PI3K comes of age

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The first ever meeting focusing exclusively on phosphatidylinositol-3 kinases (PI3Ks) in the immune system was held at the Novartis Research Centre in Horsham, UK, from 8th to 10th September 2002. Highlights of the meeting centered on new insights into the regulation and functional importance of distinct PI3K isoforms in adaptive and innate immunity. The lipid phosphatase SHIP, which tightly regulates the phosphoinositol lipid products of PI3K and downstream effector proteins that interact with PI3K-generated lipids, were also topics of hot debate.

Pharmacological and genetic experiments have established the importance of PI3Ks in lymphocytes but there has always been some doubt concerning whether TCR ligation under physiological conditions really activates PI3Ks. Doreen Cantrell (Dundee, UK) used confocal imaging to track, in real time, a lipid product of PI3K activity during T cell–APC interactions. This approach, which has recently been published in *Nature Immunology*, revealed PI3K activation predominantly (but not exclusively) at the contact zone between the T cell and APC within seconds of contact[1]. Despite rapid turnover, lipid concentrations were maintained at the contact zone, reflecting sustained PI3K activation. Pharmacological intervention with a PI3K inhibitor revealed that this sustained PI3K activation was essential for T cell proliferation.

The catalytic subunit isoforms p110γ and p110δ interest immunologists greatly because their expression is largely restricted to leukocytes. Isoforms of the Rac-specific guanine nucleotide exchange protein P-Rex, which selectively binds the lipid products of PI3K, are also predominantly expressed in peripheral blood leukocytes and lymphoid tissue, reinforcing the belief that PI3K and its downstream effectors are crucial to the immune system (Phil Hawkins, Babraham, UK)[2]. In an exciting adjunct to this idea, Bart Vanhaesebroeck (London, UK) described an innovative approach to generating mice lacking functional p110δ. The wild-type allele was replaced by knocking-in an allele encoding a kinase-dead mutant of p110δ. Mice homozygous for kinase-dead p110δ were viable and fertile, yet showed impaired B and T cell development. Both T and B cell antigen-receptor signaling was also impaired in p110δ kinase-dead lymphocytes[3]. A p110δ isoform–specific inhibitor would be expected to have a similar effect. Accordingly, several pharmaceutical companies have shown interest in this area and Joel Hayflick (Bothell, USA) described the first p110δ selective inhibitor. Although no data on lymphocyte activation was presented, he reported that this compound reduced neutrophil production of PI3K lipid products in response to the potent chemoattractant peptide formylmethionylleucylphenylalanine (fMLP), whose actions are mediated through a G protein–coupled receptor (GPCR). Importantly, the p110δ-selective inhibitor decreased fMLP-induced neutrophil chemotaxis *in vitro* and *in vivo*. This was rather surprising given that Matthias Wymann (Fribourg, Switzerland) presented some conflicting data from his p110γ-deficient mice, which suggested that the G protein βγ subunit–dependent p110γ catalytic isoform is the sole contributor to lipid accumulation in response to several different ligands that activate distinct GPCRs[4]. However, given the effects reported by Hayflick it appears that p110δ can also participate in GPCR signaling, indicating that some functional redundancy may exist between the two isoforms. Of interest, in this regard, was the report by Matthew Thomas (Horsham, UK), who demonstrated LPS-induced neutrophil influx into the lung was impaired, but not completely abrogated, in p110γ-deficient mice, supporting a role for other PI3K isoforms in leukocyte migration.

The role of PI3Ks in regulating innate immunity was also highlighted. Gerry Krystal (Vancouver, Canada) revealed that macrophages from SHIP-1–deficient mice overexpress arginase I, resulting in decreased NO production, which is a feature reminiscent of M2 suppressive macrophages. The apparent conversion of classical M1 'killing' macrophages to M2 'healing' macrophages may be an attempt to resolve the chronic inflammation resulting from sustained elevation of the phosphatidylinositol(3,4,5)trisphosphate (a major product of PI3K activity) in SHIP-1$^{-/-}$ mice. Thus, PI3K signaling may also regulate macrophage responses.

There were also cautionary tales on the assessment and quantitation of PI3K activation and insights into new high-throughput PI3K assays. Phosphorylation of Ser473 on the PI3K effector molecule protein kinase B (PKB) is the most commonly used method to detect PI3K activation. However, Brian Hemmings (Basel, Switzerland) revealed that other routes, independent of the phosphoinositol lipids formed by PI3K, could lead to PKB phosphorylation on Ser473. He suggested that detection of PKB phosphorylation on Thr308 could therefore be a better marker of PI3K activation because it is solely dependent on the phospholipid products of PI3K. Pete Downes (Dundee, UK) described a FRET-based assay that in the long-term may provide a user-friendly, high-throughput assay for measuring PI3K activation. This technological advance will be particularly helpful for reliably quantifying PI3K activity, which has, to date, been laborious and technically challenging.

Overall, the meeting established the varied and important roles for PI3Ks in adaptive and innate immunity. The challenge to the field now is to understand the roles of specific isoforms in greater detail and determine where redundancy and cross-talk exists. This is essential if the full therapeutic potential of isoform-selective PI3K inhibitors is to be realized.

1. Costello, P. *et al. Nat. Immunol.* **3**, 1082–1089 (2002).
2. Welch, H. *et al. Cell* **108**, 809–821 (2002).
3. Okkenhaug, K. *et al. Science* **297**, 1031–1034 (2002).
4. Laffargue, M. *et al. Immunity* **16**, 441–451 (2002).