

**P040** Regulation of PI3K signaling by oxidants: hydrogen peroxide selectively enhances immunoreceptor-induced recruitment of phosphatidylinositol (3,4) bisphosphate-binding PH domain proteins in lymphocytes

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Phosphoinositide 3-kinases (PI3Ks) generate several distinct lipid messengers including PI(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>) and PI(3,4)P<sub>2</sub>. PI(3,4)P<sub>2</sub> is produced with distinct kinetics and binds to distinct PH domain effector proteins. Superoxides such as hydrogen peroxide are produced during cellular activation and function in immune and inflammatory responses. We used quantitative microscopy to examine the effect of peroxide on PI(3,4)P<sub>2</sub>-mediated PH domain mobilization in B lymphocytes. Peroxide induces membrane recruitment of the PI(3,4)P<sub>2</sub>-binding PH domain proteins Bam32 and TAPP2, but not the PIP<sub>3</sub>-binding PH domain of Btk. Peroxide-induced membrane recruitment is dependant on PI3K activity, with the p110 $\delta$  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 co-stimulation 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)P<sub>2</sub>-dependant signaling.