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Regulation of phosphoinositide 3-kinase signaling by oxidants: Hydrogen peroxide selectively enhances immunoreceptor-induced recruitment of phosphatidylinositol (3,4) bisphosphate-binding PH domain proteins

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Abstract

Phosphoinositide 3-kinases (PI3Ks) generate several distinct lipid second messengers including phosphatidylinositol (3,4,5) trisphosphate (PIP3) and phosphatidylinositol (3,4) bisphosphate PI(3,4)P2. PI(3,4)P2 is produced with distinct kinetics and binds to distinct PH domain effector proteins; however, the regulation of this signaling pathway is poorly understood. Superoxides such as hydrogen peroxide are transiently produced after activation through various cell surface receptors and play important roles in immune and inflammatory responses. Here we use quantitative microscopy to examine the effect of peroxide on PI(3,4)P2-mediated mobilization of signaling proteins in B lymphocytes. Peroxide was found to induce dose-dependant membrane recruitment of the PI(3,4)P2-binding PH domain proteins Bam32, TAPP2 and Akt/PKB but not the PIP3-binding PH domain of Btk. Peroxide-induced membrane recruitment was found to be dependant on PI3K activity, with the p110δ isoform contributing much of the activity in the BJAB human B lymphoma model. Strikingly, peroxide co-stimulation enhanced antigen receptor-induced membrane recruitment of Bam32 and TAPP2, with combined stimulation exceeding the maximum achievable with either stimulus alone. Expression of the lipid phosphatase PTEN led to reduction of antigen receptor-induced membrane recruitment of TAPP2; however, peroxide costimulation could overcome the inhibitory effect of PTEN. Inhibition of the NADPH oxidase led to reduction of antigen receptor-induced membrane recruitment of TAPP2. Our results indicate that exogenous and endogenous superoxides can modulate the quality of the PI3K signal in lymphocytes by selectively increasing PI(3,4)P2-dependant signaling.

Keywords: Phosphoinositide 3-kinase; Lymphocyte; Immunoreceptor; Peroxide; PH domain; TAPP2; PTEN

1. Introduction

Phosphatidylinositol 3-kinases (PI3K) are a family of signaling enzymes activated by a variety of extracellular stimuli. PI3Ks catalyze the phosphorylation of membrane inositol lipids to generate several distinct membrane-bound second messengers [1,2]. PI3Ks have emerged as critical regulators of immunoreceptor, chemokine and cytokine signaling [3–7]. The signaling mechanisms triggered by active PI3Ks are not fully understood, but one established mechanism involves recruitment of PH domain-containing signaling proteins to membranes via their binding to specific lipid products of PI3Ks [8–10].

PI3Ks generate several distinct lipid second messengers including phosphatidylinositol (3,4,5) trisphosphate (PIP3) and phosphatidylinositol (3,4) bisphosphate (PI(3,4)P2), which are differentially regulated at the level of lipid substrate availability and specific lipid phosphatases. PIP3 is known to regulate membrane targeting and activation of Tec-family tyrosine kinases [11,12] and the serine-threonine kinase Akt/PKB [13,14]. The lipid phosphatase SHIP, a well documented regulator of immunoreceptor and cytokine receptor signaling [15], removes the D5 phosphate from PIP3, producing PI(3,4)P2. In lymphocytes, PI(3,4)P2 is known to be produced after B cell antigen receptor (BCR) stimulation [16]; however, its functional roles are not

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completely defined. There is evidence that PI(3,4)P2 is important for Akt/PKB activation [17,18]; however the relative contributions of PIP3 and PI(3,4)P2 to Akt/PKB activation remains controversial. Akt/PKB is known to phosphorylate key substrates in cascades promoting cell survival and metabolic activity [19,20] and is thought to be important for B lymphocyte activation via BCR or cytokines [13,21,22]. We and others have characterized Bam32/DAPP1, an adaptor protein that, like Akt, binds strongly to both PIP3 and PI(3,4)P2 in vitro via its PH domain [23–25]. The PH domain-dependant recruitment of Bam32 to the plasma membrane of B cells correlates with PI(3,4)P2 rather than PIP3 production [26,27]. Expression of Bam32/DAPP1 is restricted to hemopoieitic cells, and this adaptor is known to have indispensable functions in B cell activation, including linking BCR to MAPK activation, Rac1 activation and actin polymerization [28–31]. Thus, current evidence indicates that PI(3,4)P2 has signaling functions in lymphocytes by modulating localization and/or activity of Bam32 and Akt.

We and others have characterized Bam32/DAPP1-related adaptor proteins TAPP1 and TAPP2 which specifically bind PI(3,4)P2 [27,32,33], suggesting that this lipid second messenger may mediate additional unique signaling functions. TAPP1 and TAPP2 are broadly expressed; however, TAPP2 is more abundantly expressed in hematopoeitic cells. Structurally, the TAPPs are very highly homologous to one another within their N-terminal "TAPP-conserved" region, and each contains two PH domains and a C-terminal PDZ-binding motif [34]. The C-terminal PH domains were shown to bind specifically to PI(3,4)P2, while the N-terminal domains showed no specific high affinity binding to phosphoinositides [32]. TAPP1 was shown to bind to multi-PDZ domain proteins MUPP1 and PTPL1 in HEK293 cells [35,36]. TAPP1 was also recently found to associate with the PDZ domains of multiple syntrophin isoforms, and co-localized with γ1-syntrophin in membrane ruffles of PDGF-stimulated NIH-3T3 cells [37]. TAPP1 over-expression inhibited formation of circular membrane ruffles and this blockade was reversed by co-expression of syntrophins [37], suggesting a functional interaction regulating the cytoskeleton. Thus, present data indicate that Bam32/DAPP1 and the TAPPs constitute a related set of PI(3,4)P2 effector proteins involved in cytoskeletal reorganization impacting on signaling.

Recent evidence suggests that reactive oxygen species (ROS) are a major component of immunoreceptor signal amplification [38]. Production of ROS through the NADPH oxidase system is triggered by the activation of cell surface receptors on phagocytic cells, lymphocytes and other cell types [39]. B lymphocytes are known to express particularly high levels of NADPH oxidase activity [40,41], exceeded only by professional phagocytes such as macrophages and neutrophils. NADPH oxidase activity is triggered by multiple receptors in B lymphocytes, including BCR and TNF-superfamily receptors such as CD40 and Fas [42,43]. Activated NADPH oxidase produces superoxide anions, which are rapidly converted to hydrogen peroxide through the action of superoxide dismutases. Generation of ROS through NADPH oxidase is critical for promoting inflammation and intracellular killing of microbes by phagocytic cells [44]. In addition, a growing body of evidence implicates hydrogen peroxide and other ROS as second messengers activating signaling pathways intracellularly [45–47]; however, their precise roles in immune cell signaling pathways are not well defined. Hydrogen peroxide is particularly well suited as an intracellular and intercellular second messenger since it can freely diffuse through membranes to affect adjacent cells, and its elimination is specifically controlled through peroxiredoxins and catalase enzymes [48].

Here we have investigated the effect of hydrogen peroxide on membrane recruitment of PIP3- and PI(3,4)P2-specific PH domain effector proteins in B lymphocytes. We provide evidence that peroxide preferentially triggers recruitment of PI(3,4)P2-binding effectors Bam32 and TAPP2. Moreover, peroxide can synergize with immunoreceptor signaling to markedly enhance this component of PI3K signaling, and can overcome PTEN-mediated inhibition of this response. We further provide evidence that endogenous superoxide production amplifies the recruitment of PI(3,4)P2 effectors. Our results provide evidence that peroxide can potently costimulate a subset of PI3K-dependant intracellular signaling events in lymphocytes.

2. Materials and methods

2.1. Reagents and constructs

Plasmids encoding full length Bam32, full length TAPP2, or the C-terminal PH domain of TAPP2 fused to enhanced green fluorescent protein (EGFP) were generated as described previously [25,27]. Constructs encoding the Btk PH domain fused to the red fluorescent protein DsRed2 was generated by subcloning from our previously described Btk PH-EGFP-C1 vector [27] into pDsRed2-C1 (Clontech, Franklin Lakes, NJ). FLAG-PTEN (gift of Dr. J. Dixon, University of California, San Diego) was subcloned into the KpnI and BamHI sites of pcDNA3.1 vector (Invitrogen). Akt-PH-EGFP construct was a gift of Dr. D. Cantrell, University of Dundee [13]. Goat anti-human IgM $F(ab')_2$ and rabbit anti-mouse IgG $F(ab')_2$ stimulating antibodies (Jackson ImmunoResearch, West Grove, PA) were used at a final concentration of 10 μg/ml unless otherwise indicated. Hydrogen peroxide (Sigma-Aldrich, St. Louis, MO) was used at the indicated concentrations. Inhibitors used are as follows: wortmannin (50 ng/ml; Sigma), LY294002 (50 μM; Biomol, Plymouth Meeting, Pa), IC87114 (10–90 μM; ICOS, Bothell, WA), diphenyleneiodonium (15 μM; Sigma), apocynin (acetovanillone; Sigma). Cells were pretreated with the indicated inhibitors or vehicle (DMSO) for 30–60 min at 37 °C prior to stimulation.

2.2. Cell cultures and transfections

BJAB and A20 B lymphoma cells were cultured in RPMI 1640 medium (Invitrogen, Burlington, ON) containing 10% FCS and antibiotics. BJAB is a EBV-negative, IgM+human Burkitt's lymphoma that is a widely used model for lymphocyte signaling and studies of EBV infection [49,50]. The A20 line is a chemically induced murine B lymphoma, that has been widely used in studies of B cell signaling, antigen presentation, chemotaxis and lymphomagenesis [51,52]. Transfection was carried out with ElectroSquarePorator ECM 830 (BTX, San Diego, LA). 10 million cells were resuspended in 0.4 ml cold RPMI medium with 15 μg plasmid construct for 5 min in a GenePulser Cuvette (Bio-Rad, Hercules, CA) with 0.4 cm electrode gap. For BJAB cells, transfection voltage was set at 310 V using a single 10 ms pulse. For A20 cells, transfection voltage was set at 300 V using five 3 ms pulses with a 1 s pulse interval. The cuvettes were then placed on ice to incubate further for 10 min before transferring to RPMI 1640 medium containing 1% FCS and culturing overnight. For PTEN and PTEN phosphatase-dead stable transfections, 20 μg of linearized vector was electroporated as above. Cells were then selected with 2 mg/ml G418 and cloned to generate stable transfectants.