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Review

Bruton's Tyrosine Kinase is involved in innate and adaptive immunity

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Summary. Btk is a cytoplasmic tyrosine kinase, which is mainly involved in B cell receptor signalling. Gene targeting experiments revealed that Btk is important for B cell development and function. However, Btk is not only expressed in B cells, but also in most other haematopoietic lineages except for T cells and plasma cells. Recently we found that Btk is involved in Toll-like receptor signalling. Toll-like receptors play an important role in innate immunity. They are highly expressed on mast cells, macrophages and dendritic cells, which are essential for the recognition and consequently for the elimination of microbial pathogens. Therefore Btk might play an important role for the function of immunocompetent cells of innate as well as adaptive immunity.

Key words: Btk, Toll-like receptors, B cells, Myeloid cells

Introduction

Bruton's tyrosine kinase (Btk) is a cytoplasmic non-receptor tyrosine kinase that belongs to the Tec-kinase family. Tec-kinases (Btk, Bmx, Itk, Rlk, Tec) are largely expressed in cells of different lineages of the hematopoietic system. The crucial role of Btk for B cell development and function became clear when it was found that mutations in the gene coding for Btk are responsible for X-linked agammaglobulinemia (XLA) in men (Tsukada et al., 1993; Vetrie et al., 1993) and X-linked immunodeficiency (Xid) in mice (Rawlings et al., 1993; Thomas et al., 1993). Although the phenotype of *Xid* is milder than that of XLA, both diseases are characterized by dramatic defects in B cell development and function resulting in a reduction of mature B cells in the peripheral blood as well as in secondary lymphoid organs accompanied by a severe reduction of serum immunoglobulin levels. Nevertheless, the expression of Btk is not restricted to B cells, it is also expressed in myeloid cells, like mast cells (Kawakami et al., 1994; Smith et al., 1994), monocytes/macrophages (de Weers et al., 1993; Smith et al., 1994), neutrophiles (Lachance et al., 2002), dendritic cells (DCs) (Gagliardi et al., 2003), in erythroid cells (Smith et al., 1994; Robinson et al., 1998; Whyatt et al., 2000), in platelets (Quek et al., 1998), in hematopoietic stem cells (HSC), and multipotent progenitors (Phillips et al., 2000) as well as in primary neuronal cells (Yang et al., 2004). Although btk mutations cause primarily severe B cell defects, functions of btk-deficient or -mutated myeloid cells are also affected (see below).

Tec-family kinases are characterized by an N-terminal pleckstrin homology (PH) domain which is able to bind to membrane phospholipids (Salim et al., 1996; Kojima et al., 1997) as well as proteins, like heterotrimeric G-Proteins (Touhara et al., 1994; Tsukada et al., 1994; Langhans-Rajasekaran et al., 1995; Bence et al., 1997), PKC-isoforms (Yao et al., 1994; Kawakami et al., 1995; Saharinen et al., 1997; Yao et al., 1997), Stat3 (Saharinen et al., 1997), F-actin (Yao et al., 1999) and Fas (Vassilev et al., 1999). Additionally, Btk contains a Tec-homology-region (TH) implicated in the autoregulation of Tec-kinases, Src-homology regions SH3 and SH2 necessary for interaction with other proline-rich (PR) sequences and binding to sequences harbouring phosphorylated tyrosine residues, respectively. In addition, Btk contains a C-terminal kinase domain (SH1) (reviewed in (Miller and Berg, 2002)) (Fig. 1A). The multifaceted functions of Btk are realized due to its ability to form complexes with other proteins and lipids. Complex formation is a transient event in the process of signal transduction.

Btk and the adaptive immune system

Btk in B cell development and function

Mutations in the gene coding for Btk cause XLA in

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humans (Tsukada et al., 1993; Vetrie et al., 1993) and *Xid* in mice (Rawlings et al., 1993; Thomas et al., 1993). XLA is a severe inherited immunodeficiency disease, characterized by defective antibody responses and by peripheral blood B cells that are reduced in number and show an immature phenotype (Conley, 1985; Campana et al., 1990) (Fig. 1B). Although Btk is expressed very early in B cell ontogeny (de Weers et al., 1993; Smith et al., 1994), the B cell defect appears only at the pre-B1a cell stage at which the development to mature B cells takes place (reviewed in Nomura et al., 2000).

XLA becomes evident six to nine months after birth when the level of maternally-derived IgG decreases. Characteristically, affected males develop hypogammaglobulinemia and are highly susceptible to bacterial infections, which are the most common clinical manifestations. However, XLA patients have normal T cell functions and an intact cellular immunity.

More than 400 unique mutations in the Btk gene of XLA-patients have been identified that are scattered along the Btk gene (Vihinen et al., 1998). In general, no correlation could be observed between the type of mutation and the phenotype of XLA patients (Holinski-Feder et al., 1998). Even in the same family identical mutations may lead to diverse clinical effects (Bykowsky et al., 1996; Kornfeld et al., 1997).

In B cells, where Btk is expressed continuously during B cell development from the late pro-B cell stage till the mature $IgD^{high}IgM^{low}$ stage, the absence of a functional Btk leads to a failure of several signal transduction pathways, regulating important physiological processes of the cell like apoptosis, growth, cell cycle, proliferation, and particularly antigen receptor mediated signal transduction (Maas and Hendriks, 2001). B cells bearing a mutated btk gene are hyporesponsive to BCR and several other transmembrane signals and do not respond to T cell-independent type II antigens. The levels of serum IgM and IgG3 are dramatically reduced, whereas the IgG1, IgG2a and IgG2b concentrations are normal. The B cell number is reduced to approximately 50% (Wicker and Scher, 1986) (Fig. 1B). Moreover, the B1 subpopulation of B cells, characterized by the expression of the surface marker CD5, is depleted in Btk-mutant mice (Khan et al., 1995; Rawlings et al., 1996). Btk -/- mice have a phenotype that cannot be distinguished from that of Xid mice (Kerner et al., 1995; Khan et al., 1995; Hendriks et al., 1996). Xid mice are characterised by a naturally spontaneous point mutation in the btk gene leading to an amino acid substitution within the PH domain (R28C) of the Btk protein. Additionally, the phenotype of Btk-mutant mice is similar to mice bearing a mutation of components of the B cell signalosome (p85α, SLP-65 [also called BLNK or BASH], and PLCγ2) (Kurosaki, 1999, 2000). Recently a function of Btk as a tumor suppressor gene was suggested and this function was independent of Btk kinase activity (Kersseboom et al., 2003). Although Btk deficient animals do not develop tumors, the incidence of tumor development is higher in SLP-65/Btk double deficient mice, than in SLP-65 single knock animals. Moreover, overexpression of a constitutive active Btk mutant E41K in SLP-65/Btk double deficient mice prevented tumor development.

Activation of Btk by the B cell receptor (BCR)

One of the initial events that occur in response to engagement of the BCR is the activation of non-receptor protein tyrosine kinases (PTKs) like Syk, Lyn and Fyn, and the phosphorylation of the intracellular sequences of the BCR, so called immunoreceptor tyrosine-based activation motifs (ITAMs), by these kinases (reviewed in (Kurosaki, 1999) and Figure 2A). This enables the cytoplasmic Src-family kinase Syk to bind to the phosphorylated ITAMs. Thereby Syk undergoes conformational changes leading to its catalytic

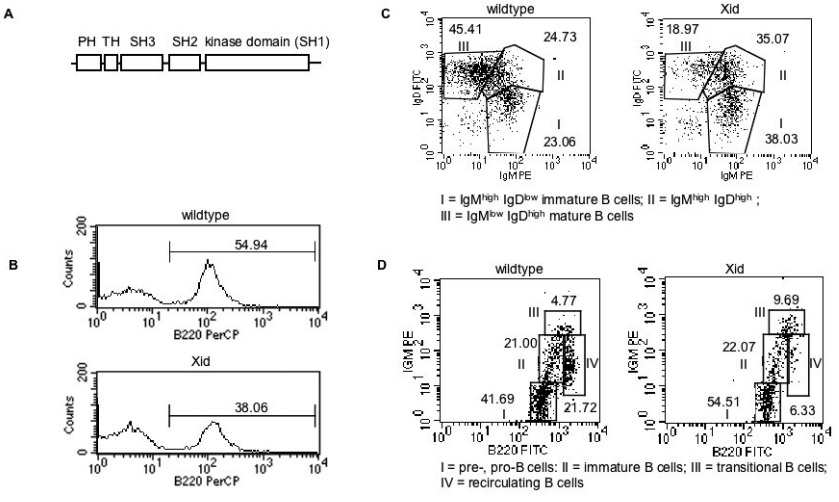

Fig. 1. The xid mutation severely affects B cell development. **A.** Domain structure of Btk. **B**. In Xid mice the overall B cell number is dramatically reduced. In the spleen of Xid mice not only the percentage of B220 positive cells is reduced, but also the splenic cellularity (data not shown) leading to a absolute reduction of splenic B cells to approximately 50%. **C.** Xid B cells exhibit an immature phenotype. Splenic lymphoid cells from wildtype and Xid mice were gated for B220+ cells and plotted for IgM and IgD. The percentage of cells within the indicated gates are given. **D.** B cell development in the bone marrow of Xid mice is affected. Bone marrow lymphocytes were gated for B220+ cells and plotted for IgM and B220. The compartment of pro- and pre-B cells is enlarged, whereas the recirculating from the periphery mature B cells are reduced resulting from an inefficient B cell maturation in secondary lymphoid organs.

activation. Syk plays a crucial role in the activation process of Btk, since in Syk -/- cells Btk activity is dramatically reduced (Kawakami et al., 2000a). Btk is phosphorylated by Src-kinases Lyn and/or Syk (Mahajan et al., 1995; Rawlings et al., 1996; Kurosaki and Kurosaki, 1997) at Y551 in the SH1 domain which increases dramatically Btk kinase activity (Mahajan et al., 1995; Rawlings et al., 1996). A second phosphorylation site is located in the SH3 domain in the Btk protein at Y223 which is autophosphorylated by Btk (Park et al., 1996). The phosphorylation on Y223 has only a minor role in Btk catalytic activity but is important for the protein binding of proline-rich sequences. Recent findings suggest that the presence of the adaptor/scaffold protein SLP-65, which binds to the Btk SH2 domain and is itself phosphorylated by Syk, is necessary for the Btk phosphorylation on Y551 by Src-family kinases (Baba et al., 2001) (Figure 1B).

Activated Syk is involved in tyrosine phosphorylation of adaptor proteins recruiting the PI3 kinase to the membrane. Activated PI3 kinase generates phosphatidylinositol-3,4,5-triphoshate (PIP3) that binds to and activates PH-domain containing proteins. The fact that the PI3 kinase p85α -/- mice have a similar phenotype to the Xid mice implies the important role of PI3 kinase in Btk function (Fruman et al., 1999; Suzuki et al., 1999). It is supposed that PI3 kinase products are necessary to recruit Btk to the plasma membrane. It was shown that the PH domain of Btk is sufficient to bind PIP3 selectively in vitro (Salim et al., 1996; Rameh et al., 1997). A mutation in the Btk gene causing *Xid* (R28C) abolishes the binding of Btk to inositol lipids in vitro (Salim et al., 1996; Rameh et al., 1997) and in vivo (Varnai et al., 1999) preventing Btk activation. Another

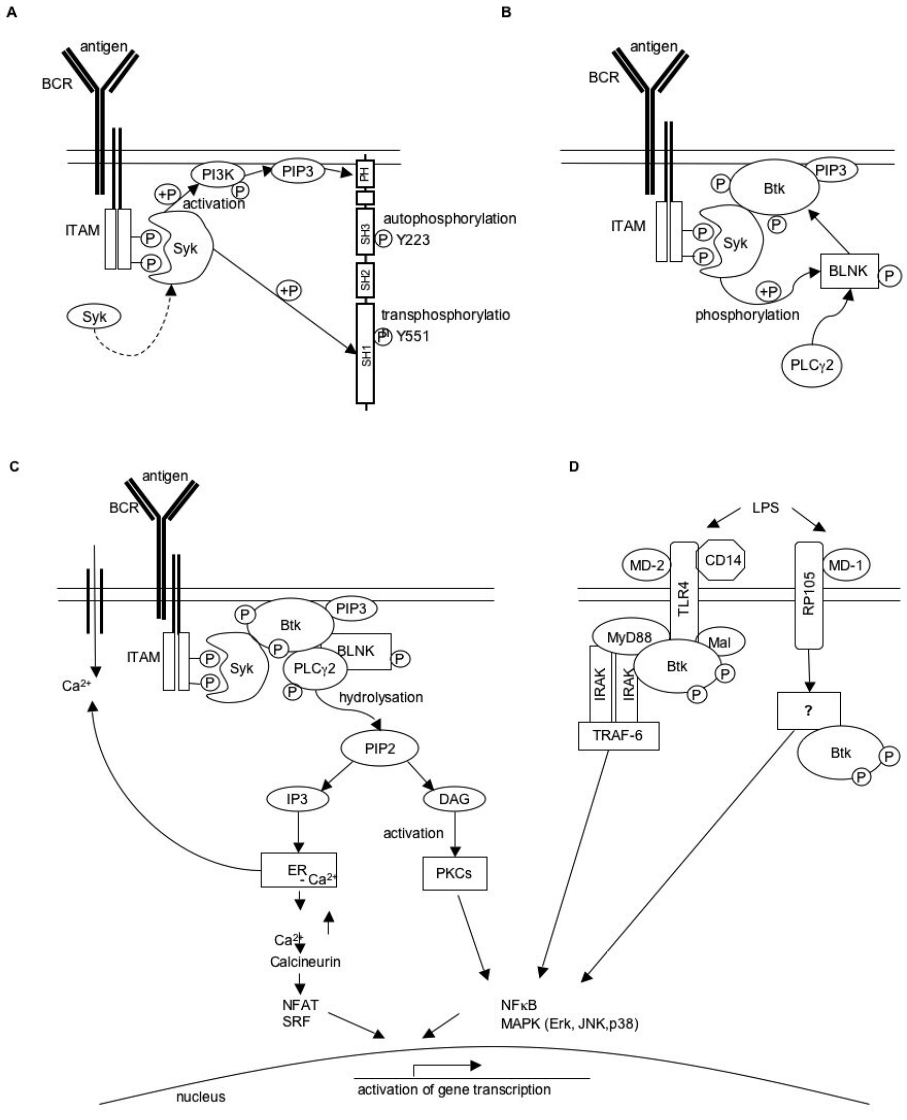

Fig. 2. Proposed model of Btk activation and Btk-dependent signalling induced upon B cell receptor (BCR) cross-linking. Details are described in the text.

mutation, E41K, also located in the PH domain, shows increased membrane association (Li et al., 1997; Varnai et al., 1999) accompanied by transforming capabilities as a result of a constitutive Btk activation (Li et al., 1997). These findings demonstrate that the binding of Btk to the membrane is mediated by its PH domain and this is critical for its kinase activity.

The phoshorylated scaffold protein termed B cell linker protein SLP-65 binds to membrane targeted Btk and provides docking sites for a variety of downstream Btk targets, like Grb2, Vav, Nck and PLCγ2. The protein complex composed of Btk, PLCγ2 and BLNK/SLP-65 facilitates Btk mediated PLCγ2 activation (Hayashi et al., 2000; Xu et al., 2000; Baba et al., 2001) by its direct phosphorylation at Y753 and Y759 (Kim et al., 2004) (Fig. 2C). Activated PLCγ2 hydrolyses PIP2 to produce IP3 and diacylglycerol (DAG). IP3 in turn activates Ca^{2+} mobilization from the intracellular stores by binding to the IP3 receptors of the endoplasmatic reticulum (ER) (Scharenberg and Kinet, 1998), whereas DAG activates protein kinase C (PKC) isoforms (Kurosaki, 2000). The resulting depletion of Ca^{2+} stored within the ER lumen drives the slow activation of store-operated channels in the plasma membrane, mediating the so called capacitative Ca^{2+} entry. Analyses of Btk mutants Y223F and Y551F showed that the transphosphorylation at Y551 by Src kinases is essential for calcium mobilization upon BCR engagement, whereas autophosphorylation at Y223 had no influence on this process (Kurosaki and Kurosaki, 1997). Recently, a second pathway of Btk mediated activation of PLCγ2 and PI3K was shown, involving phosphatidylinositol-4-phosphate 5 kinases (PIP5Ks), the enzymes that synthesize PtIns-4,5-P2 (PIP2) (Saito et al., 2003). In response to BCR activation Btk binds to PIP5Ks and shuttles them to the membrane to synthesize local PtIns-4,5-P2 – the substrate required by both the Btk activator PI3K and the Btk target PLCγ2.

Interestingly, it was shown that in vivo Btk autophosphorylation at Y223 is not required for Btk function, except for the regulation of λ light chain usage. Furthermore, during B cell development, Btk function is partially independent of its catalytic activity, probably by acting as an adapter molecule (Middendorp et al., 2003).

Downregulation of Btk activity

In contrast to Src-kinases, Btk kinase activity is not regulated by a carboxy-terminal negative regulatory domain, but by different cytoplasmic proteins. First, Sab (SH3 domain-binding protein that preferentially associates with Btk) was found to associate with Btk and thereby it downmodulates Btk kinase activity (Matsushita et al., 1998; Yamadori et al., 1999). Overexpression of Sab in B cells prevents Btk tyrosine-phosphorylation upon antigen receptor stimulation, leading consequently to a reduced InsP3 production and Ca^{2+} mobilization (Yamadori et al., 1999). More recently, in B cells another Btk interacting protein was identified interfering with Btk induced signalling processes – IBtk (inhibitor of Btk) (Liu et al., 2001). IBtk, a protein predominantly expressed in lymphoid tissues, interacts with the PH domain of Btk, downregulates its kinase activity, BCR-induced Ca^{2+} signalling and NF-κB activation. The molecular mechanisms by which these proteins interfere with Btk function are not clarified yet.

Additionally, PKCß was also shown to regulate Btk activity (Kang et al., 2001). PKCß phosphorylates a conserved serine within the Btk TH domain. Overexpression of PKCß leads to a progressive reduction of Btk phosphorylation at Y551 as well as at Y223 to basal levels. In contrast, inhibition of PKCß results in a dramatic increase of intracellular Ca^{2+} upon antigen receptor stimulation. In addition, in PKCß deficient B cells Btk is highly phosphorylated leading to an increased recruitment of Btk to the membrane (Kang et al., 2001). Thus, it was suggested that the reversible Btk translocation from the cytosol to the membrane is regulated by PKCß that in turn regulates the threshold of BCR signalling and B cell activation. Furthermore, Btk membrane localization can be inhibited by the 5'-inositol phosphatase SHIP1 that hydrolyses PIP3 (Bolland et al., 1998; Scharenberg et al., 1998), the ligand for the Btk PH domain required for Btk membrane targeting and function. SHIP1 is expressed specifically in cells of the hematopoietic system and was shown to be necessary for the downregulation of receptor mediated signalling by binding to the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) present in inhibitory receptors (reviewed in (Sly et al., 2003)). The SH2-containing tyrosine phospatase SHP-1 is also recruited to phosphorylated ITIMs, thereby it dephosphorylates Syk and Btk leading to a decrease of their kinase activities and subsequently to the inhibition of PLCγ2 mediated signalling (Maeda et al., 1999). Recently, an important phosphorylation site at Btk Y617 was identified. Phosphorylation at Btk Y617 selectively decreases the PLCγ2 dependent Ca^{2+} release upon BCR stimulation (Guo et al., 2004).

Btk activity is indirectly influenced by Cbl family proteins Cßl and Cßl-ß. Both proteins act as adaptor proteins and function as E3 ubiquitin ligases. Cßl targets the ubiquitination of Syk (Rao et al., 2001) and Lyn (Shao et al., 2004) and thereby inhibits the BCR-mediated PLCγ2 activation and Ca^{2+} release (Yasuda et al., 2000; Rao et al., 2001; Shao et al., 2004), since Syk and Lyn are necessary for Btk activation (Mahajan et al., 1995; Rawlings et al., 1996; Kurosaki and Kurosaki, 1997). In contrast, Cßl-ß acts as a scaffold protein positively on Btk-mediated PLCγ2 activation upon BCR stimulation (Yasuda et al., 2002).

Targets of Btk activation

Studies using the *Xid*-mouse model (Rawlings et al., 1993; Thomas et al., 1993) or btk gene targeted mice (Tsukada et al., 1993; Kerner et al., 1995; Khan et al.,

1995) revealed that Btk is involved in a wide array of signalling pathways that are essential for the maintenance of peripheral blood B cell numbers and for proliferation, survival and responsiveness of B cells.

One of such signalling cascades in which Btk is involved is the already discussed Ca^{2+} release pathway. Ca^{2+} elevation leads to the activation of the calmodulin-dependent protein kinase II and the calmodulin-activated serine/threonine phosphatase calcineurin. Calcineurin dephosphorylates NFAT transcription factors (Crabtree and Clipstone, 1994) and enables them to translocate into the nucleus where they activate gene transcription. In Btk deficient cells the NFAT-activity is reduced due to an inefficient activation of PLCγ2, preventing the elevation of Ca^{2+} upon BCR stimulation since the production of IP3 is reduced (Antony et al., 2003; Hao et al., 2003; Tomlinson et al., 2004). Like for NFAT in B cells, the same BCR induced signalling cascade, involving Lyn-Syk-Btk-PLCγ2 leading to Ca^{2+} release, activates the serum responsive factor SRF (Hao et al., 2003). SRF binds to the serum responsive element SRE found in the promoter region of many immediate early genes. The activation of PLCγ2 and the production of DAG are also involved in the activation of NF-κB in response to antigen receptor stimulation in B cells (Antony et al., 2003, 2004). Consequently, Btk deficient B cells show impaired NF-κB activation (Bajpai et al., 2000; Petro et al., 2000; Petro and Khan, 2001).

Another transcription factor – TFII-I – is also regulated by Btk (Sacristan et al., 2004). TFII-I is a ubiquitously expressed multifunctional transcription factor. In resting cells, TFII-I is retained in the cytosol by binding to Btk. Upon BCR cross-linking TFII-I becomes phosphorylated in a Btk independent manner, translocates into the nucleus and regulates gene transcription. Btk together with Syk was also described as a kinase essential for Akt activation in B cells (Craxton et al., 1999; Lindvall and Islam, 2002) and mast cells (Kitaura et al., 2000). Akt is a protein serine/threonine kinase that can be activated by a large array of surface receptors in different cell types, including the BCR. The cellular targets of Akt phosphorylation are involved in regulation of apoptosis, metabolism and gene transcription.

Several reports indicate that Btk is also involved in the activation of MAP kinases. Btk is required for the activation of ERK2 (Kurosaki, 1999), JNK, and p38 (Kawakami et al., 1997). MAP kinases are essential mediators of survival and apoptotic signals. Indeed, several studies indicate that Btk is involved in pro- and anti-apoptotic processes. Recently it was shown that the BCR induced upregulation of cyclin D2, responsible for G1 phase progression, is abolished in Btk and PI3K deficient primary B cells, leading to an impaired function of the cell cycle machinery and consequently to a higher apoptotic rate in response to BCR stimulation (Anderson et al., 1996; Glassford et al., 2003). The reduced expression of cyclin D2 possibly results from an impaired expression of STAT5. The STAT5 transcription factor is necessary for the activation of cyclin D2 promoter (Martino et al., 2001) and is itself a downstream target of Btk activity in B cells (Mahajan et al., 2001). In addition, Btk is required for the BCR induced NF-κB mediated upregulation of the anti-apoptotic protein Bcl-xL (Petro et al., 2002).

Additionally, it was shown that Btk is important for the control of BCR induced integrin α4ß1-mediated adhesion of B cells to VCAM-1 and fibronectin involving cytoskeletal reorganization and integrin clustering (Spaargaren et al., 2003), necessary for migration, recirculation and homing of B cells. For the controlled BCR induced inside-out signalling process leading to α4ß1 activation the successive activation of Lyn, Syk, PI3K, Btk, PLCg2, IP3 receptor mediated Ca^{2+} release and PKC are indispensable (Spaargaren et al., 2003).

Btk becomes activated by a large array of receptors

The BCR is not the only receptor, by which Btk is activated in B cells. Btk is able to interact with several receptors located on the B cell surface (Maas and Hendriks, 2001). Moreover, since Btk is not exclusively expressed in B cells but also in myeloid lineages, it can also interact with and be activated by other cell type specific receptors.

Btk has been shown to be activated by G-protein coupled receptors and binds to βγ subunits, as well as to Gαq and Gα12 subunits of the heterotrimeric G-proteins (Touhara et al., 1994; Tsukada et al., 1994; Langhans-Rajasekaran et al., 1995; Bence et al., 1997). This association increases the Btk kinase activity (Langhans-Rajasekaran et al., 1995). Since overexpression of Tec in NIH3T3 cells resulted in the activation of RhoA, a role of Btk in regulation of Rho GTPases was suggested (Mao et al., 1998).

Btk was also found to be activated and colocalized with actin fibers upon IgE receptor stimulation on mast cells (Yao et al., 1999). Therefore it was assumed that Btk is involved in the reorganization of the cytoskeleton upon receptor stimulation.

In addition, in DT40 chicken B cells and human B cell line NALM-6-UMI Btk is constitutively associated with Fas (Vassilev et al., 1999) – a member of the tumor necrosis factor (TNF) receptor family and a regulator of apoptosis in several cell types. The Fas-Btk interaction involves the PH domain of Btk and is independent of receptor activation (Vassilev et al., 1999). This suggests that in certain situations Btk modulates the susceptibility of the cell to Fas-mediated apotosis (Tumang et al., 2002).

Btk can also be activated by cytokines and their appropriate cytokine receptors. IL-5 receptor stimulation, necessary for proliferation and differentiation of B cells, leads to activation of Btk (Sato et al., 1994). Additionally in B cells, IL-6 was shown to activate Btk and Tec via Jak family kinases. Jak1 associates with Btk and directly phosphorylates it

(Takahashi-Tezuka et al., 1997).

Recently we were able to show that human Btk is phosphorylated and activated not only by BCR- but also by CD40-stimulation (Brunner et al., 2002a,b). In addition, the synergistic effect observed in response to BCR together with CD40 ligation was severely impaired by the expression of the Btk xid mutant (Haxhinasto and Bishop, 2004).

Furthermore, Btk deficient B cells show a reduced proliferation potential upon LPS stimulation (Khan et al., 1995; Klaus et al., 1997). LPS signals are recognized by TLR4 (Chow et al., 1999) and RP105 (Miyake et al., 2000; Ogata et al., 2000) on mouse B cells. We showed that Btk associates with the intracellular domains of several TLRs and that Btk activity is increased upon LPS induced TLR4 stimulation (Jefferies et al., 2003). Additionally, the importance of Btk for different signalling pathways of mast cells and macrophages, like degranulation, Ca^{2+} mobilization, NO and TNF-α production, which are induced by LPS or FcεR stimulation, was shown (Kitaura et al., 2000; Mukhopadhyay et al., 2002; Sada and Yamamura, 2003).

Btk and the innate immune system

The innate immune response in vertebrates is the first line of defence against invading microorganisms. The main players in innate immunity are phagocytes such as neutrophils, macrophages and dendritic cells. Until recently, the manner in which vertebrates respond to pathogens was obscure. It is now clear that a family of proteins, the Toll-like receptors (TLRs) (reviewed in (Iwasaki and Medzhitov, 2004)), contribute to the signal transduction induced by many pathogen-associated molecular patterns (PAMPs) – conserved motifs predominantly found in microorganisms but not in vertebrates. Stimulation of TLR causes an immediate defensive response, including the production of an array of antimicrobial peptides and cytokines.

Role of Btk in macrophages

Macrophages are large phagocytic mononuclear cells. Like DCs they develop from bone marrow derived myeloid precursor cells. Macrophages play an important role in innate immunity, as they recognize the foreign pathogen by a number of pathogen recognition receptors (PRRs) followed by phagocytosis. Macrophage effector functions lead to the production of inflammatory cytokines like TNF-α and to nitric oxide (NO) release. Since Btk is expressed in macrophages (Kaukonen et al., 1996; Weil et al., 1997) a function of Btk in those cells was suggested. Btk is activated upon LPS treatment of macrophages (Jefferies et al., 2003) leading to increased TNF-α secretion (Horwood et al., 2003). Xid mice-derived macrophages produce less NO (Mukhopadhyay et al., 1999a), TNF-α and IL1ß (Mukhopadhyay et al., 2002), but secrete higher amounts of IL-12 (Mukhopadhyay et al., 1999a). The reduced NO production correlates with the decrease of inducible NO-synthase expression in these cells (Mukhopadhyay et al., 1999a). The impaired macrophage function in Btk mutant mice could be one explanation for the delayed cure from nematode infections like microfilaria (Mukhopadhyay et al., 1999b).

Role of Btk in mast cells

Mast cells are particularly involved in the initiation of allergic reaction and chronic inflammatory processes. In addition, mast cells play an important role in host defence against bacterial pathogens which are recognized by their TLRs. Mast cells are mainly activated by cross-linking of the high affinity IgE receptor (FcεRI), leading to degranulation accompanied by release of mediators, like histamines and leukotrienes, and by cytokine secretion. Btk is expressed in mast cells and its expression is upregulated upon mast cell activation (Kawakami et al., 1994). Btk deficient mast cells develop normally (Hata et al., 1998a; Kawakami et al., 2000b). As in B cells, in mast cells Btk is involved in the regulation of JNK1, JNK2 and p38 (Kawakami et al., 1997). Since JNK activity is necessary for the induction of gene transcription of TNF-α, IL-2 (Hata et al., 1998b) and other cytokines via activation of the AP-1 transcription factor, Btk-deficient mast cells are consequently characterized by impaired proinflammatory cytokine secretion like IL-12, TNF-α and IL-6 (Hata et al., 1998a). In addition, in Btk-deficient mast cells phosphorylation of Akt (Kitaura et al., 2000), PKCß1 and PLCγ2 is impaired, therefore the generation of IP3 and intracellular Ca^{2+} elevation are reduced (Hata et al., 1998a; Kawakami et al., 2000b). Thus, these findings suggest an impaired FcεRI induced function of Btk-mutant mast cells.

Role of Btk in DCs

DCs are bone marrow derived leukocytes and constitute the most potent antigen presenting cells. Immature DCs are able to recognize and to capture foreign antigens. After maturation and migration to secondary lymphoid structures they present antigenic peptides on the relevant MHC molecules. DCs are the central players in activation of naïve $CD4^+$ T cells and drive them into TH1 or TH2 lineages. Although expression of Btk was detected in DCs (Gagliardi et al., 2003), so far no influence of Btk on DC differentiation, maturation and antigen presentation has been detected (Gagliardi et al., 2003). Since Btk is the only member of Tec family kinase detected in DCs a phenotype of Btk mutant DCs is expected. To elucidate finally the function of Btk in DCs more studies are required.

Btk and Toll-like Receptors

To achieve more insight into the function of Btk we had performed a Yeast-two-hybrid screen to search for

Btk interaction partners. Among several potentially interacting proteins we identified the receptor TLR8, a member of the TLR family (Jefferies et al., 2003). The interaction of TLR8 with Btk was analysed in more detail. We identified the TLR8-Btk interaction domain comprising the intracellular TIR-domain, which is common and highly conserved in all TLRs. Additional interaction studies revealed that Btk interacts not only with TLR8, but also with other members of the TLR-family, namely TLRs 4, 6, and 9. We demonstrated that Btk is a member of the multiprotein complex that is recruited to the TLR TIR domain (the TLR4 was studied) upon LPS stimulation. Btk does not only specifically interact with the TIR domain, but also with other proteins within the multiprotein complex, like Mal, MyD88 and IRAK (Jefferies et al., 2003) (Fig. 2D). Moreover, we were also able to show, that the overexpression of a dominant negative Btk mutation prevents LPS induced NF-κB activation (Jefferies et al., 2003).

TLRs are expressed mainly on mast cells, macrophages and dendritic cells, but also on B cells. They recognize microbial pathogenes and activate signalling pathways resulting in the induction of the immune response directed against the pathogen.

A second study using human Btk-deficient mononuclear cells from XLA patients also showed that Btk is involved in TLR signalling and responsible for LPS induced TNF-α production. Moreover, overexpression of Btk led to an increase of TNF-α production due to the stabilization of TNF-α mRNA via the 3' untranslated region (Horwood et al., 2003).

TLR on B cells

Several recent studies have suggested a role of TLRs in the stimulatory effects by PAMPs on human B cells (Krieg et al., 1995; Werling and Jungi, 2003). In a recent study the expression as well as the function of TLRs on human B cells has been extensively and systematically investigated (Bourke et al., 2003) showing that TLRs 1, 6, 7, 8, 9, and 10 are expressed on human B cells. On mouse B cells TLR4 is expressed and responsible for LPS signalling (Miyake et al., 2000; Ogata et al., 2000). The highest expression was observed for TLRs 9 and 10, and their expression was further increased in activated germinal center B cells. It is supposed, that in addition to their role as antibody-producing cells during the adaptive immune response, B cells may also respond to pathogens in a non-specific manner associated with the innate branch of the response. Two recent studies have directly explored the collaboration between TLR and the BCR in activating B cells (Leadbetter et al., 2002; Bernasconi et al., 2003). In these studies it was shown that stimulation of TLR9 by bacterial DNA potentiates BCR-induced B cell activation. In addition, TLR9 also cooperates together with the CD40 receptor to induce IL-12 secretion by activated B cells, which in turn resulted in an increased IFNγ production by in that way polarized TH1 cells (Wagner et al., 2004).

Another pathogen recognition receptor, namely RP105, is expressed on B cells and cooperates with TLR4 in recognition of LPS (Miyake et al., 2000; Ogata et al., 2000). Its extracellular domain is very similar to Drosophila Toll although its intracellular domain is without similarity to other TLRs. Its expression is largely restricted to B cells and macrophages (Miyake et al., 1995). Stimulation of B cells by LPS enhances their antigen-presenting capacity and is associated with increased B cell proliferation and antibody secretion (Kearney and Lawton, 1975; Oliver et al., 1999). As already mentioned, the response of Btk deficient B cells to LPS is impaired (Khan et al., 1995; Klaus et al., 1997). Stimulation of the RP105 receptor on murine B cells with anti-RP105 antibodies leads not only to increased proliferation of these cells, but also protects them from apoptosis. B cells from Xid mice failed to respond to RP105 stimulation indicating the involvement of Btk in RP105 dependent signalling (Miyake et al., 1994). Interestingly, like for BCR signalling activation, the requirement of an intact PI3K was also described for the LPS response of mouse B cells (Hebeis et al., 2004). Whether the PI3K activity induced by LPS treatment of B cells is necessary for Btk activation and membrane recruitment still needs to be elucidated. The molecular mechanisms leading to the activation of Btk via TLR triggering as well as the signal transduction pathways from the receptor into the cell initiated by an active Btk likewise remain to be resolved.

Taken together, these findings indicate a general role of Btk in innate and adaptive immunity signalling.

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