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## PI3K $\delta$ and primary immunodeficiencies

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### Abstract

Primary immunodeficiencies are inherited disorders of the immune system, often caused by the mutation of genes required for lymphocyte development and activation. Recently, several studies have identified gain-of-function mutations in the phosphoinositide 3-kinase (PI3K) genes *PIK3CD* (which encodes p110 $\delta$ ) and *PIK3R1* (which encodes p85 $\alpha$ ) that cause a combined immunodeficiency syndrome, referred to as activated PI3K $\delta$  syndrome (APDS) or p110 $\delta$ -activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency (PASLI). Paradoxically, both loss-of-function and gain-of-function mutations that affect these genes lead to immunosuppression, albeit via different mechanisms. Here, we review the roles of PI3K $\delta$  in adaptive immunity, describe the clinical manifestations and mechanisms of disease in APDS and highlight new insights into PI3K $\delta$  gleaned from these patients, as well as implications of these findings for clinical therapy.

## Introduction

Activated PI3K $\delta$  syndrome (APDS; also known as PASLI) is among a growing number of newly defined primary immunodeficiency (PID) syndromes in which the causal mutations have been identified by next-generation sequencing. The clinical manifestations of APDS are diverse and heterogeneous (**Box 1**), but the majority of patients present with recurrent respiratory infections, often associated with airway scarring (bronchiectasis) and ear and sinus damage, which is suggestive of antibody (B cell) deficiency. Severe, recurrent or persistent infections with herpes family viruses, indicating defective T cell function, are also common in this condition, and may cause early death in some affected individuals. Many patients develop benign lymphadenopathy, often associated with hepatosplenomegaly, and there is a substantially increased risk of B cell lymphoma associated with APDS (**Box 1**). Increased susceptibility to viral infection and poor recall responses of memory T cells differentiate APDS from isolated hypogammaglobulinemia<sup>1-4</sup>, hence APDS should be considered a combined immunodeficiency<sup>5</sup>. More than 100 patients have been reported to date with APDS, but the precise incidence is not yet known<sup>6,7</sup>.

APDS is caused by heterozygous gain-of-function (GOF) mutations in *PIK3CD* or *PIK3R1* that induce hyperactivation of the protein products p110 $\delta$  or p85 $\alpha$ , respectively<sup>1-4</sup>. The p85 $\alpha$  regulatory subunit and p110 $\delta$  catalytic subunit together form the heterodimeric lipid kinase PI3K $\delta$ , which is engaged by multiple receptors in cells of the immune system, including the B cell receptor (BCR) and the T cell receptor (TCR), as well as cytokine and costimulatory receptors. Homozygous loss-of-function (LOF) mutations in these same subunits cause a distinct and much rarer form of immunodeficiency in humans, which can be re-capitulated in mice<sup>8-10</sup>, and this apparent dichotomy, together with the clinical features of the affected patient groups, has informed our understanding of the role of PI3K $\delta$  in immune cell development and function.

In this review, we will summarise what is known about PI3K $\delta$ , focusing on its regulation of adaptive immune responses. Much of this knowledge derives from studies using gene-targeted mice. We will then summarise the two cases that have been reported on PI3K $\delta$ -deficiency in humans, before describing in greater detail the clinical and immunological manifestations of APDS.

## Overview of class I PI3Ks

The class IA PI3Ks are heterodimeric proteins composed of (and named after) a p110 $\alpha$ , p110 $\beta$  or p110 $\delta$  catalytic subunit that constitutively associates with a p85 regulatory subunit; the sole class IB PI3K is composed of the p110 $\gamma$  catalytic subunit that interacts with a p101 or p84 regulatory subunit (**Table 1**). p110 $\alpha$  and p110 $\beta$  are broadly expressed, whereas p110 $\gamma$  and p110 $\delta$  are predominantly expressed by leukocytes. Although there is substantial potential for redundancy among the catalytic

subunits, unique roles for each individual p110 isoform have been described, reflecting their different expression patterns as well as how they are engaged by their respective receptors<sup>8, 11</sup>. For example, p110 $\alpha$  is activated by insulin-like receptors and regulates growth, metabolism and angiogenesis<sup>11</sup>, whereas p110 $\beta$  contributes to metabolic signalling and has been shown to regulate responses of mouse neutrophils to immune complexes<sup>12, 13</sup>. P110 $\gamma$  is highly expressed in myeloid cells and contributes to chemotactic responses, as well as reactive oxygen species (ROS) production in neutrophils<sup>14</sup>. Together with p110 $\delta$ , p110 $\gamma$  is also important during pre-T cell development in the thymus<sup>15</sup>. p110 $\delta$ , which is the focus of this review, is highly expressed both in lymphocytes and myeloid cells and is activated by antigen receptors, costimulatory receptors, cytokine receptors and growth factor receptors<sup>8</sup>.

Class I PI3Ks catalyse the phosphorylation of PtdIns(4,5)P<sub>2</sub> to generate PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>), which acts as a membrane tether for cell signalling proteins with pleckstrin homology (PH) domains. Prominent among these are PDK1 and AKT, which act in concert to phosphorylate substrates such as the FOXO transcription factors (which become inactivated) and regulators of the mTOR complex 1 (which becomes activated). Therefore, activation of class I PI3Ks results in inactivation of FOXO transcription factors. In lymphocytes, BTK and ITK are PIP<sub>3</sub>-responsive tyrosine kinases that contribute to the activation of phospholipase C-gamma (PLC $\gamma$ ) and other downstream signalling proteins (**Figs 1, 2**). The lipid phosphatase PTEN converts PIP<sub>3</sub> back to PtdIns(4,5)P<sub>2</sub><sup>8</sup>.

Class IA PI3K regulatory subunits are encoded by three different genes (*PIK3R1*, *PIK3R2* and *PIK3R3*) (Table 1). *PIK3R1* encodes p85 $\alpha$ , p55 $\alpha$  and p50 $\alpha$  (each from an alternative transcription start site), *PIK3R2* encodes p85 $\beta$ , and *PIK3R3* encodes p55 $\gamma$ <sup>16</sup>. These regulatory subunits have SH2 domains, which bind phosphorylated YXXM motifs of cell surface receptors and membrane-associated proteins. p85 $\alpha$ , p55 $\alpha$ , p50 $\alpha$  and p85 $\beta$  are ubiquitously expressed, whereas p55 $\gamma$  is mainly expressed in the brain and testes<sup>16</sup>. Any of the class IA PI3K regulatory subunits can bind to p110 $\alpha$ , p110 $\beta$  and p110 $\delta$  without apparent selectivity. PI3K $\delta$  is best understood to comprise p85 $\alpha$  with p110 $\delta$ , but association between p110 $\delta$  and any of the other class IA PI3K regulatory subunits is also possible. It is also important to recognise that p85 $\alpha$  has many p110 $\delta$ -independent functions, as it can also bind p110 $\alpha$  and p110 $\beta$ <sup>16</sup>.

The class IA PI3K regulatory subunits influence the p110 catalytic subunits in three ways<sup>17</sup>: they prevent proteolytic degradation of p110; they inhibit p110 catalytic activity; and they recruit the p110 subunit to tyrosine phosphorylated proteins at the plasma membrane.

Once the SH2 domains of p85 $\alpha$  are engaged by phosphotyrosines, the inhibitory contacts with p110 are relieved<sup>17</sup>. Thus, mutations in the *PIK3R1* gene can influence PI3K activity by allowing the

degradation of p110 $\delta$  or by diminishing its recruitment to receptors (in the case of *PIK3R1* null or LOF mutations), or by releasing the inhibitory action of p85 $\alpha$  on p110 $\delta$  (in the case of *PIK3R1* GOF mutations). In addition to the regulatory subunits, p110 $\alpha$  and p110 $\delta$  can bind RAS and p110 $\beta$  binds RAC or CDC42. These small GTPases help tether the p110 subunit to the membrane once it has been recruited to a receptor via its regulatory subunit<sup>17, 18</sup>.

### PI3K $\delta$ and immunity: lessons from mice

Prior to the description of APDS, most of our knowledge of the role of PI3K $\delta$  in immunity and infection was based on genetic and pharmacological studies using mouse models. The GOF mutations that cause APDS have recently been shown to result in increased basal and stimulated PIP<sub>3</sub> levels and PIP<sub>3</sub>-dependent signalling cascades in patient-derived lymphocytes<sup>1-4</sup>, and the study of these patients may give us new insights into how the balance of PI3K $\delta$  activity regulates immune cell functions. Here, we summarise what these studies in mice have taught us, before describing the immunological phenotypes of human patients with mutations in *PIK3R1* or *PIK3CD*.

#### *Loss of PI3K $\delta$ function in mouse B cells*

In mice, early B cell development in the bone marrow is only mildly affected by the loss of p85 $\alpha$  or p110 $\delta$ <sup>19-23</sup>, whereas the combined loss of p110 $\alpha$  and p110 $\delta$  leads to a near-complete development block at the pro-B cell stage<sup>24</sup>. However, mice lacking the p85 $\alpha$  or p110 $\delta$  subunits have fewer follicular B cells, lack marginal zone (MZ) B cells and peritoneal B1 B cells, have reduced serum immunoglobulins, and respond poorly to vaccination<sup>19-23</sup>. PI3K $\delta$  couples BCR activation with both PIP<sub>3</sub> production and signalling events downstream of the BCR (**Fig 1**). PI3K $\delta$ -deficient B cells fail to respond to mitogenic stimuli, but undergo class-switch recombination (CSR) in response to interleukin-4 (IL-4) and lipopolysaccharide (LPS) *in vitro*<sup>19-26</sup>. However, mice lacking p110 $\delta$  selectively in B cells can produce high-affinity IgG antibodies in response to immunisation with the T cell-dependent (TD) antigen NP-CGG<sup>27</sup> (but as discussed later, germline loss of *PIK3R1* or *PIK3CD* leads to attenuated TD antibody responses). By contrast, PI3K $\delta$  activity within B cells is required for T cell-independent (TI) antibody responses. This may be due in part to the loss of B1 and MZ B cell subsets, which are the dominant B cell subsets that respond to TI antigens, in PI3K $\delta$ -deficient mice<sup>21, 22, 27, 28</sup>.

#### *Consequences of hyperactive PI3K $\delta$ signalling in B cells in mice*

While there are several mouse models of LOF mutations in *Pik3cd*, the phenotype of *Pik3cd* GOF-mutant mice remains to be described. We can however, make inferences from other models of hyperactive PI3K signalling (in which *Pten* or *Foxo1* is ablated in the germline or in B cells) or from mice expressing a membrane-bound form of p110 $\alpha$  in B cells. PTEN antagonises PI3K signalling and

hence its ablation leads to elevated PIP<sub>3</sub> levels. FOXO transcription factors are negatively regulated by PI3K-AKT, and hence, their loss mimics some of the effects of hyperactive PI3K-AKT signalling. FOXO transcription factors induce the expression of genes involved in immunoglobulin gene recombination and development such as *Rag1*, *Rag2*, *Ikaros* and *Il7a* (Fig 1)<sup>29-31</sup>. Failure to undergo VDJ recombination because of elevated PI3K signalling and subsequent inactivation of FOXO1 can lead to a partial block of B cell development in the bone marrow<sup>29,30</sup>. In addition, elevated PI3K signalling can increase the sensitivity of developing *Pten*-null B cells to negative selection by self antigens<sup>32</sup>. Interference with RAG expression and/or negative selection may lead to the development of B cells with aberrant phenotypes, as observed in patients with APDS (see later).

Activation-induced cytidine deaminase (AID; encoded by *Aicda*) is the master regulator of CSR and somatic hypermutation (SHM)<sup>33</sup>. Deletion of *Pten* or *Foxo1* in B cells impairs immunoglobulin class switching<sup>26,30,34,35</sup>, suggesting that increased PI3K signalling in B cells antagonise this process. Indeed, addition of a PI3K $\delta$  inhibitor can restore CSR in *Pten*<sup>-/-</sup> cells *in vitro*<sup>35</sup>. AID is induced by FOXO1 and *in-vitro* activated *Foxo1*<sup>-/-</sup> B cells (which mimic B cells with GOF PI3K $\delta$  mutations) exhibit impaired CSR, due partially to the loss of *Aicda* transcription; however, inefficient CSR was still observed in *Pten*<sup>-/-</sup> B cells in the presence of ectopic AID, suggesting that PI3K signalling also regulates CSR by affecting AID function at the post-transcriptional level<sup>26,34,35</sup>. During the germinal centre reaction, B cells cycle between the light zone and dark zone. B cells interact with cognate T cells in the light zone, and if they receive the appropriate signals, undergo CSR and then traffic to the dark zone where they proliferate and undergo SHM<sup>36</sup>. When, *Foxo1* was deleted specifically in germinal centre B cells, CSR was impaired despite normal *Aicda* transcription and AID protein expression. This suggests that FOXO1 regulates the targeting of AID to the immunoglobulin gene locus, that FOXO1 targets other genetic loci required for CSR and SHM, and/or that *Foxo1* deletion in germinal centre B cells affects the expression of other proteins required for CSR<sup>37,38</sup>. Moreover, *Foxo1* ablation or induction of PI3K activity in germinal centre B cells led to loss of germinal centre dark zones due to aberrant trafficking of B cells, at least in part as a consequence of lost expression of CXC-chemokine receptor 4 (*Cxcr4*), which is a target of FOXO1<sup>37,38</sup>. Hence, failure to expand antigen-specific B cells that have undergone selection in the germinal centre light zone is an additional cause of impaired high affinity class-switched antibody production.

Together, these findings contrast the effects of impaired PI3K signalling versus unrestrained PI3K signalling in B cells: PI3K $\delta$  deficiency in mature B cells impairs TI antibody responses but does not affect CSR or SHM<sup>27</sup>, whereas, hyperactivation of PI3K signalling in mature B cells interferes with CSR and SHM and promotes the expansion of antigen-specific B cell populations in the germinal centre dark zones (Fig 2)<sup>26,34,35</sup>.

PI3K $\delta$  is required for mouse CD4<sup>+</sup> T cell differentiation and Treg cell function

If PI3K $\delta$ -deficient B cells can undergo CSR, then why do PI3K $\delta$ -deficient mice fail to respond to T cell-dependent vaccines? The answer relates to the provision of T cell help for B cell development and immunoglobulin class switching. ICOS is a T cell costimulatory receptor and a potent activator of PI3K $\delta$ . Mutant mice in which ICOS has been uncoupled from PI3K $\delta$  lack follicular helper T (Tfh) cells<sup>39</sup>. Similarly, deletion of the p110 $\delta$  subunit in T cells interferes with the development of Tfh cells, leading to a dramatic attenuation of T cell-dependent immune responses, including the induction of CSR and SHM in B cells<sup>27</sup>. These results highlight a dual role for PI3K $\delta$  in antibody production: inactivation of PI3K $\delta$  in B cells, which leads to activation of FOXO transcription factors, is a prerequisite for CSR and SHM<sup>26, 34, 35</sup>, whereas the activation of PI3K $\delta$  in Tfh cells is a prerequisite for the provision of help to supports CSR and SHM in B cells<sup>27</sup>.

Naïve CD4<sup>+</sup> T cell differentiation towards the Th1, Th2 and Th17 cell lineages is delayed or attenuated when PI3K $\delta$  is inhibited<sup>40-42</sup>. This may reflect a key role for FOXO transcription factors in the suppression of Th cell differentiation, for example by suppressing the *Ifng* gene<sup>43</sup>, as well as the requirement for mTOR activity to promote Th cell differentiation<sup>44</sup>. A reduction in Th2 cell responses underpins the resistance of PI3K $\delta$ -deficient mice to experimentally induced asthma, despite elevated IgE levels<sup>25, 45</sup>. In addition, reduced Th17 cell responses may protect PI3K $\delta$ -deficient mice from experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis<sup>46</sup>. Although PI3K $\delta$ -deficient mice only develop a partial Th1 cell response to *Leishmania major* infections, PI3K $\delta$ -deficient mice control *Leishmania major* infections more effectively than wild-type mice, likely due to defects in a regulatory immune cell population<sup>47</sup>.

PI3K $\delta$  is required for FOXP3<sup>+</sup> regulatory T (Treg) cell homeostasis and function<sup>48</sup>. PI3K $\delta$ -deficient mice develop colitis because of inappropriate activation of effector T cells by gut microbes, and PI3K $\delta$ -deficient Treg fail to suppress experimental colitis<sup>22, 48</sup>. Patients taking the PI3K $\delta$  inhibitor idelalisib (Zydelig; Gilead) also develop colitis, probably in part as a result of reduced Treg cell function<sup>49, 50</sup>. However, PI3K $\delta$ -deficient mice and mice lacking p110 $\delta$  only in Treg cells mount a more effective immune response against a broad range of tumours than wild-type mice<sup>51</sup>. As with antibody production, these data highlight the dual nature of PI3K $\delta$ , which is required both for optimal cytokine production by effector T cells and for effective Treg cell-mediated tolerance. Whether PI3K $\delta$  inhibition results in impaired or enhanced cell-mediated immune responses is context dependent and therefore difficult to predict (**Fig 3**). Interestingly, inactivation of PI3K $\delta$  results in the hyperresponsiveness of dendritic cells and macrophages to Toll-like receptor ligands, resulting in increased IL-12 production, which may further contribute to increased cell-mediated immune responses upon LOF of PI3K $\delta$ <sup>52</sup>.

PI3K $\delta$  regulates mouse CD8<sup>+</sup> T cell effector functions

PI3K $\delta$ -deficient CD8<sup>+</sup> T cells stimulated *in vitro* are characterised by a reduced abundance of mRNAs encoding proteins associated with inflammation and cytotoxicity, such as IFN $\gamma$ , granzyme B and perforin<sup>51, 53, 54</sup>. By contrast, the expression of genes regulating the homing of T cells to the lymph nodes, such as *Sell* (which encodes CD62L), *Ccr7* and *Klf2* are increased in PI3K $\delta$ -deficient CD8<sup>+</sup> T cells stimulated *in vitro*<sup>55</sup>. Thus, PI3K $\delta$  can regulate the homeostatic trafficking of T cells to the lymph nodes and contributes to the reprogramming of CD8<sup>+</sup> T cells to acquire full effector functions and migrate to peripheral tissues.

PI3K $\delta$  is required to reach the optimal magnitude of CD8<sup>+</sup> T cell responses *in vivo*<sup>53, 56</sup>. Nevertheless, PI3K $\delta$ -deficient CD8<sup>+</sup> T cells can become fully differentiated cytotoxic T cells that produce IFN $\gamma$  and GZMB required for the killing of virus-infected cells or tumours; this suggests that the transcriptional defects described *in vitro* can, at least in part, be overcome by strong inflammatory stimuli *in vivo*<sup>51, 53</sup>. Moreover, long-term CD8<sup>+</sup> T cell memory responses are intact in PI3K $\delta$ -deficient mice<sup>53</sup>. This is partially because the generation of CD8<sup>+</sup> effector T cells is reduced during recall responses, whereas the generation of long-term memory CD8<sup>+</sup> T cells in the lymph nodes and bone marrow is preserved<sup>53</sup>. Similarly, the inhibition of the downstream kinase mTOR with low-dose rapamycin during vaccination or infection augments the generation of memory CD8<sup>+</sup> T cells at the expense of effector CD8<sup>+</sup> T cells<sup>57</sup>. Hence, by promoting mTOR activity, PI3K $\delta$  skews CD8<sup>+</sup> T cell differentiation in favour of effector T cells, but antagonises the generation of memory CD8<sup>+</sup> T cells. Thus, strong PI3K $\delta$  activity is associated with effector CD8<sup>+</sup> T cell differentiation, whereas the maintenance of CD8<sup>+</sup> T cell memory requires the suppression of PI3K signalling (Fig 4).

Consequences of hyperactive PI3K signalling in mouse T cells

Similar to B cells, the consequence of PI3K $\delta$  hyperactivation in mouse T cells can be inferred from experiments using PTEN-deficient or FOXO1-deficient T cells. Loss of PTEN expression in early T cell development leads to the development of an immature T cell lymphoma and a hyperactivated T cell phenotype, characterised by the increased secretion of effector T cell cytokines and autoimmunity<sup>58</sup>. Similar results were observed in mice expressing a mutant p85 $\alpha$  protein that lacked inhibitory contacts with the p110 catalytic subunit<sup>59</sup>. Deleting PTEN in mature CD4<sup>+</sup> T cells also resulted in enhanced cytokine production and Th cell function, but did not induce T cell transformation or autoimmunity<sup>60</sup>. Furthermore, loss of *Foxo1* leads to a loss of memory CD8<sup>+</sup> T cell development after infection<sup>61</sup>. Together, these data indicate a unique sensitivity of thymocytes to PI3K $\delta$ -dependent T cell transformation, and suggest that PI3K $\delta$  signalling also affects central tolerance to self-peptides. Overall, these studies suggest that unrestrained PI3K signalling in T cells lowers their threshold of activation.

## Alterations in PI3K $\delta$ signalling leads to PIDs in humans

Both LOF and GOF mutations in PI3K genes that cause PIDs in humans have been described. Our understanding of the underpinning causes of these PIDs has been greatly aided by the use of mouse models (as described in the previous section), but have also furthered and challenged our understanding of the functions of PI3K $\delta$  (**Box 2** and below).

### Loss of function of p85 $\alpha$ or p110 $\delta$ in humans

As with mouse T cells, inhibition of PI3K $\delta$  in human T cells suppresses the expression of effector cytokines such as IFN $\gamma$ , IL-4 and IL-17<sup>41</sup>. A single patient with a homozygous *PIK3R1* mutation that generated a premature stop codon (resulting in the loss of p85 $\alpha$  and markedly decreased expression of p110 $\delta$ ) presented with recurrent pneumonia associated with agammaglobulinemia and severe B cell lymphopenia due to a block in early B cell development<sup>10</sup>. The development of colitis in this patient was attributed to antibody-deficiency and the consequent outgrowth of gut pathogens, but could also be due to Treg cell deficiency<sup>10</sup>. Similarly, one patient lacking p110 $\delta$  as a result of the inheritance of two different non-functional alleles has been described, and this patient presented with sinopulmonary infections, septic arthritis, inflammatory bowel disease and autoimmune hepatitis, associated with hypogammaglobulinemia<sup>9</sup>. Loss of p110 $\delta$  was again associated with severe B cell lymphopenia and fewer memory T cells<sup>9</sup>. Thus, the two reported patients with a loss of PI3K $\delta$  suffer infections associated with the lack of B cells. Interestingly, in mice, a complete block in B cell development and severe mature B cell lymphopenia are only observed when both the p110 $\alpha$  and p110 $\delta$  are inactivated in the B cell lineage<sup>24</sup>, suggesting a redundancy between these isoforms in mice that is not reflected in humans. The inflammatory and autoimmune manifestations in PI3K $\delta$ -deficient humans, possibly associated with reduced Treg cell function, underscore the importance of PI3K $\delta$  in maintaining self-tolerance. PI3K $\delta$  is also required for the generation of ROS by human neutrophils and treatment of patients with the PI3K $\delta$  inhibitor idelalisib can lead to neutropenia and increased risk of infections<sup>49, 62</sup>.

### Activating PI3K $\delta$ mutations that underlie human APDS

In 2013, groups in Cambridge (UK) and Bethesda (US) reported whole-exome sequencing studies of patients with uncharacterised PID, which revealed causal heterozygous activating mutations in *PIK3CD*<sup>1, 2</sup>. The UK patients were identified by screening cohorts of PID patients with a high frequency of recurrent chest infections and bronchiectasis, features suggestive of antibody deficiency, although frequent herpes viral infections and an increased proportion of effector T cells were also noted<sup>1</sup>. The US cohort were identified on the basis of persistent viremia with herpes-family viruses, which are commonly associated with altered T cell or natural killer (NK) cell function,

in addition to frequent airway infections<sup>2</sup>. Because both B cells and T cells are affected in these patients, APDS should be characterised as a combined immunodeficiency<sup>1-5</sup>.

This immunodeficiency had previously been noted in a Taiwanese boy by targeted sequencing of the *PIK3CD* gene in children with B cell immunodeficiency, although the nature of the mutation (GOF or LOF) was not elucidated<sup>63</sup>. Subsequently, a number of additional studies have identified APDS patients with mutations in *PIK3CD*<sup>5, 7, 64-69</sup> or *PIK3R1*<sup>6, 70-73</sup>. Patients with GOF mutations in either of these genes appear to largely phenocopy each other, despite the fact that p85 $\alpha$  is ubiquitously expressed and can pair with p110 $\alpha$  and p110 $\beta$  in addition to p110 $\delta$ . There is some evidence for effects of the *PIK3R1* mutation outside the immune system (for example, short stature, Box 1)<sup>74</sup>, but detailed analyses of the effects of this p85 $\alpha$  defect on p110 $\alpha$  or p110 $\beta$  have not yet been reported. The biochemical and clinical symptoms of patients with APDS1 (*PIK3CD* mutations) or APDS2 (*PIK3R1* mutations) are similar, suggesting that the pathological features of both syndromes are a consequence of aberrant and hyperactive PI3K $\delta$  signalling<sup>1-4</sup>. Here we use the generic term APDS unless referring specifically to either. A milder form of APDS-like immunodeficiency has been described in Cowden disease, caused by heterozygous loss of PTEN, although the increases in PIP<sub>3</sub> and pAKT levels from these patient T cells was less obvious than observed in the T cells from patients with APDS<sup>69, 75</sup>.

The most frequent mutation in *PIK3CD* (c.3061G>A; OMIM 602839 <http://www.omim.org/entry/602839#0001>) encodes a glutamic acid for lysine substitution at position 1021 (E1021K) of p110 $\delta$  (Table 1). To date, this mutation has only been found in APDS patients and their affected family members but not among healthy unrelated subjects<sup>1</sup>. Patients with the E1021K mutation have been found across continents and ethnicities. Genetic analysis showed no founder effect, demonstrating that E1021K is a recurrent mutation that appeared *de novo* independently in multiple unrelated families<sup>1</sup>.

p110 $\delta$  with the E1021K mutation has increased lipid kinase activity, as shown using recombinant proteins *in vitro* and by measuring PIP<sub>3</sub> and AKT phosphorylation levels in patient-derived T cells<sup>1, 2</sup>. The E1021K mutation is located in the C-terminal lobe of the kinase domain of p110 $\delta$ , similarly to the oncogenic H1047R mutation of p110 $\alpha$ , and enhances the membrane association of p110 $\delta$  *in vitro*, facilitating more effective phosphorylation of its lipid substrate PtdIns(4,5)P<sub>2</sub>; this increases accumulation of PIP<sub>3</sub> and lowers the activation threshold of PI3K $\delta$ <sup>1, 17</sup> (Fig 3). Other missense p110 $\delta$  mutations — N334K, C416R and E525K — have also been shown to cause APDS, although they are less frequent than E1021K<sup>2</sup> (Table 1). Interestingly, GOF mutations of the homologous amino acid residues of p110 $\alpha$  (N345, C420 and E545, respectively), have been identified in tumors

(<http://www.sanger.ac.uk/genetics/CGP/cosmic/>) and are thought to interfere with the inhibitory contacts imposed by p85 $\alpha$  and hence increase the lipid kinase activity of the p110 subunit<sup>17</sup>; this implies that a similar mechanism may lead to enhanced PIP<sub>3</sub> accumulation in cells from patients with APDS with the equivalent mutations and hence the immune modulation seen in APDS (Fig 4). APDS is thus distinct from most other PIDs in that it is the hyperactivation of signaling pathways, rather than their inhibition, that leads to immune dysfunction. This distinction offers unique therapeutic opportunities (see below).

A heterozygous splice site mutation before exon 11 of the *PIK3R1* gene leads to an in-frame fusion of exon 10 with exon 12, resulting in the deletion of 42 amino acids in p85 $\alpha$  (del p.434 – 475, **OMIM: <http://omim.org/entry/616005>**) p55 $\alpha$  and p50 $\alpha$ <sup>3, 4</sup>(Fig 3). These amino acids lie in the inter-SH2 domain that regulates the activity of the catalytic p110 subunits<sup>76</sup>. Oncogenic mutations in this region result in mutant proteins that can bind p110 subunits but are less effective at inhibiting their enzymatic activity<sup>76, 77</sup>. Similar to mutations in the p110 $\delta$  subunit, this is thought to lower the threshold of activation for PI3K $\delta$ . The mutant p85 $\alpha$ <sup>del434–475</sup> protein (Fig 4,  $\Delta$ Ex11) was shown to stabilize p110 $\delta$ , which was expressed at near normal levels in patient cells, but its inhibitory function was impaired, leading to increased PI3K $\delta$  activity<sup>3, 4</sup>. Because the net effect of the p85 $\alpha$  (del p.434 – 475) is increased PI3K $\delta$  activity, we consider it as a GOF mutation, even though it is strictly speaking a mutation resultin in loss of *inhibitory* function.

Thus, a number of different mutations in *PIK3R1* or *PIK3CD* lead to increased activity of PI3K $\delta$ , either by disrupting inhibitory contacts between p85 $\alpha$  and p110 $\delta$  or by increasing the affinity of p110 $\delta$  for the plasma membrane, promoting its interaction with the lipid substrate and hence facilitating phosphorylation of PtdIns(4,5)P<sub>2</sub>.

Activating PI3K $\delta$  mutations lead to impaired B cell function and vaccine responses

Immunoglobulin levels in patients with APDS are variable, ranging from isolated specific antibody deficiency or IgG subclass deficiency to severe hypogammaglobulinemia, often with increased IgM levels. In one cohort, 10% of a heterogeneous PID cohort who suffered recurrent infections were found to have APDS<sup>1</sup>, whereas in a second cohort of mainly antibody-deficient PID patients, fewer than 1% had *PIK3CD* mutations<sup>5</sup>.

Most APDS patients have increased proportions of circulating transitional B cells , reduced class-switched memory B cells, and impaired vaccine responses<sup>1,3</sup>. *In vitro*, APDS patient-derived B cells showed impaired CSR (consistent with the observed tendency for these patients to have reduced IgG and increased IgM levels), but in contrast to the findings in mouse cells, this was not associated with reduced *AICDA* mRNA levels<sup>2</sup>. As noted above, it is possible that PI3K regulates AID function by post-

transcriptional mechanisms as well as by regulation of mRNA expression<sup>35</sup>. Alternatively, the defective CSR in APDS patients could be due to defects in germinal centre Tfh cells<sup>78</sup>, aberrant B cell maturation and/or defective migration of B cells during the germinal centre reaction in the spleen, as shown for FOXO1-deficient B cells in mice<sup>37, 38</sup>. The basis for the increased percentage of circulating transitional B cells in patients with APDS remains incompletely understood, but is likely to be a consequence of impaired B cell maturation and/or an increased propensity for mature B cells to undergo apoptosis<sup>1</sup>. These findings are in marked contrast to the dramatic loss of B cells and agammaglobulinemia seen in the rare patients with LOF mutations in *PIK3R1* or *PIK3CD*.

Encapsulated bacteria (*Haemophilus influenzae* and *Streptococcus pneumoniae*) are the most frequent respiratory isolates from patients with APDS (Box 1), which is consistent with a substantial defect in antibody-mediated immunity. However, the severity of respiratory infections and resulting structural damage in the lungs do not correlate well with the reduction in B cell numbers or the extent of immunoglobulin deficiency<sup>6, 7</sup>, and immunoglobulin replacement therapy alone does not appear to limit the progress of lung damage in patients with APDS<sup>6, 7</sup>. One explanation for this apparent discrepancy is that PI3K $\delta$  hyperactivation causes additional defects (such as altered T cell functions or innate immune cell dysfunction) that also contribute to an increased susceptibility to respiratory bacterial infections. As mentioned above, PI3K $\delta$  has been shown to promote ROS production by human neutrophils, which could cause collateral damage if excessively produced during infections<sup>62</sup>. However, analysis of APDS patient neutrophils did not reveal an obvious increase in ROS production, or indeed in PIP<sub>3</sub> production, in response to stimulation with microbial peptides<sup>1</sup>. However, the increased susceptibility of patients with APDS to staphylococcal skin infections and abscess formation<sup>1, 65</sup>, as well as defective killing of mycobacteria by macrophages from an APDS patient<sup>64</sup>, suggest that abnormalities may indeed exist in the innate immune system which remain to be more completely investigated. Increased PI3K activity has been shown to compromise the migratory accuracy of neutrophils, and hence prolong their tissue-transit time, leading to increased opportunities for bystander tissue injury mediated by surface-associated neutrophil proteases<sup>79</sup>. Hence a wide range of impaired immune cell functions, affecting both innate and adaptive immune responses, may contribute to recurrent infection and bronchiectasis in patients with APDS.

Activating PI3K $\delta$  mutations cause T cell senescence

Peripheral blood analysis revealed an increase in effector-type T cells with a severe reduction in naïve T cell numbers<sup>1-4</sup>. Freshly isolated peripheral blood cells demonstrated reduced secretion of cytokines and increased apoptosis upon TCR restimulation<sup>1-3</sup>. Unexpectedly, acute PI3K $\delta$  inhibition in T cells from APDS patients reduced TCR-triggered apoptosis, suggesting a previously unappreciated role for PI3K $\delta$  signalling in pro-apoptotic pathways<sup>1, 3</sup>. However, T cell blasts that had escaped

apoptosis and expanded after activation *in vitro* showed increased production of IFN $\gamma$ , TNF and granzyme B<sup>2</sup>. Thus, chronic hyperactivation of PI3K $\delta$  signalling promotes T cell differentiation into terminal effector cells with increased sensitivity to TCR-induced cell death and dysregulation of cytokine secretion.

Notably, the expression of CD57, which is a marker of senescence on CD8<sup>+</sup> T cells<sup>80</sup>, was consistently high on patient cells<sup>2,4</sup>. Subsequent analyses confirmed shortening of telomere length in APDS patient lymphocytes<sup>4</sup>, suggesting T cell senescence contributes to immune dysfunction in APDS patients. Patients free from viraemia also presented with increased numbers of CD57<sup>+</sup>CD8<sup>+</sup> T cells<sup>2</sup>; therefore, CD8<sup>+</sup> T cell senescence in APDS is likely to be distinct from T cell exhaustion driven by chronic viral infections. T cell senescence due to telomere shortening results in cell cycle arrest while maintaining most other responses to antigen<sup>81</sup>, whereas T cell exhaustion from chronic antigen stimulation results in the upregulation of co-inhibitory receptors that broadly dampen TCR signalling and antigen responsiveness<sup>82</sup>. These findings point to *in vivo* hyperproliferation (which is consistent with enlarged spleen and lymph nodes) as the underlying cause of the T cell senescence and short telomeres in APDS patients and support the connection between cell division and effector T cell differentiation.

T cells from APDS patients exhibit increased activity of mTOR<sup>2</sup>, a key mediator of the switch from a catabolic naïve state to an anabolic effector state during a T cell response<sup>83</sup>. Increased glucose uptake is also observed in T cells from APDS patients compared with healthy subjects<sup>2,4</sup>. These findings indicate that changes in T cell metabolism induced by hyperactive PI3K $\delta$  signalling may underlie the hyperproliferation associated with T cell senescence in APDS patients. Further studies will be needed to determine if elevated mTOR activity is a direct consequence of increased PI3K $\delta$  activity or whether it also reflects the skewed effector phenotype of T cells in APDS patients. PI3K $\delta$  inhibition reduced, but did not ablate, phosphorylation of S6K (a component of the mTOR signalling pathway) in APDS T cells, confirming that PI3K $\delta$  contributes to mTOR activity in these cells<sup>4</sup>. Therefore, unrestrained and prolonged PI3K $\delta$  and mTOR activity may drive APDS T cells towards senescence rather than allowing T cells to revert to a metabolically quiescent phenotype after antigen exposure (Fig 3).

The main clinical manifestation of abnormal T cell function in APDS is herpes viral infection. All of the patients with *PIK3CD* mutations that were described by Lucas *et al.*<sup>3</sup> experienced chronic Epstein–Barr virus (EBV) and/or cytomegalovirus (CMV) viremia; in other studies the occurrence of CMV/EBV

was lower<sup>1, 4-7</sup>, although herpes simplex virus and varicella zoster virus infections were also noted. These inter-study differences may reflect the case-finding strategies, immune profiles and/or pathogen exposure of the patients. Surprisingly, given the abnormal T cell profiles, few other opportunistic infections have been reported. Some cases of problematic viral warts and molluscum contagiosum have been identified<sup>7</sup>, perhaps suggesting impaired NK cell function, though this has yet to be confirmed experimentally.

### **Treatment options for patients with APDS**

As APDS patients often present with reduced IgG levels or respond poorly to vaccines, many are treated with immunoglobulin replacement therapy that is often supplemented with prophylactic antibiotics. While this may have been effective in some patients, it has not prevented the acquisition or progression of bronchiectasis in others, even when the treatment was initiated in childhood<sup>6, 7</sup>. Haematopoietic stem cell transplantation (HSCT) is a treatment option, particularly for younger patients. HSCT could also help prevent or treat malignant B cell transformation, which occurs in 10–15% of patients. Several patients have undergone HSCT and, although significant improvements have been noted<sup>6, 7</sup>, the follow up of these patients is too short to make a definitive conclusion.

#### Rapamycin

Lucas and colleagues reported use of the mTOR inhibitor rapamycin in one patient, who showed a dramatic reduction in lymphadenopathy and hepatosplenomegaly and improvement in T cell subset defects<sup>2</sup>. Similar improvements have been noted in a recent case report of a four year old boy also treated with rapamycin<sup>68</sup>. However, the effect of rapamycin on B cell homeostasis and humoral immune responses in APDS patients remains to be determined. It is important to keep in mind that PI3K $\delta$  regulates other pathways in addition mTOR, and conversely, that mTOR is also regulated by PI3K-independent pathways<sup>8</sup>. Moreover, mTOR regulates the expression of *PTEN* such that treatment of T cells with rapamycin can actually increase PI3K signalling in T cells<sup>84</sup>, potentially exacerbating aspects of hyperactive PI3K $\delta$  signalling in APDS.

#### PI3K $\delta$ inhibitors

The PI3K $\delta$  inhibitor idelalisib is licenced for use in chronic lymphocytic leukaemia and non-Hodgkin lymphoma<sup>85, 86</sup>. However, idelalisib has a considerable side-effect profile, including pneumonitis, pneumonia, transaminitis and colitis in up to 42% of patients treated<sup>49</sup>. Histologically, the colitis in these patients is reminiscent of that seen in mice lacking functional PI3K $\delta$ , suggesting it is an on-target effect rather than a compound-specific effect<sup>49</sup>. It is possible that APDS patients will benefit from lower doses of PI3K $\delta$  inhibitors, which are effective for the treatment of B cell lymphomas, and

hence may be spared some of the more severe side effects. Another possibility is that topical administration of the PI3K $\delta$  inhibitor may avoid some of the adverse effects.

Two clinical trials of PI3K $\delta$  inhibitors in patients with APDS have recently been announced: [NCT02435173](#) sponsored by Novartis for an oral PI3K $\delta$  inhibitor and [NCT02593539](#) sponsored by GlaxoSmithKline for an inhaled PI3K $\delta$  inhibitor. To correct systemic immune defects, including lymphoproliferation and lymphoma, an oral inhibitor is more likely to be effective; however, an inhaled inhibitor is expected to have a better safety profile and may be appropriate for patients who are primarily affected by airway infections and potentially may limit progression of bronchiectasis.

## Conclusions

GOF mutations in PI3K $\delta$  lead to a range of B and T cell developmental and functional defects that compromise host defence, leading to recurrent bacterial and viral infections (Box 1). This distinguishes APDS patients from patients with LOF of PI3K $\delta$  who present with much more severe B cell lymphopenia and agammaglobulinemia, but not T cell senescence. In general, GOF mutations are unusual causes of immune deficiency<sup>87</sup>. The therapeutic options for LOF of PI3K $\delta$  may be limited to immunoglobulin replacement therapy, bone marrow transplants and perhaps gene therapy. Although these are also options for APDS, existing (mTOR inhibitors) and emerging (PI3K $\delta$  inhibitors) therapeutics offer the additional possibility of correcting the biochemical defects that arise from APDS-associated mutations, and the impact of these agents is currently being explored.

The fact that both LOF and GOF PI3K $\delta$  mutations lead to immunodeficiencies highlights the concept that this pathway must be precisely and dynamically modulated for optimal immune cell function: too much, too little or the inability to turn the pathway on or off as needed, has detrimental consequences (Fig 3)<sup>8</sup>. These considerations raise the possibility that aberrant PI3K signalling in immune cells may also occur in non-genetic diseases or conditions that lead to increased susceptibility to infections.

Many fundamental questions remain to be answered. How common is APDS among PID patients? What are some of the genetic or environmental influences that lead to the clinical heterogeneity of APDS patients? Are there mutations in other genes that lead to hyperactivation of PI3K $\delta$  and APDS-like syndromes? Why do APDS T cells undergo apoptosis when stimulated? Why does recurrent airway infection lead to bronchiectasis more frequently in APDS patients than in other PIDs? Can PI3K $\delta$  inhibitors restore normal immune function in APDS? The answers to these and further questions will require more detailed analysis of APDS patient cohorts, genetic screening of larger PID cohorts, and establishment of mouse models that mimic this intriguing new disease and help evaluate different therapeutic strategies.



**Table 1. PI3K subunits and APDS mutations**

PI3K class	Gene	Protein	Expression	Selected functions	APDS-associated mutations**
<b>Catalytic subunits</b>					
Class IA	PIK3CA	p110 $\alpha$	Ubiquitous	<ul style="list-style-type: none"> <li>Metabolism</li> <li>Angiogenesis</li> </ul>	ND
	PIK3CB	p110 $\beta$	Ubiquitous	<ul style="list-style-type: none"> <li>Metabolism</li> <li>Neutrophil activation</li> </ul>	ND
	PIK3CD	p110 $\delta$	Haematopoietic cells and CNS	<ul style="list-style-type: none"> <li>Immunity</li> </ul>	
Class IB	PIK3CG	p110 $\gamma$	Haematopoietic cells and heart	<ul style="list-style-type: none"> <li>Immunity</li> <li>Metabolism</li> <li>Cardiac</li> </ul>	N334K (1) <sup>2</sup> C416K (2) <sup>65</sup> E525K (7) <sup>2</sup> , E525A (3) <sup>69</sup> E1021K (63) <sup>1, 2, 5, 7, 63-69</sup>
<b>Regulatory subunits</b>					
Class IA	PIK3R1	p85 $\alpha$ , p55 $\alpha$ , p50 $\alpha$	Ubiquitous	<ul style="list-style-type: none"> <li>Metabolism</li> <li>Immunity</li> </ul>	del p.434-475 (43) <sup>3, 4, 6, 70-74</sup>
	PIK3R2	p85 $\beta$	Ubiquitous	<ul style="list-style-type: none"> <li>Metabolism</li> <li>Immunity</li> </ul>	ND
	PIK3R3	p55 $\gamma$	Brain and testes	<ul style="list-style-type: none"> <li>Unknown</li> </ul>	ND
<b>Class IB</b>	PIK3R5	p101	Haematopoietic cells	<ul style="list-style-type: none"> <li>Immunity</li> </ul>	ND
	PIK3R6	p84*	Haematopoietic cells	<ul style="list-style-type: none"> <li>Immunity</li> </ul>	ND

\*Also known as p87. \*\* Number of cases reported in brackets. ND, none described

### Box 1. Clinical features of APDS

Patients with APDS display features of both immune deficiency and of immune dysregulation:

- Recurrent lung, ear and sinus infections (with encapsulated bacteria such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, which require opsonisation for effective killing) are near-universal and are associated with a high incidence of organ damage including hearing impairment and bronchiectasis (permanent airway scarring)<sup>1-4</sup>.
- Severe, recurrent or persistent infections with herpes family viruses are common, in particular chronic EBV or CMV viremia, and HSV and VZV infections<sup>1, 3-7</sup>. Frequent isolates of some respiratory viruses such as adenovirus and echovirus have also been described<sup>1</sup>.
- Opportunistic infections are rare, although a few patients have experienced recurrent viral warts or molluscum contagiosum infections<sup>49</sup>.
- An increased incidence of abscess formation, lymphadenitis and cellulitis with gram-positive bacteria (mainly *Staphylococcus aureus*), and defective killing of mycobacteria by macrophages isolated from a patient with APDS suggest a mild deficit in innate immunity<sup>1, 64</sup>.
- Benign lymphoproliferation (lymphadenopathy, hepatosplenomegaly and focal nodular lymphoid hyperplasia) is a common feature of all patients with APDS that have been studied to date.
- Histopathological analysis of lymphoid tissue from affected patients demonstrates atypical follicular hyperplasia with attenuation of mantle zones in APDS1, and small B cell follicles in APDS2. Germinal centres were disrupted by infiltrating T cells (often PD1-positive) in both APDS1 and APDS2<sup>6, 7</sup>.
- There is a high frequency of lymphoma associated with APDS, encompassing a wide range of histopathological patterns<sup>1, 2, 7, 65, 67</sup>.
- Immune cytopenias (thrombocytopenia, haemolytic anaemia and neutropenia) and autoimmune-like solid organ conditions (such as juvenile arthritis, glomerulonephritis, thyroiditis and sclerosing cholangitis) have also been reported<sup>7, 66</sup>, with a frequency of 34% in a cohort of 53 patients with APDS1<sup>7</sup> and 17% in a cohort of 36 patients with APDS2<sup>6</sup>.
- Mild developmental delays has been observed in both APDS1 and APDS2 cohorts, with a higher incidence in APDS2 (31% versus 19%)<sup>6, 7</sup>.
- Growth retardation is common in patients with APDS2<sup>6, 73, 74</sup> but does not seem to be a feature of APDS1 and may relate to the association of heterozygous mutations in *PIK3R1* with SHORT syndrome (short stature, hyperextensibility of joints, hernia, ocular depression, Rieger anomaly and teething delay)<sup>88-91</sup>.

**Box 2: Lessons learned from APDS**

Although the normal physiological role of PI3K $\delta$  has been extensively studied in mouse models, investigation of patients with APDS has provided important new insights about the biology of this kinase in humans.

- Mutations causing LOF or GOF of PI3K $\delta$  lead to immunodeficiency. This illustrates how this pathway needs to be dynamically regulated for normal immune cell function.
- The previously reported roles for PI3K $\delta$  in B cell function and humoral immunity did not predict the increase of transitional B cell numbers that have been observed in APDS patients.
- Defects in CSR that are not attributable to defective *AICDA* mRNA expression (encoding AID) remain to be fully understood.
- Augmented PI3K $\delta$  results in a loss of naïve T cells and an *in vivo* proliferative burst that causes lymphoproliferative disease and drives the T cells toward cellular senescence (a phenotype that is poorly mimicked in mouse models due to long telomeres).
- Moreover, patient T cells are highly susceptible to TCR restimulation-induced cell death, indicating a previously unappreciated role for PI3K $\delta$  in a pro-apoptotic signalling pathway.
- The high proportion of patients with severe respiratory infections and bronchiectasis suggests a role for PI3K $\delta$  in promoting inflammation of the lungs by mechanisms that are incompletely understood, but which may indicate a key role for PI3K $\delta$  in airway-associated innate immune responses, in addition to its role in humoral immunity.
- Previously, LOF point mutations in *PIK3R1* were shown to cause SHORT syndrome<sup>88-91</sup>. It is unclear why the  $\Delta$ Ex11 mutations that cause APDS2 manifest primarily as PID; however, it is of interest to note at least one case where this mutation was suggested to relate to SHORT syndrome<sup>73</sup>. This indicates that *PIK3R1*  $\Delta$ Ex11 may have distinct effects on different p110 isoforms in different tissues.

### Figure 1. BCR signaling

PI3K $\delta$  is a heterodimeric enzyme, typically composed of a p85 $\alpha$  regulatory subunit and a p110 $\delta$  catalytic subunit. In B cells, PI3K $\delta$  is activated upon cross-linking of the BCR, after stimulation with IL-4 or by the chemokine CXCL13 via CXCR5. The BCR co-opts the co-receptor CD19 or the adapter protein BCAP, both of which have YXXM motifs to which the p85 $\alpha$  SH2 domains can bind. The IL-4R co-opts IRS1, which also has YXXM motifs. The mechanism whereby CXCR5 is coupled to PI3K $\delta$  remains to be defined (indicated by a dotted line). PI3K $\delta$  signalling through AKT promotes the activation of mTOR and suppresses FOXO1 function (via phosphorylation-dependent nuclear export). FOXO1 is a transcription factor that activates the genes encoding RAG proteins involved in V(D)J recombination, IKAROS which is required for early B cell development, CD62L which is required for homing to lymph nodes and AID, which is required for CSR and SHM. The amino acid sensor mTOR contributes to the growth and proliferation of B cells. All proteins coloured in green have been affected by LOF mutations causing PID. Of these, only p85 $\alpha$  and p110 $\delta$  have also been affected by GOF mutations causing APDS.

### Figure 2. TCR signaling

PI3K $\delta$  is a heterodimeric enzyme, typically composed of a p85 $\alpha$  regulatory subunit and a p110 $\delta$  catalytic subunit. In T cells, the TCR, the costimulatory receptor ICOS and the IL-2R can activate PI3K $\delta$ . ICOS contains a YXXM motif in the cytoplasmic domain which is essential for ICOS-mediated co-stimulation. Precisely how the TCR activates PI3K $\delta$  remains incompletely understood, though TCR ligation is known to induce ZAP70-mediated phosphorylation of LAT. Whether PI3K binds LAT directly or via other adapter proteins remains to be established. Mechanisms of PI3K $\delta$  activation downstream of IL-2R are even less clear, but a role for JAK3 has been implicated. PI3K $\delta$  contributes to the downregulation of the expression of IL-7R $\alpha$  and CD62L, via the AKT-dependent inactivation and nuclear export of FOXO1, preparing the T cell to exit the lymph nodes and circulate through the vascular systems and organs. PI3K $\delta$  also increases metabolism and contributes to T cell effector-associated phenotypes by promoting activation of mTOR.

### Figure 3. Dynamic regulation of PI3K $\delta$ signaling in the immune system

PI3K $\delta$  activity needs to be dynamically regulated for normal immune cell function, as some cell types and processes require high PI3K $\delta$  activity, while other depend on low PI3K $\delta$  activity (e.g., if they require FOXO1-dependent gene transcription). Problems arise if cells cannot increase or suppress PI3K $\delta$  due to mutations, and have chronically low or high PI3K $\delta$  activity. Immunosuppression is associated with loss-of function and gain-of-function in *PIK3CD*, which encoded the PI3K $\delta$  subunit

p110 $\delta$ . Illustrated are some key cell types and processes affected by high or low PI3K $\delta$  activity, and the consequences of being locked in one state or the other.

In the healthy state (top), PI3K $\delta$  signalling is low in naïve and memory T cells, which are characterised by low mTOR and metabolic activity and high expression of FO XO1-dependent lymph node homing receptors. In activated effector T cells, PI3K $\delta$  activity is high as a consequence of TCR, IL2R and ICOS signalling. Effector T cells are also characterised by high mTOR and metabolic activity, whereas FO XO1-dependent expression of lymph node homing receptors is reduced.

Inhibition of PI3K signalling during thymic development is thought to favour the development of Treg. However, PI3K $\delta$  activity is required to maintain normal numbers of Treg cells in the peripheral lymphoid tissues and for Treg to adopt an effector phenotype, especially peripheral tissues.

Maintenance of low-level signaling (also referred to as tonic signalling) via PI3K $\delta$  (and to a lesser extent PI3K $\alpha$ ) maintains survival of naïve follicular B cells. Upon activation, PI3K is increased and this contributes to B cell proliferation. However, for B cells to undergo CSR in the GC, PI3K $\delta$  signalling needs to be tuned back down to allow higher FO XO1 transcription and proper AID targeting.

In disease states (bottom) caused by gain-of-function or loss-of-function in PI3K $\delta$ , the proper dynamics of signalling result in cellular defects associated with immunodeficiency. Chronically high PI3K $\delta$  activity leads to T cell (more senescence, death, and Tregs) and B cell (more transitional B cells and less CSR and SHM) abnormalities with increased susceptibility to B cell lymphoma, infections, and lymphoproliferative disease. Chronically low PI3K $\delta$  activity leads to a different set of T cell (poor responses and low Tregs) and B cell (low numbers) abnormalities resulting in prevalent infections and colitis.

#### Figure 4. APDS mutations lower the threshold of PI3K $\delta$ activation

**a** | Schematic diagram of the protein domains in the p85 $\alpha$  regulatory and p110 $\delta$  catalytic subunits with mapped interactions shown with lines, where the black line indicates the binding interaction mediated constitutive interaction and the red lines indicate inhibitory contacts. The locations of the described amino acid substitutions caused by APDS mutations are indicated. ABD: adaptor-binding domain, RBD: RAS-binding domain, SH3: SRC-homology 3 domain, P: proline-rich region, BH: breakpoint-cluster region homology domain, SH2: SRC-homology 2 domain, N-: N-terminal, C-: C-terminal, i-: inter-.

**b** | Class IA PI3Ks are activated by their recruitment to tyrosine kinase-associated receptors at the plasma membrane. The p85 $\alpha$  regulatory subunit (p50 fragment containing the N-SH2–i-SH2–C-SH2 domains, shown here in blue) stabilizes the p110 $\delta$  catalytic subunit (orange) through constitutive binding of the p85 $\alpha$  i-SH2 domain (coiled portion) to the p110 $\delta$  adaptor-binding domain (ABD). Binding of the p85 $\alpha$  SH2 domains to tyrosine-phosphorylated residues on an activated receptor releases the inhibitory contacts between the p85 $\alpha$  SH2 domains and the p110 $\delta$  C2, helical and kinase domains (shown in red in part a). It is possible that the  $\Delta$ Ex11 mutation (red) that truncates the p85 $\alpha$  inter-SH2 domain affects p110 $\delta$  more than it affects p110 $\alpha$ , hence the lack of more dramatic pleiotropic effects on growth and metabolism in individuals with this deletion. Ras–GTP further tethers p110 $\delta$  to the membrane by binding to the Ras-binding domain (RBD) of p110 $\delta$ . GOF mutations in *PIK3R1* and *PIK3CD* increase kinase activity by interfering with inhibitory interactions between the p85 $\alpha$  regulatory and p110 $\delta$  catalytic subunit ( $\Delta$ Ex11, N334K, C416R and E525K), or by increasing the affinity of p110 $\delta$  for the plasma membrane (E1021K). The E1021K mutations may also interfere with inhibitory contacts from the p85 $\alpha$  C-SH2 domain<sup>1</sup>. See ref (<sup>17</sup>) and references therein for further details of the structures and mechanisms of regulation of PI3K $\delta$ .

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**Conflicts of interest.**

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## Glossary terms

### Activated PI3K $\delta$ syndrome (APDS)

The term APDS encompasses two syndromes: APDS1 (also known as PASLI-CD), which result from a mutation in the *PIK3CD* gene that lead to the hyperactivation of the p110 $\delta$  subunit of PI3K $\delta$ ; and APDS2 (also known as PASLI-R1), which results from splice mutations in *PIK3R1* that lead to exon skipping and produces a truncated p85 $\alpha$  protein with reduced inhibition of p110 $\delta$ .

### Activation-induced cytidine deaminase (AID)

An enzyme that is required for two crucial events in the germinal centre: somatic hypermutation and class-switch recombination.

### Germinal centre reaction

Germinal centres are specialised structures within spleens and lymph nodes where B cells present antigen to T cells and in return, are selected to undergo CSR and SHM.

### Hypogammaglobulinemia

An immune disorder characterised by low serum IgG levels.

### Immune complexes

Complexes of antigen bound to antibody and, sometimes, components of the complement system. The levels of immune complexes are increased in many autoimmune disorders, in which they become deposited in tissues and cause tissue damage.

### Class-switch recombination (CSR)

The process by which proliferating B cells rearrange their DNA to switch from expressing IgM (or another class of immunoglobulin) to expressing a different immunoglobulin heavy-chain constant region, thereby producing antibody with different effector functions.

### T cell-independent (TI) antibody response

An antibody response to polymeric antigens, such as polysaccharides and lipids, that does not require T cell help.

### Primary immunodeficiency (PID)

An inherited disorder of the immune system that leads to recurrent infections and/or immune dysregulation. Currently there are around 84,000 patients diagnosed worldwide with PID.

### Somatic hypermutation (SHM)

A unique mutation mechanism that is targeted to the variable regions of rearranged immunoglobulin gene segments. Combined with selection for B cells that produce high-affinity antibody, SHM leads to affinity maturation of B cells in germinal centres.

### T follicular helper cells (Tfh cells)

CD4<sup>+</sup> T helper cells that are essential for the induction of class switching in the germinal centres of secondary follicles during antibody responses to T cell-dependent antigens.

### Transitional B cells

Immature B cells that have left the bone marrow for the spleen and are precursors of follicular B cells, marginal zone B cells and B1 B cells.

### Senescence

A state in which a cell fails to progress through the cell cycle due to activation of the DNA damage response, which can occur upon extreme shortening of telomeres.

### Author Biographies

#### Carrie Lucas

Carrie L. Lucas is an Assistant Professor of Immunobiology at Yale University whose laboratory investigates signaling in T cells from healthy subjects and patients with inherited immune disorders to dissect pathways critical for adaptive immunity. Carrie trained as a PhD student at Harvard University and as a postdoc at NIAID/NIH.

#### Anita Chandra

Anita Chandra graduated in Medical Sciences from the University of Cambridge, UK and went on to specialise in Clinical Immunology. She undertook her PhD at the Laboratory of Molecular Biology and is currently working as a Wellcome Trust funded Clinician Scientist between the Babraham Institute and Addenbrooke's Hospital, Cambridge.

#### Sergey Nejentsev

Sergey Nejentsev is a Wellcome Trust Senior Research Fellow and a medical geneticist at the University of Cambridge specialising in immune-mediated disorders. Currently, his group investigates genetic and functional mechanisms of susceptibility to infection. His studies led to the discovery of several novel primary immunodeficiencies, including APDS.

#### Alison Condliffe

Alison Condliffe is Professor of Respiratory Medicine at the University of Sheffield, where she leads the clinical Respiratory Immunology service. Her research focuses on PI3 kinases in immunity, particularly in the setting of APDS. She trained at Cambridge and Edinburgh, and undertook a Wellcome Fellowship at the Babraham Institute, Cambridge.

#### Klaus Okkenhaug

Klaus Okkenhaug is a group leader at the Babraham Institute in Cambridge. His research focuses on the role of PI3K in infection, immunity and cancer. Recent work from his lab has identified PI3K $\delta$  as a potential target for cancer immunotherapy and helped characterise the role of PI3K $\delta$  in APDS.

- PI3K $\delta$  is a key signal transduction node in cells of the immune system. This kinase complex is acutely activated in B cells and T cells after exposure to antigen and controls many aspects of lymphocyte development and differentiation, in part via the AKT, FOXO1 and mTOR pathways.
- Rare loss-of-function mutations in PI3K $\delta$  also cause immunodeficiency and immune-mediated pathologies, including colitis. The PI3K $\delta$  inhibitor idelalisib causes frequent colitis at doses tested in leukaemia/lymphoma trials, possibly due to effects on Treg.
- Activated PI3K $\delta$  Syndrome (APDS) is a newly described primary immunodeficiency caused by hyperactive PI3K $\delta$  signalling and resultant T cell senescence/death and impaired antibody responses. APDS is generally characterized by recurrent sinopulmonary infections with structural lung damage, viremia with herpes family viruses, lymphoproliferative disease, and increased risk of B cell malignancies.

- APDS1 patients have a heterozygous mutation in PIK3CD, the gene encoding the p110 $\delta$  catalytic subunit of PI3K $\delta$ , whereas APDS2 patients have a heterozygous mutation in PIK3R1, the gene encoding the p85 $\alpha$  regulatory subunit of PI3K $\delta$ . Both sets of mutations lead to higher intrinsic activity of PI3K $\delta$ .
- To date, most APDS patients have been treated with antibody replacement therapies and some with the mTOR inhibitor rapamycin. In the future, PI3K $\delta$  inhibitors may be used to treat APDS patients, possibly as the first example of targeted therapy against a hyperactive mutant kinase in primary immunodeficiency.