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PI3Kδ and primary immunodeficiencies

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Abstract

Primary immunodeficiencies are inherited disorders of the immune system, often caused by mutation of genes required for lymphocyte development and activation. Recently, several studies have identified gain-of-function mutations in the PI3K genes *PIK3CD* (p110δ) and *PIK3R1* (p85α) that cause a combined immunodeficiency syndrome, referred to as Activated PI3K Delta Syndrome (APDS) or p110δ-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI). Paradoxically, both loss- and gain-of-function mutations affecting these genes lead to immunosuppression, albeit via different mechanisms. Here we review the roles of PI3Kδ in adaptive immunity, describe the clinical manifestations and mechanisms of disease in APDS and highlight new insights into PI3Kδ gleaned from these patients, as well as implications of these findings for clinical therapy.

Introduction

APDS is among a growing number of newly defined primary immunodeficiency (PID) **[G]** 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 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 (Box 1). Increased susceptibility to viral infection and poor recall responses of memory T cells differentiate APDS from isolated hypogammaglobulinemia[1-4], hence APDS should be considered a combined immunodeficiency[5]. More than 100 patients have been reported so far with APDS, but the precise incidence is not yet known[6, 7].

APDS is caused by heterozygous gain-of-function (GOF) mutations in *PIK3CD* or *PIK3R1* that induce hyperactivation of the protein products p110δ or p85α, respectively[1-4]. The p85α regulatory subunit and p110δ catalytic subunit together form the heterodimeric lipid kinase PI3Kδ, which is engaged by multiple receptors in the immune system, including the BCR, TCR, and 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[8-10], and this apparent dichotomy, together with the clinical features of the affected patient groups, has informed our understanding of the role of PI3Kδ in immune cell development and function.

Overview of class I PI3Ks

The class IA PI3Ks comprise p110α, p110β or p110δ catalytic subunits, which form heterodimeric complexes with a p85 regulatory subunit; the sole class IB PI3K, p110γ, interacts with a p101 or p84 regulatory subunit (Table 1). P110α and p110β are broadly expressed, whereas p110γ and p110δ are found predominantly in leukocytes. Although there is significant 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[8, 11]. For example, p110α is activated by insulin-like receptors and regulates growth, metabolism and angiogenesis[11]. P110β can also contribute to metabolic signalling and has been shown to regulate responses of mouse neutrophils to antigen-antibody complexes[12, 13]. P110γ is highly expressed in myeloid cells and contributes to chemotactic responses as well as reactive oxygen species production in neutrophils[14]. Together with p110δ, p110γ is also important during pre-T cell development in the thymus[15]. P110δ, which is the focus of this review, is expressed highly both in lymphocytes and myeloid cells and is activated by antigen receptors, costimulatory, cytokine receptors and growth factor receptors[8].

Class I PI3Ks catalyse phosphorylation of PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3 (PIP_3), 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). In lymphocytes, BTK and ITK are PIP_3-responsive tyrosine kinases that contribute to the activation of phospholipase C-gamma (PLCγ) and other downstream signalling proteins (Figs 1, 2). The lipid phosphatase PTEN converts PIP_3 back to PtdIns(4,5)P_2 [8].

Class IA PI3K regulatory subunits are encoded by three different genes (*PIK3R1*, *PIK3R2* and *PIK3R3*)(Table 1). *PIK3R1* encodes p85α, p55α and p50α (each from an alternative transcription start

site), *PIK3R2* encodes p85β, and *PIK3R3* encodes p55γ [16]. These regulatory subunits have SH2 domains, which bind phosphorylated YXXM motifs of cell surface receptors and membrane-associated proteins. p85α, p55α, p50α and p85β are widely expressed, whereas p55γ is mainly expressed in the brain and testes [16]. Any of the class IA PI3K regulatory subunits can bind to p110α, p110β or p110δ without apparent selectivity. For simplicity, we refer to the different heterodimeric proteins as PI3Kα, PI3Kβ, PI3Kδ or PI3Kγ based on the constituent p110 subunit. PI3Kδ is best understood to comprise p85α with p110δ, but association between p110δ and any of the other class IA PI3K regulatory subunits is also possible. It is also important to recognise that p85α has many p110δ-independent functions as it can also bind p110α and p110β [16].

The class IA PI3K regulatory subunits influence the p110 catalytic subunits in three ways (Fig 3)[17]:

1) they prevent proteolytic degradation of p110
2) they inhibit p110 catalytic activity
3) recruit p110 to tyrosine phosphorylated proteins at the plasma membrane

Once the p85α SH2 domains are engaged by phosphotyrosines, the inhibitory contacts with p110 are relieved[17]. Thus, mutations in the *PIK3R1* gene can influence PI3K activity by allowing degradation of p110 or diminishing its recruitment to receptors (*PIK3R1* null or LOF mutations), or by releasing the inhibition of p110 (*PIK3R1* GOF mutations)(Fig. 3). In addition to the regulatory subunits, p110α and p110δ can bind Ras, whereas p110β binds to Rac or Cdc42. These small GTPases help tether p110 to the membrane once recruited to a receptor via its regulatory subunit[17, 18].

PI3Kδ in immune cell function: lessons from mouse studies

Prior to the description of APDS, most our knowledge of the role of PI3Kδ in immunity and infection was based on genetic and pharmacological studies using mouse models. APDS GOF mutations increase basal and stimulated PIP_3 levels and PIP_3-dependent signalling cascades in patient-derived lymphocytes[1-4], and the study of these patients may give us new insights into how the balance of PI3K activity regulates immune cellular functions. Herein we summarise what these studies in mice have taught us, before describing the immunological phenotypes of human patients with mutations in p85α or p110δ.

Loss of function of PI3Kδ in mouse B cells

In mice, early B cell development in the bone marrow is only mildly affected by the loss of p85α or p110δ[19-23], while combined loss of p110α and p110δ leads to a near complete block at the pro-B cell stage[24]. Nevertheless, mice lacking the p85α or p110δ subunits have fewer follicular B cells, lack marginal zone (MZ) and peritoneal B1 B cells, have reduced serum immunoglobulins, and respond poorly to vaccination[19-23]. PI3Kδ couples BCR activation to both PIP_3 production and downstream signalling events (Fig 1). PI3Kδ-deficient B cells fail to respond to mitogenic stimuli, but undergo class-switching (CSR) **[G]** in response to IL-4 and LPS *in vitro*[19-26]. However, mice lacking p110δ selectively in B cells are competent to produce high-affinity IgG antibodies in response to immunisation with NP-CGG (T cell-dependent, TD antigen **[G]**)[27] (but as we shall see later, germline-loss of p85α or p110δ leads to attenuated TD antibody responses). PI3Kδ activity intrinsic to B cells is required for T cell-independent (TI) antibody responses, however. This may be due in part to loss of B1 and MZ B cell subsets (which are the dominant B cell subsets that respond to TI antigens) in PI3Kδ-deficient mice[21, 22, 27, 28].

Consequences of hyperactive PI3Kδ signalling in B cells in mice

While there are several mouse models of LOF mutations in PI3Kδ, the phenotype of the GOF mutation in mouse PI3Kδ remains to be described. We can however, make inferences from other

models of hyperactive PI3K signalling in which Pten or Foxo is ablated in the germline or specifically in B cells or from mice expressing a membrane-bound form of p110α in B cells. FOXO transcription factors regulate the expression of genes involved in immunoglobulin gene recombination and development such as *RAG1, RAG2, IKAROS* and *IL7*α (Fig 1)[29-31]. Failure to undergo VDJ recombination because of elevated PI3K signalling can lead to a partial block during B cell development in the bone marrow[29, 30]. In addition, elevated PI3K signalling can increase the sensitivity of developing B cells to negative selection by self antigens[32]. Interference with RAG expression and/or negative selection may lead to the development of B cells with aberrant phenotypes, as discussed later for APDS patients.

Activation-Induced Cytidine Deaminase (AID) is the master regulator of CSR and somatic hypermutation **[G]** (SHM) [33]. Deletion of *Pten* or *Foxo1* in B cells impairs Ig class switching[26, 30, 34, 35], demonstrating that increased PI3K signalling in B cells antagonises this process. In *Foxo1*-deficient B cells activated *in vitro*, impaired CSR is partially due to negative regulation of AID transcription through the PI3K-FOXO1-AID axis; however, inefficient CSR was still observed in *Pten*-deficient B cells in the presence of ectopic AID, suggesting that PI3K signalling also regulates CSR by affecting AID function at the post-transcriptional level[26, 34, 35]. During the germinal centre (GC) 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 somatic hypermutation[36]. When, *Foxo1* was deleted specifically in (GC) **[G]** B cells, CSR was impaired despite normal *AICDA* transcription and AID protein expression. This suggests that either Foxo1 regulates targeting of AID to the immunoglobulin gene locus, that Foxo1 targets other genetic loci required for CSR and SHM, or that Foxo1 deletion in the GC affects expression of other proteins required for CSR[37, 38]. Moreover, *Foxo1* ablation or induction of PI3K activity in germinal centre B cells led to loss of GC dark zones due to aberrant trafficking of B cells at least in part as a consequence of lost expression of *Cxcr4* which is a target of Foxo1[37, 38]. Hence, failure to expand antigen-specific B cells that have undergone selection in the GC 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. PI3Kδ deficiency in mature B cells impairs TI antibody responses but does not affect CSR or SHM[27]. By contrast, hyperactivation of PI3K signalling in mature B cells interferes with CSR and SHM and expansion of antigen-specific B cells in the GC dark zones (Fig 4) [26, 34, 35].

PI3Kδ is required for mouse CD4$^+$ T cell differentiation and Treg function

If PI3Kδ-deficient B cells can undergo CSR, then why do PI3Kδ-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 PI3Kδ. Mutant mice in which ICOS has been uncoupled from PI3Kδ fail to develop follicular helper T cells (Tfh) **[G]** [39]. Similarly, deletion of the p110δ subunit in T cells interferes with the development of Tfh, leading to dramatic attenuation of T cell-dependent immune responses, including CSR and SHM by the B cells activated in absence of Tfh[27]. These results highlight a dual role for PI3Kδ in antibody production: inactivation of PI3Kδ in B cells leading to activation of Foxo transcription factors is a prerequisite for CSR and SHM[26, 34, 35], whereas the activation of PI3Kδ in Tfh is prerequisite for the provision of B cell help supporting CSR and SHM[27].

Naïve CD4 T cell differentiation towards Th2, Th17 and Th1 lineages is delayed or attenuated when PI3Kδ is inhibited[40-42]. This may reflect a key role for Foxo transcription suppressing Th differentiation, for instance by suppressing the *Ifng* gene[43], as well as the requirement for mTOR

activity to promote Th differentiation[44]. A reduction in Th2 responses underpins the resistance of PI3Kδ-deficient mice to experimental asthma, despite elevated IgE levels[25, 45]. Reduced Th17 responses may protect PI3Kδ-deficient mice from experimental autoimmune encephalitis, a mouse model of multiple sclerosis[46]. Although PI3Kδ deficient mice raise an impaired Th1 response after infections with *Leishmania monocytogenes*, PI3Kδ-deficient mice control Leishmania infections more effectively than WT mice, likely due to defects in a regulatory immune cell population[47].

PI3Kδ inhibition interferes with Foxp3$^+$ regulatory T cell (Treg) homeostasis and function[48]. PI3Kδ-deficient mice develop colitis because of inappropriate activation of effector T cells by gut microbes and PI3Kδ-deficient Treg fail to suppress experimental colitis[22, 48]. Patients taking the PI3Kδ inhibitor Idelalisib also develop colitis, probably in part as a result of reduced Treg function[49, 50]. However, PI3Kδ-deficent mice and mice lacking p110δ only in Treg mount a more effective immune response against cancer than do WT mice[51]. As with antibody production, these data highlight the dual nature of PI3Kδ, which is required both for optimal cytokine production by effector T cells and for effective Treg-mediated suppression. Whether PI3Kδ inhibition results in impaired or enhanced cell-mediated immune responses is context dependent and therefore difficult to predict (Fig 4). Interestingly, PI3Kδ inactivation results in hyper-responsiveness to toll like receptor ligands in DC and macrophages resulting in increased IL-12 production, which may further contributed to increased cell-mediated immune responses upon LOF of PI3Kδ [52].

PI3Kδ regulates mouse CD8$^+$ T cell effector functions

PI3Kδ-deficient CD8$^+$ T cells stimulated *in vitro* are characterised by reduced abundance of mRNAs associated with inflammation and cytotoxicity, such as *IFNγ, Gzmb* and *Pfn*[51, 53, 54]. By contrast, the expression of genes regulating the homing of T cells to the lymph nodes, such as *Sell* (CD62L), *Ccr7* and *Klf2* are increased in PI3Kδ-deficient CD8$^+$ T cells stimulated *in vitro*[55]. Thus, PI3Kδ can regulate the homeostatic trafficking of T cells to the lymph nodes and contributes to the reprogramming of CD8$^+$ T cells to acquire full effector functions and migrate to peripheral tissues.

PI3Kδ is required for optimal magnitude of CD8$^+$ T cell responses *in vivo*[53, 56]. Nevertheless, PI3Kδ-deficient CD8$^+$ T cells can respond to infection and become fully differentiated cytotoxic T cells that produce IFNγ and granzyme B required for the killing of virus-infected cells or tumours; this suggests that the transcriptional defects described *in vitro* can be, at least in part, be overcome by strong inflammatory stimuli *in vivo*[51, 53]. Moreover, long-term CD8$^+$ T cell memory responses are intact in PI3Kδ-deficient mice[53]. This is in part because during recall responses, the generation of CD8 effector T cells is reduced, whereas the generation of long-term memory T cells in the lymph nodes and bone marrow is preserved[53]. Similarly, the inhibition of the downstream kinase mTOR with low-dose rapamycin during vaccination or infection augments CD8 T cell memory at the expense of effector CD8+ T cells[57]. Hence, by promoting mTOR activity, PI3Kδ skews CD8$^+$ T cell differentiation in favour of effector T cells, but antagonises the generation of memory T cells. Thus, strong PI3Kδ activity is associated with effector T cell differentiation, whereas the maintenance of T cell memory requires suppression of PI3K signalling (Fig 4).

Consequences of hyperactive PI3K signalling in mouse T cells

The consequence of PI3Kδ hyperactivation in mouse T cells can be inferred from experiments using Pten-deficient or Foxo-deficient T cells. Pten deletion in early T cell development leads to immature T cell lymphoma and a hyperactivated T cell phenotype, characterised by increased secretion of effector T cell cytokines and autoimmunity[58]. Similar results were observed in a mouse expressing a deletion mutation of p85α which lacked inhibitor contacts with p110 [59]. Deleting Pten in mature CD4$^+$ T cells also engenders enhanced cytokine production and Th function, but did not induce T cell

transformation or autommunity[60]. Furthermore, genetic loss of *Foxo1* leads to the absence of memory $CD8^+$ T cells after infection[61]. Together, these data indicate a unique sensitivity to PI3Kδ-dependent T cell transformation during thymic development, where PI3Kδ signalling may also affect central tolerance to self-peptides. Overall, these studies suggest that unrestrained PI3K signalling in T cells lowers their threshold of activation.

Alterations in PI3Kδ signalling leads to PIDs in humans

Both LOF and GOF mutations that cause PID in humans have been described. Our understanding of the underpinning causes of these PIDs has been greatly aided by the investigations using mouse models described in the previous section, but have also furthered and challenged our understanding of PI3Kδ, as highlighted in Box 2 and described further below.

Loss of function of p85α or p110δ in humans

As with mouse T cells, inhibition of PI3Kδ activity in human T cells suppressed the expression of effector cytokines such as IFNγ, IL-4 and IL-17 [41]. A single patient with a homozygous *PIK3R1* mutation that generated a premature stop codon (resulting in the loss of p85α and markedly decreased expression of p110δ) presented with recurrent pneumonia associated with agammaglobulinemia and severe B cell lymphopenia due to a block in early B cell development[10]. Development of colitis in this patient was attributed to antibody-deficiency and consequence outgrowth of gut pathogens, but could also be due to Treg deficiency[10]. Similarly, one patient lacking p110δ 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[9]. Loss of p110δ was again associated with severe B cell lymphopenia and fewer memory T cells[9]. Thus, the two reported patients with loss of PI3Kδ 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 p110α and p110δ are inactivated in the B cell lineage[24], suggesting a redundancy between these isoforms in mice that is not reflected in humans. The inflammatory and autoimmune manifestations in PI3Kδ-deficient humans underscore the importance of PI3Kδ in maintaining self-tolerance, possibly associated with reduced Treg function. PI3Kδ is also required for the generation of reactive oxygen species (ROS) [G] by human neutrophils and treatment of patients with the PI3Kδ inhibitor idelalisib can lead to neutropenia and increased risk of infections [49, 62].

Activating PI3Kδ 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*[1, 2]. 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[1]. The US cohort were identified on the basis of persistent viremia with herpes-family viruses, which are commonly associated with altered T cell or NK cell function, in addition to frequent airway infections[2]. Because both B cells and T cells are affected, APDS should be characterised as a combined immunodeficiency[1-5].

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 GOF nature of the mutation was not elucidated[63]. Subsequently, a number of additional studies have identified APDS patients with mutations in PIK3CD[5, 7, 64-67] or PIK3R1[6, 68-71]. Patients with GOF mutations in either of these genes appear to largely phenocopy each other, despite the fact that PIK3R1 is ubiquitously expressed and

can pair with other catalytic subunits besides p110δ. There is some evidence for effects of the PIK3R1 mutation outside the immune system (e.g, short stature, Box 1)[72], but detailed analyses of effects of this p85α truncation on p110α or p110β have not yet been reported. In order to distinguish the PID caused by PIK3CD and PIK3R1 mutations respectively, the terms APDS1/PASLI-CD and APDS2/PASLI-R1 have been proposed[1-4]. Patients with APDS1 or APDS2 resemble each other biochemically and clinically, suggesting that the pathological features are a consequence of aberrant and hyperactive PI3Kδ signalling[1-4]. Here we use the generic terms APDS unless referring specifically to either. A milder form of APDS-like immunodeficiency has been described in Cowden disease, caused by heterozygous loss of the PIP3 phosphatase PTEN, though the increases in PIP3 from these patient T cells was less obvious than observed in APDS[73].

The most frequent mutation in *PIK3CD* (c.3061G>A) encodes a glutamic acid for lysine substitution at position 1021 (E1021K) of p110δ (Table 1). To date, this mutation has only been found in APDS patients and their affected family members but not among healthy unrelated subjects[1]. 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[1].

Increased lipid kinase activity of p110δ carrying the E1021K mutation was shown using recombinant proteins *in vitro* and by measuring PIP_3 and AKT phosphorylation in patient-derived T cells[1, 2]. The E1021K mutation is located in the C-terminal lobe of the kinase domain of p110δ, similarly to the oncogenic H1047R mutation of p110α, and enhances the membrane-association of p110δ *in vitro*, facilitating more effective phosphorylation of its lipid substrate PIP_2, hence lowering the threshold of activation for PI3Kδ[1, 17] (Fig 3). Other missense p110δ mutations, N334K, C416R and E525K, have also been shown to cause APDS, although they are less frequent than E1021K[2](Table 1). Interestingly, GOF mutations of the homologous amino acid residues of p110α (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 and hence increase p110 lipid kinase activity[17]; by implication a similar mechanism leads to enhanced PIP_3 accumulation in cells from APDS patients with the equivalent mutations and hence the immune modulation seen in APDS (Fig 3). APDS is thus distinct from most other PIDs in that it is the hyperactivation of signaling pathways, rather than inhibition, that leads to immune dysfunction. This distinction offers a therapeutic opportunity (see below).

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 p85α (del p.434 – 475), p55α and p50α[3, 4](Fig 3). These amino acids lie in the inter-SH2 domain that regulates activity of the catalytic p110 subunits[74]. Oncogenic mutations in this region result in mutant proteins that can bind p110 subunits but are less effective at inhibiting their enzymatic activity[74, 75]. Similar to mutations in the p110δ subunit, this is thought to lower the threshold of activation for PI3Kδ. The mutant $p85\alpha^{del434–475}$ protein (Fig 3, ΔEx11) could stabilize p110δ, which was expressed at near normal levels in patient cells, but its inhibitory function was impaired, leading to increased PI3Kδ activity[3, 4].

Thus, a number of different mutations in *PIK3R1* or *PIK3CD* lead to increased activity of the p85α/p110δ heterodimeric protein, either by disrupting inhibitory contacts between p85α and p110δ or by increasing affinity for the plasma membrane, promoting interaction with its lipid substrate and hence facilitating phosphorylation.

Activating PI3Kδ mutations lead to Impaired B cell function and vaccine responses

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

Most APDS patients have increased proportions of circulating transitional B cells **[G]**, reduced class switched memory B cells, and impaired vaccine responses[1,3]. *In vitro*, patient-derived B cells showed impaired CSR (consistent with the observed tendency to reduced IgG and increased IgM levels), but in contrast to the findings in mouse cells, this was not associated with reduced AID mRNA levels[2]. As noted above, it is possible that PI3K regulates AID function by post-transcriptional mechanisms as well as by regulation of expression[35]. Alternatively, the defective CSR in APDS patients could be due to defects in germinal centre Tfh cells[76], aberrant B cell maturation and/or defective migration of B cells during the germinal centre reaction in the spleen, as shown for *Foxo*-deficient B cells in mice[37, 38]. The basis for the increased percentage of circulating transitional B cells in APDS patients 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[1]. These findings are in marked contrast with the dramatic loss of the 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 APDS patients (Box 1), compatible with a significant defect in antibody-mediated immunity. However, the severity of respiratory infections and consequent structural lung damage do not correlate well with total B cell numbers or extent of immunoglobulin deficiency[6, 7] and immunoglobulin replacement therapy alone does not appear to limit progression of lung damage in APDS. One explanation for this apparent discrepancy is that PI3Kδ hyperactivation causes additional defects (such as the altered T cell functions described above, or innate immune cell dysfunction) not directly related to antibody production that also contribute to respiratory bacterial infections. For instance, PI3Kδ has been shown to promote ROS production by human neutrophils which could cause collateral damage if excessively produced during infections[62]. However, analysis of APDS patient neutrophils did not reveal obvious increase in ROS, or indeed in PIP_3 production, in response to stimulation with microbial peptides[1]. However, Staphylococcal skin infections and abscess formation[1, 65], as well as defective killing of mycobacteria by macrophages from an APDS patient[64] suggest abnormalities may indeed exist in the innate immune system which remain to be more completely qualified. Increased PI3K activity has been shown to compromise neutrophil migratory accuracy, and hence prolong the tissue-transit time, leading to increased opportunities for bystander tissue injury mediated by surface-associated neutrophil proteases[48]. Hence a wide range of impaired cellular functions, affecting innate and adaptive immune responses, may contribute to recurrent infection and bronchiectasis in APDS.

Activating PI3Kδ mutations cause T cell senescence

Peripheral blood analysis revealed a preponderance of effector-type T cells with a severe reduction in naïve T cells[1-4]. Freshly isolated peripheral blood cells demonstrated reduced secretion of cytokines and increased apoptosis upon TCR restimulation[1-3]. Unexpectedly, acute PI3Kδ inhibition in APDS patient cells reduced the induction of apoptosis, suggesting that PI3Kδ signalling contributes to previously unappreciated pro-apoptotic pathways in APDS T cells[1, 3]. However, T cell blasts that had escaped apoptosis and expanded after activation *in vitro* showed increased production of IFNγ, TNFα and granzyme B [2]. Thus, chronic hyperactivation of PI3K signalling promotes T cell differentiation

into terminal effectors with increased sensitivity to TCR-induced cell death and derangements in cytokine secretion.

Notably, the expression of CD57, a surface marker on CD8 T cells in a senescent state due to extreme telomere shortening[77], was consistently high on patient cells[2, 4]. Subsequent analyses confirmed shortening of telomere length in APDS patient lymphocytes[4], suggesting T cell senescence **[G]** contributes to immune dysfunction in APDS patients. Patients free from viraemia also presented with increased $CD57^{+}CD8$ T cells[2]; therefore, T cell senescence in APDS is likely to be distinct from T cell exhaustion driven by chronic viral infections. T cell senescence from telomere shortening results in cell cycle arrest while maintaining most other responses to antigen[78], whereas T cell exhaustion from chronic antigen stimulation results in upregulation of co-inhibitory receptors that broadly dampen TCR signalling and antigen responsiveness[79]. These findings point to *in-vivo* hyperproliferation (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 T cell effector differentiation.

T cells from APDS patients exhibit increased activity of mTOR[2], a key mediator of the switch from a catabolic naïve state to an anabolic effector state during a T cell response[80]. Increased glucose uptake is also observed in T cells from APDS patients as compared to healthy subjects[2, 4]. These findings indicate changes in T cell metabolism induced by hyperactive PI3K signalling may underlie the hyperproliferation that is associated with T cell senescence in APDS patients. Further studies will be needed to determine if the elevated mTOR activity is a direct consequence of increased PI3K activity or whether it also reflects the skewed effector phenotype of T cells in APDS patients. PI3Kδ inhibition did reduce, but not ablate, S6 phosphorylation in APDS T cells, confirming that PI3Kδ contributes to mTOR activity in these cells[4]. Unrestrained and prolonged PI3K and mTOR activity may hence drive APDS T cells towards senescence rather than allowing T cells to revert to a metabolically quiescent phenotype after antigen exposure.

The main clinical manifestation of abnormal T cell function in APDS is herpes viral infection. All *PIK3CD* mutation patients reported by Lucas *et al.*[3] experienced chronic EBV and/or CMV viremia; in other studies the occurrence of CMV/EBV was lower[1, 4-7], although Herpes simplex virus and Varicella-zoster virus infections were also noted. These inter-study differences may reflect the case-finding strategies, immune profiles or pathogen exposure. 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[7], perhaps suggesting impaired NK cell function, though this has yet to be confirmed experimentally.

Treatment options for APDS

As APDS patients often present with reduced IgG and/or respond poorly to vaccines, many are treated with immunoglobulin replacement therapy 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 initiated in childhood[6, 7]**.** Haematopoietic stem cell transplant (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[6, 7], the follow up of these patients is too short to make a definitive conclusion.

Rapamycin

Lucas and colleagues reported one patient treated with the mTOR inhibitor rapamycin, who showed a dramatic reduction in lymphadenopathy and hepatosplenomegaly[2]. 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, however, that PI3Kδ regulates other pathways besides mTOR, and conversely, that mTOR receives input from PI3K-independent pathways[8]. Moreover, mTOR regulates the expression of *Pten* such that treatment of T cells with rapamycin can actually increase PI3K signalling in T cells, potentially exacerbating aspects of hyperactive PI3Kδ signalling in APDS[81].

PI3Kδ inhibitors

The PI3Kδ inhibitor Idelalisib is licenced for use in chronic lymphocytic leukaemia and non-Hodgkin lymphoma[82, 83]. However, Idelalisib has a significant side-effect profile, including pneumonitis/pneumonia, transaminitis and colitis in up to 42% of patients treated[49]. Histologically the colitis is reminiscent of that seen in mice lacking functional PI3Kδ, suggesting it is an on-target class-effect rather than compound-specific[49]. It is possible that APDS patients will benefit from lower doses of PI3Kδ inhibitors than are required for 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 PI3Kδ inhibitor may avoid some of the adverse effects.

Two clinical trials of PI3Kδ inhibitors in APDS have been announced to date: NCT02435173 sponsored by Novartis for an oral PI3Kδ inhibitor and NCT02593539 sponsored by GSK for an inhaled PI3Kδ 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, potentially to limit progression of bronchiectasis.

Conclusions

GOF mutations in PI3Kδ lead to a range of T and B 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 PI3Kδ 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[84]. The therapeutic options for LOF of PI3Kδ may be limited to immunoglobulin replacement, bone marrow transplants and perhaps gene-therapy. Although these are options for APDS as well, existing (mTOR inhibitors) and emerging (PI3Kδ 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 of PI3Kδ leads to immunodeficiencies highlights the concept that this pathway must be modulated precisely and dynamically 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 4) [8]. 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 clinical heterogeneity of APDS patients? Are there mutations in other genes that lead to hyperactivation of PI3Kδ 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

PI3Kδ inhibitors restore normal immune function in APDS? The answers to these and further questions will required 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

	Catalytic Subunit	Tissue	Selected functions	
	Gene	**Protein**		
Class IA	PIK3CA	p110α	Ubiquitous	Metabolism, angiogenesis
	PIK3CB	p110β	Ubiquitous	Metabolism, neutrophil activation
	PIK3CD	p110δ	Haematopoietic, CNS	Immunity
Class IB	PIK3CG	p1110γ	Haematopoietic, heart	Immunity, metabolism, cardiac
	Regulatory Subunit		**Tissue**	**Selected functions**
	Gene	**proteins(s**		
Class IA	PIK3R1	p85α, p55a, p50a	Ubiquitous	Metabolism, Immunity
	PIK3R2	p85β	Ubiquitous	Metabolism, Immunity
	PIK3R3	p55γ	Brain, testes.	Unknown
Class IB	PIK3R5	p101	Haematopoietic	Immunity
	PIK3R6	p84 (aka p87)	Haematopoietic	Immunity
	APDS		**Number of cases reported**	**References**
APDS1	PIK3CD	N334K	1	[2]
		C416R	2	[65]
		E525K	7	[2]
		E1021K	60	[1, 2, 5, 7, 63-67]
APDS2	PIK3R1	del p.434-475	43	[3, 4, 6, 68-72]

There are three class IA PI3K catalytic subunits (p110α, p110β and p110δ) which can bind one of 5 SH2-domain containing regulatory subunits (p85α, p55 α, p50 α, p85β and p55γ). There is little evidence for any preferential binding by either of the catalytic subunits for either of the regulatory subunits. The class IB PI3K catalytic subunit p110γ can bind to either p84 or p101. The latter lack SH2 domains and are instead recruited to G-protein coupled receptors. To date, PIDs have been described with LOF or GOF mutations in *PIK3R1* or *PIK3CD*, affecting p85α and p110δ, respectively. Several amino acid substitution mutations have been described in PIK3CD. In PIK3R1, all mutations described that cause APDS2 results in alternative splicing such that the mature mRNA lacks sequence from exon 11. Although this is strictly speaking a loss of *inhibitory* function mutation, we describe it as a GOF mutation in this review as it leads to gain of PI3Kδ activity and is distinct from the complete LOF of PIK3R1 which leads to loss of PI3Kδ.

Figure 1. BCR signaling

In B cells, PI3Kδ is activated upon cross-linking of the BCR, after stimulation with IL-4 or by the chemokine CXCL13. The BCR coopts the co-receptor CD19 or the adapter protein BCAP, both of which have YXXM motifs to which the p85α SH2 domains can bind. The IL-4R coopts IRS1, which also has YXXM motifs. The mechanism whereby CXCR5 is coupled to PI3Kδ remains to be defined. B cells are unusual in that most cells use PI3Kγ or PI3Kβ to transmit signals from GPCRs. Key targets for PI3K signalling are FOXO1 and mTOR. FOXO1 is a transcription factor that activates the genes for 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, AICDA which encoded AID. AID is required for CSR and SHM. The amino acid sensor mTOR contributes to the growth and proliferation of B cells. All proteins labelled in blue have been affected by LOF mutations causing PID. Of these, only p85α and p110δ have also been affected by GOF mutations causing PID.

Figure 2. TCR signaling

In T cells, the TCR, the costimulatory receptor ICOS and the IL-2R can activate PI3Kδ, whereas PI3Kγ is activated by CXCR4 and other chemokine receptors. ICOS contains a YXXM motif in the cytoplasmic domain which is essential for ICOS-costimulation. How the TCR and IL-2R activate PI3Kδ remains incompletely understood. Via the inactivation of FOXO1, PI3Kδ contributes to the downregulation of the expression of IL7Ra and CD62L, preparing the T cell to exit the lymph nodes and circulate through the vascular systems and organs.

Figure 3. APDS mutations lower threshold of activation for PI3Kδ

Class IA PI3Ks are activated by recruitment to tyrosine kinase-associated receptors at the plasma membrane. The p85α regulatory subunit (p50 fragment containing the nSH2-iSH2-cSH2) shown here in gold) stabilizes the p110δ catalytic subunit (blue) through constitutive binding of the p85α inter-SH2 domain (coiled portion) to the p110δ adaptor-binding domain (ABD). Binding of the p85α SH2 domains to tyrosine-phosphorylated residues releases inhibitory contacts between the p85α SH2/inter-SH2 domains and the p110δ C2, helical, and kinase domains. Both the p85α N-and C-SH2 domains impose negative regulation of p110δ, whereas p110α is only affected by the N-SH2 domain (not shown). It is possible that the ΔEx11 mutation (purple) truncating the p85α inter-SH2 domain affects p110δ more than it affects p110α, hence the lack of more pleiotropic effects on growth and metabolism. Ras-GTP further tethers p110δ to the membrane by binding to the Ras-binding domain (RBD) of p110δ. GOF mutations in *PIK3R1* and *PIK3CD* increase kinase activity by interfering with inhibitory interactions between the p85α regulatory and p110δ catalytic subunit (ΔEx11, N334K, C416R, E525K), or by increasing the affinity of p110δ for the plasma membrane (E1021K). The E1021 K mutations may also interfere with inhibitor contacts from the p85α C-terminal SH2 domain (not shown)[1]. See ref ([17]) and references therein for further details of the structures and mechanisms of regulation of PI3Kδ.

Figure 4. Dynamic regulation of PI3Kδ signaling in the immune system

PI3Kδ activity needs to be dynamically regulated for normal immune cell function as some cell types and processes require high PI3Kδ activity, while other depend on low PI3Kδ activity, eg if they require Foxo1-dependent transcription of genes. Problems arise if cells cannot increase or suppress PI3Kδ activity, but rather, have chronically low or high activity as a consequences of mutations. In both cases, immunosuppression occurs. Illustrated are some key cell types and processes affected by high or low PI3Kδ activity, and the consequences of being locked in one state or the other.

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 associated with a high incidence of organ damage including hearing impairment and bronchiectasis (permanent airway scaring)[1-4]
- Severe, recurrent or persistent infections with herpes family viruses are common, in particular chronic EBV or CMV viremia, and HSV and VZV infections[1, 3-7]. Frequent isolates of some respiratory viruses such as adeno- and echovirus have also been described [1]
- Opportunistic infections are rare although a few patients have experienced recurrent viral warts or Molluscum contagiosum infections[49]
- An increased incidence of abscess formation, lymphadenitis and cellulitis with gram-positive bacteria (mainly *Staphylococcus aureus*), and defective killing of mycobacteria by macrophages from an APDS patient suggest a mild deficit in innate immunity[1, 64].
- Benign lymphoproliferation (lymphadenopathy, hepatosplenomegaly and focal nodular lymphoid hyperplasia) is a common feature of all studies of APDS to date.
- Histopathologically, lymphoid tissue demonstrates atypical follicular hyperplasia with attenuation of mantle zones in APDS1, and small B cell follicles in APDS2. Germinal centres were disrupted in both APDS1 and APDS2 by infiltrating T-cells (often PD1-positive)[6, 7].
- There is a high frequency of lymphoma in APDS, encompassing a wide range of histopathological patterns[1, 2, 7, 65, 67]
- Immune cytopenias (thrombocytopenia, haemolytic anaemia and neutropenia, and auto-immune-like solid organ conditions (e.g. juvenile arthritis, glomerulonephritis, thyroiditis and sclerosing cholangitis) have also been reported[7, 66], with a frequency of 34% in a cohort of 53 patients with APDS1 [7] and 17% in a cohort of 36 patients with APDS2 [6].
- Mild developmental delay has been observed in both APDS1 and APDS2 cohorts, with a higher incidence in APDS2 (31% versus 19%) [6, 7]
- Growth retardation is common in APDS2 [6, 71, 72] but does not seem to be a feature of APDS1, and may relate to the association of heterozygous PIK3R1 mutations with SHORT syndrome (Short stature, hyperextensibility of joints, hernia, ocular depression, Rieger anomaly, and teething delay)[85-88].

Box 2: Lessons learned from APDS

Although the normal physiological role of PI3Kδ has been extensively studied in mouse models, investigation of APDS patients has provided important new insights about the biology of this kinase in humans.

- Mutations causing LOF or GOF of PI3Kδ lead to immunodeficiency. This illustrates how this pathway needs to be dynamically regulated for normal immune cell function
- The previously reported roles for PI3Kδ in B cell function and humoral immunity did not predict the preponderance of transitional B cells observed in APDS patients.
- Defects in CSR not attributable to defective AID mRNA expression remain to be fully understood.

- Augmented PI3K results in loss of naïve cells and an *in-vivo* proliferative burst that causes lymphoproliferative disease and pushes 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 pro-apoptotic signalling role for PI3Kδ.
- The high proportion of patients with severe respiratory infections and bronchiectasis suggests a role for PI3Kδ in promoting inflammation of the lungs by mechanisms that are incompletely understood, but which may indicate a key role for PI3Kδ 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 [85-88]. It is unclear why the ΔEx11 mutations that cause APDS2 manifest primarily as PID; however, it is of interest to note at least one case where this mutation was linked with SHORT syndrome [71]. This indicates that PIK3R1 ΔEx11 may have distinct effects on different p110 isoforms in different tissues.

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Conflicts of interest.

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Glossary terms

APDS/PASLI

APDS stands for Activated PI3K Delta Syndrome whereas PASLI stands for P110δ-Activating mutation causing Senescent T cells, Lymphadenopathy, and Immunodeficiency. Both refer to mutation in the PIK3CD gene that lead to hyperactivation of the p110δ subunit of PI3Kδ. **APDS-2/PASI-R1** refer to splice mutations in PIK3R1 that lead to exon skipping resulting in truncated p85α protein with reduced inhibition of p110δ.

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

Immunoglobulin class-switch recombination (CSR)

The process by which a heavy-chain variable region gene segment attached to one heavy-chain constant region gene segment in the expressed heavy-chain gene is recombined with a downstream constant region gene segment to express a new antibody class.

T cell-independent antibody response (TI)

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

Primary Immunodeficiency (PID)

Inherited disorders of the immune system that lead to recurrent infections and/or immune dysregulation. Currently there are around 84,000 PID patients diagnosed worldwide.

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)

$CD4^+$ 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.

T_H0 cells

Transitional B cells

Immature B cells that have left the bone marrow for the spleen and are precursors for follicular B cells, Marginal Zone B cells and B1 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.