

**P023** Novel G protein-independent activation of PI3K $\gamma$  via Ca<sup>2+</sup> and PKC regulates allergy

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Mast cells control allergy and inflammation through the release of histamine, cytokines and lipid mediators. During antigen/IgE stimulation, phosphoinositide 3-kinase  $\gamma$  (PI3K $\gamma$ ) relays paracrine and autocrine inflammatory signals and thus plays a central role in mast cell function<sup>1</sup>. Quantitatively, half of the PI3K $\gamma$ -dependent activation is mediated by adenosine and other G<sub>i</sub>-protein coupled receptor (GPCR) ligands. The other half is controlled by a novel, Ca<sup>2+</sup>-dependent pathway, which can be triggered separately by Ca<sup>2+</sup>-ionophores or thapsigargin and is resistant to *B. pertussis* toxin and adenosine deaminase. This, and the fact that also phorbol ester (PMA) triggered protein kinase B (PKB/Akt) phosphorylation in a PI3K $\gamma$ -dependent way, suggested that diacylglycerol-binding molecules could be involved. As such, guanine nucleotide exchange factor RasGRP4 activates Ras in mast cells<sup>2</sup>. In 32D cells expressing RasGRP4, however, PI3K $\gamma$ /PKB was not activated by PMA, while MAPK responded. Using a panel of protein kinase C (PKC) inhibitors we could dissect classical and novel PKC members, which are upstream of PI3K $\gamma$ . PI3K $\gamma$  thus integrates signals from GPCRs via G protein  $\beta\gamma$  subunits and relays a Ca<sup>2+</sup>-triggered, PKC-dependent pathway, which plays an important role in allergen-mediated mast cell stimulation.

<sup>1</sup>Laffargue et al. (2002) "Phosphoinositide 3-Kinase  $\gamma$  is an Essential Amplifier of Mast Cell Function". *Immunity*, 16:441.

<sup>2</sup>Yang et al. (2002) "RasGRP4, a new mast cell-restricted Ras guanine nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs..." *J. Biol. Chem.* 277:25756.