Regulation of B-lymphocyte activation by the PH domain adaptor protein Bam32/DAPP1

A.J. Marshall1, T. Zhang and M. Al-Alwan
Department of Immunology, University of Manitoba, 730 William Avenue, Winnipeg, MB, Canada, R3E 0W3

Abstract
PI3Ks (phosphoinositide 3-kinases) play critical roles in BCR (B-cell receptor) signalling via the generation of 3-phosphoinositide second messengers. Recruitment of PH domain (pleckstrin homology domain)-containing signal transduction proteins to the plasma membrane through binding to 3-phosphoinositide second messengers represents a major effector mechanism for PI3Ks. Here, we review data on the PH domain-containing adaptor protein Bam32 (B-cell adaptor molecule of 32 kDa)/DAPP1 (dual adaptor for phosphotyrosine and 3-phosphoinositides 1), focusing on its functions in B-lymphocyte activation. Present results support the view that Bam32/DAPP1 mediates multiple PI3K-dependent responses in B-cells through membrane-proximal mechanisms involving Src kinases, Rac1, F-actin and mitogen-activated protein kinases, resulting in selective effects on BCR-mediated proliferation, antigen presentation and generation of antibody responses.

Bam32/DAPP1 translocation to the plasma membrane
Bam32 (B-cell adaptor molecule of 32 kDa)/DAPP1 (dual adaptor for phosphotyrosine and 3-phosphoinositides 1) is a 32 kDa signalling protein possessing an N-terminal SH2 (src homology 2) domain and a single centrally located tyrosine phosphorylation site (Tyr139) in addition to its C-terminal PH domain (pleckstrin homology domain) [1,2].

In vitro binding studies indicated that the PH domain can bind with high affinity to PI(3,4,5)P3 (phosphatidylinositol 3,4,5-trisphosphate) and its 5-dephosphorylation product PI(3,4)P2 (phosphatidylinositol 3,4-bisphosphate) [1]. Competitive binding studies suggest that the Bam32 PH domain may preferentially bind to PI(3,4)P2 over PI(3,4,5)P3 [3].

Consistent with the in vitro lipid-binding characteristics of Bam32, its positively charged phosphoinositide-binding pocket differs from that of selective PI(3,4,5)P3 binders such as Grp1 and Btk, in that it forms more hydrogen bonds with the 4-phosphate of the lipid headgroup and no hydrogen bonds with the 5-phosphate [3].

Experiments using EGFP (enhanced green fluorescent protein)-tagged Bam32/DAPP1 found that Bam32 translocates to the plasma membrane of B-lymphoma cells upon BCR (B-cell receptor) cross-linking [2]. This translocation is PI3K (phosphoinositide 3-kinase)-dependent and PH domain-mediated. Similar translocation was observed in PDGF (platelet-derived growth factor)-treated endothelial cells and to some extent in DT40 chicken B-cells [4]. In the BJAB human B-lymphoma model, BCR-driven translocation of Bam32 is a relatively slow and sustained response compared with translocation of Btk PH domain, which is rapid and transient [5,6]. In the BJAB model, Bam32 recruitment kinetics correlate well with PI(3,4)P2 production, while Btk recruitment correlates with PI(3,4,5)P3 production [6]. Lastly, overexpression of the lipid phosphatase SHIP (SH2-containing inositol phosphatase), which dephosphorylates PI(3,4,5)P3 to produce PI(3,4)P2 [7,8], was found to abolish membrane recruitment of the Btk PH domain, while enhancing the recruitment of Bam32 [5], suggesting that PI(3,4)P2 may have a dominant role in driving Bam32 translocation.

Bam32/DAPP1 phosphorylation and signalling
Bam32 is phosphorylated upon BCR cross-linking or pervanadate stimulation of B-cells [2], PDGF stimulation of endothelial cells [4], insulin, IGF1 (insulin-like growth factor 1) or EGF (epidermal growth factor) stimulation of HEK-293 cells (human embryonic kidney cells) [9] or when co-expressed with Src-family kinases [10]. Tyr139 appears to be the only phosphorylation site [9]. Phosphorylation in response to EGF or BCR stimulation is blocked by inhibitors of Src-family kinases [9,11]. Bam32 phosphorylation requires PI3K activation and PH domain-mediated membrane recruitment [4,9]. These studies in different cell types and systems largely concur with the model that PH domain-mediated membrane recruitment facilitates Bam32 phosphorylation at Tyr139 by Src family kinases.

To date, the strongest evidence for Bam32 function in signal transduction comes from studies on antigen receptor signalling in B-lymphocytes. Bam32-deficient B-cells show...
reduced BCR-induced proliferation, whereas proliferation induced by a variety of other stimuli is not impaired [12,13]. The major signalling deficiency identified in both Bam32-deficient mouse B-cells and DT40 chicken B-cells, is reduced BCR-induced activation of MAPK (mitogen-activated protein kinase) pathways [12,14]. Bam32-deficient cells show reduced activation of JNK (c-Jun N-terminal kinase) and ERK (extracellular-signal-regulated kinase), and we have also found that JNK activation is impaired in BJAB cells overexpressing Y139F mutant Bam32 [15]. Significantly, BCR-induced activation of upstream MAP2Ks and MAP3Ks, such as MEK1/2 (MAPK/ERK kinases 1/2) and MEKK1 (MEK kinase 1), were also reduced in Bam32-deficient mouse cells. This suggests that Bam32 affects MAPK pathways at a receptor-proximal level, by directly or indirectly influencing the activation of MAP4Ks.

Bam32/DAPP1 and the cytoskeleton
Several observations suggest a link between Bam32/DAPP1, cytoskeletal rearrangement and receptor trafficking. During PDGF stimulation of endothelial cells, Bam32 is recruited to the membrane and subsequently accumulates in intracellular vesicles, many of which coincide with internalized PDGF receptors [4]. In the BJAB model, Bam32 associates with filamentous-actin-rich membrane ruffles [6]. At later time points after BCR cross-linking, Bam32 becomes polarized on the cell surface and associates with BCR patches and caps, as well as internalized BCR aggregates [11]. Overexpression of wild-type Bam32 in BJAB cells enhanced both F-actin formation and Rac1 activity, while Y139F mutant Bam32 inhibited both F-actin formation and Rac1 activity [15], suggesting that Bam32 phosphorylation is key for this functional activity. Rac1 activation is also reduced in Bam32-deficient DT40 cells [15] and Bam32-deficient murine B-cells (S. Hon and A.J. Marshall, unpublished work). In addition to its roles in actin remodelling, Rac1 is known to play an important role in activation of JNK; thus it is possible that Bam32 regulates both actin remodelling and JNK activation through Rac1.

The co-localization of Bam32 with BCR during antigen-induced capping and internalization prompted studies to assess whether this molecule regulates internalization, trafficking and presentation of antigens via the BCR. Bam32-deficient DT40 cells or BJAB cells overexpressing Y139F mutant Bam32 showed delayed kinetics of BCR internalization, suggesting that Bam32 has a functional role in rapid BCR-mediated Ag uptake [11]. However, in recent work with Bam32-deficient murine splenic B-cells we have not seen impairment in BCR internalization, suggesting that the regulatory role of Bam32 depends on the cellular context. We did, however, find that Bam32 is required for efficient BCR-mediated antigen presentation, suggesting that Bam32 may regulate post-internalization trafficking of BCR-antigen complexes (M. Al-Alwan, T. Zhang and A. Marshall, unpublished work). In parallel studies, we found that PI3K signalling is not required for BCR internalization, but is required for efficient BCR-mediated antigen presentation by primary murine B-cells [16], consistent with a function for 3-phosphoinositide-dependent signalling complexes in B-cell antigen presentation.

Bam32/DAPP1 functions in generation of antibody responses
Bam32-deficient mice were found to generate markedly reduced antibody responses to TI-2 (T-cell-independent type II) antigens [12,13] consistent with the defect in BCR-induced proliferation reported in these studies. In contrast, these mice appeared to mount normal antibody responses when immunized with T-cell-dependent antigens. We have recently re-examined antibody responses in these mice, focusing on aspects thought to be highly dependent on B-cell antigen presentation to T-cells. We found that Bam32 is required for maintenance of germinal centre responses following immunization with the T-dependent antigen NP-OVA (T. Zhang, M. Al-Alwan and A. Marshall, unpublished work). While overall levels of antigen-binding IgG antibody generated are relatively normal, consistent with previous reports, we found selective deficits in production of switched immunoglobulin isotypes, as well as impaired antibody affinity maturation. These defects may be partly accounted for by our observation that Bam32-deficient B-cells have impaired BCR-mediated antigen presentation capability.

References

Received 30 November 2006