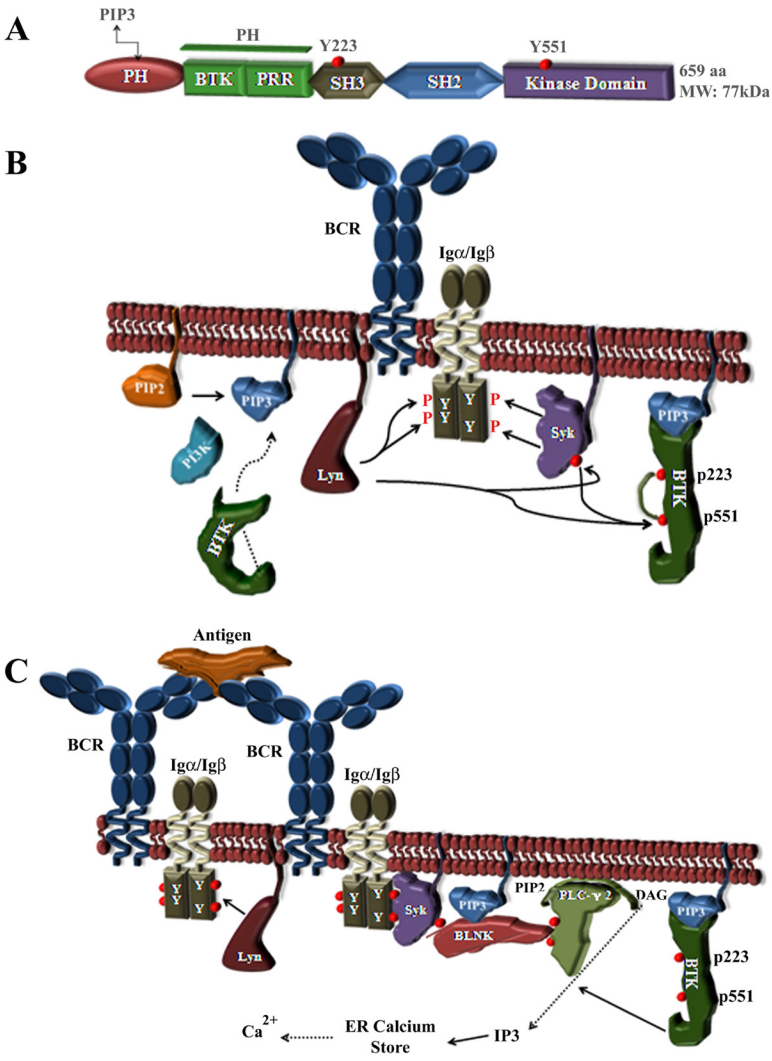

Figure 1. Btk in the signaling pathway mediated by the BCR. (A) Btk structure showing the PH, TH, SH3, SH2, and the catalytic SH1 domains. The TH domain consists of two motifs, i.e., a proline-rich region (PRR) and Btk. (B) Signaling through BCR initiates after cross-linking by the antigen and subsequent phosphorylation of ITAMs located in the Igα/Igβ cytoplasmic region. Lyn and Fyn are recruited to these ITAMs through interaction with their SH2 domains. Then, Btk is phosphorylated at residue Y551. Subsequently, Btk is autophosphorylated at Y223 to be fully active. Btk is recruited to the plasma membrane through interaction of its PH domain with PIP3. Targets of Btk include PLCγ2 and PI3K. (C) Btk induces the p-PLCγ2 after BCR cross-linking; PLCγ2, in turn, catalyzes the hydrolysis of PIP2 into IP_3 and DAG. IP_3 induces calcium mobilization from the ER to induce full B cell activation. P, phosphate; Y, tyrosine residue.

Syk at Y551 in the SH1 domain, which increases Btk activity dramatically. A second phosphorylation site is located in the SH3 domain in Btk at Y223, which is autophosphorylated (Fig. 1B) [44]. Once activated, Btk can interact with BLNK, which interacts with other proteins that function as Btk substrates; for a review, see ref. [45].

Following antigen-receptor engagement in B cells, Btk induces tyrosine p-PLCγ2 with subsequent production of IP_3 and mobilization of intracellular Ca^{2+} (Fig. 1C) [43]. This Btk-dependent tyrosine p-PLCγ2 requires an intact SH2 domain and is inhibited specifically by the fungal metabolite wortmannin, indicating the involvement of PI3K [46]. Btk is also activated by a wide variety of other receptors, including GPCRs, IL-3, IL-5, TLRs, and platelet receptors [47, 48]. In fact, Btk has been described as being involved in various signaling pathways, most of which will be discussed in this review.

BTK IN MYELOID CELLS

Btk is expressed in lymphoid and myeloid cells. In recent years, its role in monocytes and neutrophils has been studied, and there is growing evidence that Btk is involved in the control of cytokine production upon TLR stimulation. The importance of Btk in macrophage function was observed initially in microfilaria-infected Xid mice. Those experiments demonstrated a delayed microfilaria clearance that was associated with reduced NO production and low levels of TNF-α, IL-1β, and IL-12 production [49]. The role of Btk in the production of proinflammatory cytokines in murine cells was also explored in experiments involving splenocytes and peritoneal macrophages that were stimulated with peptidoglycan, polyinosinic:polycytidylic acid, LPS, and CpG (agonists for TLR2, TLR3, TLR4, and TLR9, respectively). These experiments demonstrated that Btk-deficient murine cells produced increased levels of proinflammatory cytokines, such as IL-6, TNF-α, and IL-12p40 and decreased levels of the immunoregulatory cytokine IL-10. Such defects are the result of altered signaling, as low levels of the regulatory phosphatase MAPK phosphatase-1 and high levels of p-JNK and -ERK1/2 were reported [50, 51]. Additional experiments using the human monocytic THP1 cell line demonstrated that TLR8 and -9 could interact with Btk [52, 53]. Furthermore, coimmunoprecipitation exper-

iments using different human and mouse cell lines of myeloid origin suggest that Btk interacts with MyD88, TIRAP, and IL-1R-associated kinase-1 but not with TRAF-6 [53]. In fact, MyD88- and Btk-deficient mice show a reduced myeloid compartment, which is even deeper in double-deficient mice, indicating the importance of both proteins in myeloid development [54]. In addition, LPS activates Btk, and inactive mutant forms of Btk inhibit NF-κB activation, suggesting that Btk is an important regulator in TLR4 signaling [53]. Furthermore, using transfection experiments, it was shown that TLR2 and TLR4 stimulation induces Btk-dependent TIRAP phosphorylation and promotes interaction with suppressor or cytokine signaling-1; such interaction results in TIRAP polyubiquitination and subsequently, its degradation [55].

The role of Btk in human myeloid cells from XLA patients has been examined by several groups with differing results. For example, Horwood et al. [56, 57] reported reduced TNF-α production in LPS- and palmitoyl-S-glyceryl cysteine-stimulated XLA PBMCs. In agreement with that study, Sochorova et al. [58] reported that XLA-derived DCs did not display IL-6 and TNF-α production after stimulation with ssRNA; similar data were obtained by Taneichi et al. [59]. In contrast, Marron et al. [60] reported recently that human, nondifferentiated cells from XLA patients displayed a proinflammatory profile when stimulated through TLR4, -7, and -8. Significantly increased TNF-α and IL-6 levels were detected when purified monocytes and mDCs were stimulated with LPS, loxoribine, and CL097 (a derivative of the imidazoquinoline compound R848). Under these conditions, there were no differences of p38 and IκBα phosphorylation or TIRAP degradation [48]. These data were supported later by Gonzalez-Serrano et al. [24], who demonstrated that PBMCs from XLA patients produced increased levels of TNF-α, IL-10, IL-1β, and IL-6 in response to LPS stimulation, supporting the importance of Btk in the regulation of inflammation. **Figure 2** summarizes the signaling pathway in which Btk is involved after TLR4 activation. Such differences might be a result of the biological material used in the experiments, i.e., in vitro-differentiated cells, THP1 cells, or fresh PBMCs. Other factors that may contribute to these differences include the B cell depletion procedure (which should be performed in samples from healthy controls), the occurrence of infections, or the Ig replacement when the samples are obtained.

Human neutrophil experiments have provided additional information, suggesting a role for Btk in the regulation of innate immune functions. Recently, Marron et al. [60] suggested that Btk-deficient neutrophils have normal oxidative burst and phosphorylation patterns (p-ERK1/2, p-p38, and p-JNK) after stimulation with TLR4 and -7/8 agonists; however, a report by Honda et al. [61] demonstrated significantly increased production of ROS when XLA neutrophils were stimulated with TLR ligands, together with a neutrophil chemoattractant (fMLP). This study also described augmented apoptosis in XLA neutrophils after stimulation and increased levels of cleaved caspase 3, indicating that Btk deficiency in human neutrophils results in them being prone to apoptosis and exacerbated ROS production [61]. In addition, XLA neutrophils display increased membrane localization of Rac-2, a regulator of NADPH oxidase activity, and higher baseline activity of protein tyrosine kinases, such as Fak, Lyn, Src, Syk, Vav, and PI3K. Lastly, in Btk-deficient neutrophils, TIRAP interacts with PI3K-p85 at the plasma membrane, whereas in control neutrophils, Btk interacts with TIRAP, suggesting that in normal neutrophils, Btk is involved in confining TIRAP to the cytoplasm and preventing its binding to PI3K, thus maintaining enzyme inactivity [61].

Additional experiments in mice have demonstrated altered myeloid differentiation. In a work published by Fiedler et al. [62], Btk-deficient mice presented increased proportions of

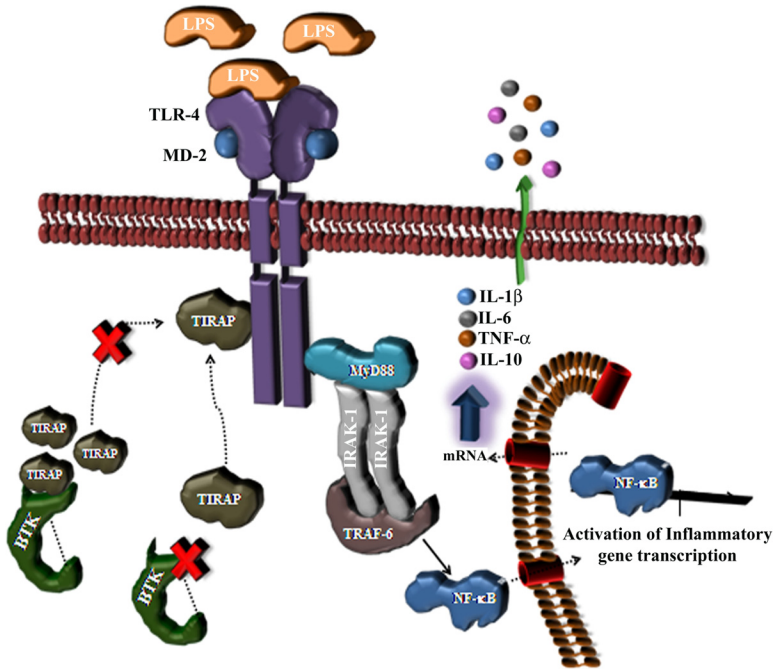

Figure 2. Btk is involved in the signaling pathway mediated by TLRs. TLR4 interaction with its coreceptor myeloid differentiation protein 2 (MD-2) leads to full sensitivity to LPS, inducing a signaling cascade that leads to the recruitment of adaptor proteins (such as TIRAP and MyD88) and the activation of NF-κB to induce the production of proinflammatory cytokines. BTK acts as regulator of TLR signaling as it interacts with TIRAP. Such interaction may induce TIRAP degradation or confine it to the cytoplasm. In both cases, it blocks further TLR signaling activation, down-regulates the production of proinflammatory cytokines by decreasing NF-κB activation, and induces an increase in the production of anti-inflammatory cytokines.

myeloid and granulocyte progenitors and immature granulocytes, whereas the number of mature granulocytes was decreased in BM samples. In addition, Btk-deficient BM granulocytes expressed significantly lower levels of certain transcripts, e.g., neutrophil elastase, lactoferrin, and MPO, and presented reduced activity of some granule proteins, e.g., elastase and gelatinase [62]. Currently, there are no data available regarding defects in neutrophil maturation in XLA patients. However, it is interesting to note that 10–20% of XLA patients experience neutropenia, which is associated with viral and mild bacterial infections [17, 18, 63]. The pathogenesis of neutropenia in XLA is unclear. The fact that neutrophils display enhanced apoptosis following TLR ligation is one possible explanation; however, there is no association between the type of mutation in *BTK* and the occurrence of neutropenia. Lastly, recent findings suggest an association between the FcγRIIa and TLR4 in human neutrophils [64].

Several studies indicate a role for Btk in cell migration. Probably the earliest report regarding this hypothesis is the work published by Cariappa et al. [65] in 1999, who demonstrated that Xid mice show accelerated migration of newly formed B cells in vivo. Additional data were reported by de Gorter et al. [66] in 2007. showing reduced migration and adhesion to VCAM-1 in response to stromal-derived factor-1, which led to p-PLCγ in different Btk-deficient cell lines, suggesting a direct role of Btk in the signaling pathway. A role of Btk in cell recruitment is also reported in myeloid cells. For example, the Btk inhibitor LFM-A13 induced reduced chemotaxis, adhesion, and ROS production in response to fMLP [67]. In addition, Lachance et al. [68] showed in 2002 that Tec-kinases (Btk, Tec, and Bmx) were recruited to the plasma membrane and became phosphorylated after stimulation of human neutrophils with fMLP. This event was dependent on G-proteins and PI3K, as PTX and wortmannin inhibited recruitment and phosphorylation of these kinases to the plasma membrane [68]. Further experiments demonstrated that Btk is involved in β2-integrin activation and subsequently, slow rolling after E-selectin engagement in murine leukocytes. It was suggested that Btk participates downstream of Syk and activates PLCγ2 and PI3K, as Btk-deficient neutrophils showed reduced p-PLCγ2 and p-PI3K but not Syk [69]. In vivo experiments, using a murine model of acute kidney injury, demonstrated that the SLP-76 (a paralog of BLNK) showed reduced neutrophil adhesion, migration, and recruitment to the kidney; the same study demonstrated that SLP-76 is upstream of Btk, in murine neutrophils, after E-selectin engagement [70].

Kuehn et al. [71] reported a deficiency in murine mast cell migration and suggested that Btk regulates actin polymerization and Rac activation after stimulation through the FcεRI, cKit, or the PGE2R. A broader review regarding the involvement of Btk in leukocyte recruitment was reported by Block and Zarbock [72] in 2012.

Several reports have explored the role of Btk in DC signaling, although the study of DCs in humans is difficult, as in most studies, the differentiation to mDCs is induced by using purified monocytes isolated from PBMCs. A report by Gagliardi et al. [73] suggests that the functions of mDCs, in terms of T cell stimulation and expression of costimulatory molecules, are similar between XLA patients and healthy controls; similar observations were reported by Liu et al. [74]. However, studies using BMDCs obtained from mice pointed out interesting differences between Btk-deficient and WT mice. It has been observed that LPS-stimulated BMDCs from Btk-deficient mice show an increased ability to induce T cell proliferation and cytokine secretion (e.g., IL-4, IFN-γ, IL-5, IL-13); in addition, a reduced production of IL-10 by BMDCs from Btk-deficient mice was observed, which was suggested to be the central mechanism to explain why T cells are overstimulated in the absence of Btk [75]. Further experiments supported the hypothesis that the IL-10 production in DCs is dependent on Btk, which signals through the c-MET, essential for various functions in immune cells [76, 77]. The authors also showed that c-MET-dependent activation of Btk occurs in DCs from human and mice (BMDCs, splenic DCs, and mDCs); interestingly, they proposed that Btk is upstream of the c-Src-PI3K-mammalian target of rapamycin pathway that leads to the inhibition of NF-κB activation as a result of the induction of IL-10 expression [78].

There are other receptors that may signal through Btk. For example, in human plasmacytoid DCs, CD303 cross-linking (a c-type lectin receptor) induces a signaling pathway similar to that induced by the BCR [79]. Cross-linking of CD303 leads to reduced p-IκBα and inhibits IFN-I production induced by CpG stimulation [79].

BTK AND PLATELETS

Btk is also expressed by platelets. Whether XLA patients possess alterations in platelet functionality is an intriguing question, as platelets, in addition to being responsible for blood clotting in damaged tissues, also have an important function in inflammation [80]. Platelets enhance leukocyte infiltration through direct interactions among platelets, leukocytes, and activated endothelial cells. Platelet adhesion molecules that mediate such interactions are P-selectin, β3-integrin, GPVI, and GPIV [81]. Platelet-mediated leukocyte recruitment has been shown to promote viral clearance in mouse models of acute hepatitis and lymphocytic choriomeningitis [80]. Btk has been shown to participate in the signaling cascade mediated by GPVI, which is a collagen receptor and forms a complex together with FcγR [82]. Signaling through GPVI-FcγR is similar to that mediated by the BCR. Briefly, the exposure of platelets to collagen surfaces results in the tyrosine p-FcγR. The Src-family tyrosine kinases Fyn and Lyn are responsible for p-FcγR in ITAMs, providing a docking site for Syk. Consequently, linker for activation of T cells is phosphorylated, which recruits SLP-76 to activate PI3K, and PI3K acts on membrane molecules producing PIP3 and PIP2. These molecules interact with Btk and Btk then activate PLCγ2 to produce DAG and IP_3 [81]. Other signaling molecules, e.g., Grb2-related adapter protein 2, Grb2, Cbl, SLP-76-associated phosphoprotein 130, and Vav, are also recruited to the signaling complex. This signaling pathway is necessary for platelet secretion and aggregation. Vav1-deficient mice display slightly diminished, GPVI-stimulated aggregation responses [81]. The relevance of Btk in platelet activation by collagen is uncertain, as

platelets also express Tec, which has been reported to be activated in the absence of Btk [83]. In addition, aggregation, dense granule secretion, and calcium mobilization are diminished significantly but not abolished in platelets from XLA patients [84]. Recently, Btk has been suggested as being required in the generation of platelet microparticles, which are small, membrane-coated vesicles released from platelets after activation. In patients with rheumatoid arthritis, the interaction of platelet microparticles with synoviocytes induces the release of proinflammatory cytokines and exacerbates joint destruction. Btk inhibition blocks the generation of platelet microparticles and consequently, proinflammatory cytokine production in cocultured platelets with fibroblast-like synoviocytes [85]. Whether this occurs in vivo in XLA patients is unknown.

BTK AND OTHER CELLS

There are a few reports describing Btk functions in cells other than B cells, monocytes, neutrophils, and platelets. For example, Btk appears to have an interesting role in NK cells, as Btk-deficient mice display defects in NK cell activation, including decreased cytotoxicity and reduced expression of IFN-γ, perforin, and granzyme B after TLR3 ligation [86]. Moreover, osteoclasts from Btk/Tec double-deficient mice present severe osteopetrosis and altered PLCγ activation mediated by RANK [87]. XLA patients do not present any bone alteration, but Danks et al. [88]reported that osteoclasts, differentiated from $CD14^+$ cells from XLA donors, displayed defects when $CD14^+$ cells were cultured in the presence of dentine, RANK, and M-CSF, including reduced actin ring formation and reduced resorption clusters (pits); similar results were reported for osteoclasts from Xid mice [89].

CONCLUDING REMARKS

The role of Btk has been studied extensively in the last two decades. It was initially considered to be a protein essential for B cell development. Growing evidence now indicates important roles for this protein in other cells. These findings may have relevance in XLA pathogenesis, possibly facilitating future therapies, in addition to γ-globulin replacement in these patients. The importance of Btk expression in myeloid cells is unclear, and the related signaling pathways remain to be explored.

AUTHORSHIP

G.L-H. wrote and reviewed parts of the manuscript. A.V-H. and J.C.R-A. wrote a review of BCR signaling and mutations in *BTK*. L.B-R. wrote the Btk and other cells' section. M.E.G-S. and F.E-R. reviewed the clinical findings in XLA. L.S-A. contributed with intellectual perspectives regarding the quality of the information presented.

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DISCLOSURES

The authors declare no conflict of interest.

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