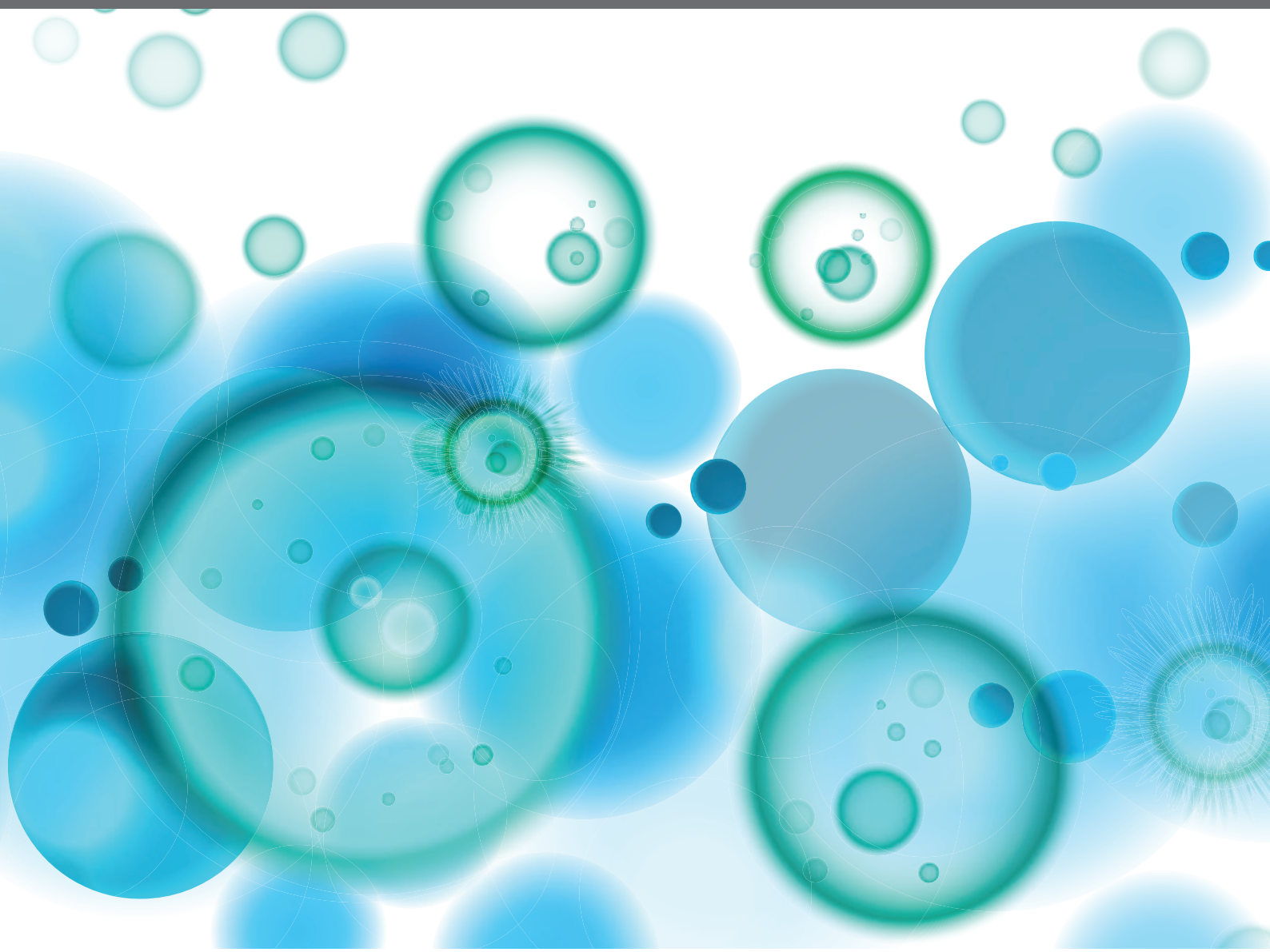
HUMAN DISORDERS OF PI3K BIOLOGY

EDITED BY: Carrie L. Lucas and Stuart G. Tangye
PUBLISHED IN: Frontiers in Immunology, Frontiers in Pediatrics and Frontiers in Genetics



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-378-1
DOI 10.3389/978-2-88966-378-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

HUMAN DISORDERS OF PI3K BIOLOGY

Topic Editors:
Carrie L. Lucas, Yale University, United States
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Citation: Lucas, C. L., Tangye, S. G., eds. (2021). Human Disorders of PI3K Biology. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-378-1

Table of Contents

frontiers in Immunology

EDITORIAL
published: 26 November 2020
doi: 10.3389/fimmu.2020.617464

Editorial: Human Disorders of PI3K Biology

Carrie L. Lucas[1]* *and Stuart G. Tangye*[2]*

[1] Department of Immunobiology, Yale University School of Medicine, New Haven, CT, United States, [2] Immunity & Inflammation Theme, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Keywords: PI3K, primary immunodeficiencies, Activated PI3Kδ Syndrome, immunology, human genetics

Editorial on the Research Topic

Human Disorders of PI3K Biology

The aim of this Research Topic was to bring together experts in basic, translational, and clinical research relating to phosphoinositide 3-kinase (PI3K) biology. The monogenic human immune disease "Activated PI3Kδ Syndrome" (APDS) has shed new light on immune functions of this kinase as well as its therapeutic targeting. PI3Ks have pleotropic effects across all cell types by phosphorylating PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3, a second messenger that recruits and activates signaling proteins to trigger cell growth, proliferation, and survival. PI3Kδ is comprised of the p110δ catalytic and the p85α regulatory subunits, encoded by *PIK3CD* and *PIK3R1*, respectively. This kinase complex can be targeted by multiple small molecule inhibitors, many of which are currently at various stages of clinical development. Heterozygous variants in *PIK3CD* or *PIK3R1* have been found to cause APDS, primarily by affecting inhibitory contacts between the two proteins. Defining the genetic etiology of this disorder has enabled rational targeted therapy to tune down PI3Kδ signaling.

These and related discoveries have not only advanced our understanding of human disease but also pointed to gaps in basic science knowledge regarding regulation of PI3K complexes and their activity. In this Research Topic, 13 manuscripts cover a range of subjects, mostly centered around findings in APDS and cancer. Michalovich and Nejentsev discuss genetic discovery as a basis for treatment of APDS, which includes hematopoietic stem cell transplantation (HSCT), management of infections/lymphoproliferation, and targeted inhibition of PI3Kδ in clinical trials. Dornan and Burke review structural biology concepts for Class IA PI3K variants in cancer and immunodeficiency. Various biochemical and biophysical studies have shown the intricate molecular mechanisms by which class IA PI3Ks are regulated via intra- and inter-subunit interactions between the catalytic and regulatory subunits. The differential expression of PI3Ks, in addition to their varied response to upstream activating stimuli, contributes to their regulation. Thus, further elucidation of these mechanisms is crucial as PI3Ks are linked to various human diseases ranging from developmental disorders, to cancer and immunodeficiencies.

Condliffe and Chandra review respiratory manifestations of APDS. The vast majority of APDS patients present with early-onset recurrent respiratory infections of bacterial and viral origin due to compromised immune responses, leading to complications such as bronchiectasis and small airway disease. Malignant or benign lymphoproliferative disease, along with other non-infectious conditions such as growth impairment, are also common in APDS patients. Maccari et al. provide a perspective piece on the European Society for Immunodeficiencies (ESID) APDS registry and highlight disease evolution and response to rapamycin. The chronology of

Edited and reviewed by:
Isabelle Meyts,
KU Leuven, Belgium

***Correspondence:**
Carrie L. Lucas
carrie.lucas@yale.edu
Stuart G. Tangye
s.tangye@garvan.org.au

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *14 October 2020*
Accepted: *04 November 2020*
Published: *26 November 2020*

Citation:
Lucas CL and Tangye SG (2020) Editorial: Human Disorders of PI3K Biology. Front. Immunol. 11:617464. doi: 10.3389/fimmu.2020.617464

presentation for these conditions usually begins with recurrent respiratory infections very early in life, followed by lymphoproliferative disorders, and then with gastrointestinal conditions and autoimmune cytopenias. Inhibition of mechanistic target of rapamycin (mTOR), a regulator of cell proliferation and growth downstream of PI3Kδ, with rapamycin (sirolimus) is effective at mitigating lymphoproliferative disease in APDS but has had limited effect on managing other features of disease. Coulter and Cant discuss in more depth potential therapeutic approaches. APDS patients have various clinical manifestations with some patients being asymptomatic while others exhibiting recurrent infections and antibody defects. Historically, conventional therapies such as immunoglobulin replacement therapy, HSCT, and antimicrobial prophylaxis have been used as treatments. However, the heterogeneity of disease presentation requires a more tailored approach which can be achieved through the use of selective PI3Kδ inhibitors such as Leniolisib.

Wentink et al. provide new data on the phenotype of $CD8^+$ T cells in APDS as it relates to exhaustion. As a contributing mechanism for increased susceptibility to infections and dysregulated immune responses, $CD8^+$ T cell exhaustion due to chronic T cell stimulation and proliferation is relevant for APDS pathology. Cannons et al. provide a perspective piece on the survival, differentiation, and function of $CD8^+$ T cells in APDS. Despite having a normal or even elevated frequency of Epstein-Barr Virus (EBV)–specific $CD8^+$ T cells, APDS patients have defects in controlling EBV and cytomegalovirus viremia. While seemingly not affecting the development of antigen-specific T cells, hyperactive PI3Kδ impacts $CD8^+$ T cell proliferation, differentiation, and survival, which have direct relevance for their function *in vivo*. Cohen covers consequences of herpesvirus infections in APDS in more depth. Herpesviruses can directly bind surface receptors that activate the PI3Kδ pathway and further modulate signaling through viral proteins. Together with compromised antibody production, cytokine secretion, and phagocytosis in APDS, these effects may contribute to the prevalence of uncontrolled herpesviruses in this disorder.

Mace addresses natural killer (NK) cells in the context of PI3K signaling and its role in the migration, activation, signaling and cytotoxicity of NK cells. Dysregulation of these pathways has important impacts on viral infections and malignancy. Fan and Turka discuss PI3K in regulatory T cells. In addition to the effects of APDS on the function of B cells, macrophages, and various other T cell compartments, recent studies have begun to elucidate the nuanced relationship between metabolic pathways and the function and lineage maintenance of Tregs as directed by IL-2 signaling through effects of PI3Kδ on FOXP3 expression. This highlights the possibility of targeting particular subsets of T cells based on their preferred metabolic pathways, potentially allowing for either strengthening or dampening Treg suppressive activities to combat autoimmune conditions or boost immune activation, respectively.

Asano et al. provide new data on APDS B cells and hyperactive PI3Kδ in this key cell type. The level of phosphorylated AKT (pAKT) is reported to be higher in unstimulated circulating B cells of patients with APDS compared to healthy controls, as assessed by phospho-flow cytometry. This may allow for the differentiation between the various forms of APDS resulting from different pathogenic variants. Since this assay does not require culturing patient cells, it opens the possibility for using the level of pAKT as a rapid diagnostic tool. Jhamnani et al. discuss class switch recombination (CSR) phenotypes in APDS. Since APDS patients exhibit defects in CSR with elevated IgM and low IgG, IgA, and IgE, it can be categorized within the spectrum of hyper-IgM syndromes.

Jung et al. review a set of immune diseases affecting function of mTOR. They discuss variants in genes encoding components of the PI3K/AKT/mTOR/S6 kinase (S6K) signaling pathway(s) that have been associated with primary immunodeficiencies. The overlapping immunodeficiency phenotypes observed in patients with impairment in these pathways led to the suggested disease category of "immune TOR-pathies."

Together, these articles address findings related to genetics, structural biology, clinical manifestations and treatments, CD8 T cell responses, NK cell biology, T regulatory cells, B cell abnormalities, and multiple precision treatment perspectives.

AUTHOR CONTRIBUTIONS

CLL and SGT wrote and edited the editorial. All authors contributed to the article and approved the submitted version.

FUNDING

CLL was funded by Yale University and NIAID/NIH grants R01AI138141 and R21AI144315. SGT is supported by grants awarded by the National Health and Medical Research Council of Australia.

ACKNOWLEDGMENTS

The topic editors thank the contributing authors as well as Saba Tegegne and Daniel Murdock for input.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 29 January 2018
doi: 10.3389/fimmu.2018.00069

Immunometabolism and PI(3)K Signaling As a Link between IL-2, Foxp3 Expression, and Suppressor Function in Regulatory T Cells

*Martin Y. Fan[1,2] and Laurence A. Turka[1,2]**

[1] Center for Transplantation Sciences, Department of Surgery, Massachusetts General Hospital, Boston, MA, United States, [2] Program in Immunology, Division of Medical Sciences, Harvard Medical School, Boston, MA, United States

CD4$^+$ Foxp3$^+$ regulatory T cells (Tregs) are an essential component of immune homeostasis. Modulation of Treg function has been proposed as a means of treating autoimmune conditions and preventing rejection of organ transplants, although achieving this goal will require a detailed understanding of Treg signaling pathways. Signaling within Tregs is known to differ considerably from that observed in other T cell subsets. Of note, Tregs are the only cell type known to constitutively express CD25, the main ligand-binding subunit of the IL-2 receptor. The PI(3)K/Akt/mTOR cascade constitutes a major signaling pathway downstream of IL-2 and is closely tied to cellular metabolism. Due to increasing recognition of the links between cellular fuel usage and immune cell function, the interplay between IL-2 signaling and Treg metabolism represents an important space for exploration and a potential approach for immunomodulation. Here, we discuss how IL-2 may affect Treg metabolism *via* PI(3)K signaling, as well as the effects of altered metabolism on Treg lineage stability and suppressor function.

Keywords: regulatory T cells, CD25, IL-2, metabolism, aerobic glycolysis, fatty acid oxidation, PI(3)K

OPEN ACCESS

Edited by:
Carrie L. Lucas, Yale University, United States

Reviewed by:
Megan K. Levings, University of British Columbia, Canada
Jose R. Regueiro, Complutense University of Madrid, Spain

****Correspondence:***
Laurence A. Turka
lturka@partners.org

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *26 November 2017*
Accepted: *10 January 2018*
Published: *29 January 2018*

Citation:
Fan MY and Turka LA (2018) Immunometabolism and PI(3)K Signaling As a Link between IL-2, Foxp3 Expression, and Suppressor Function in Regulatory T Cells. Front. Immunol. 9:69. doi: 10.3389/fimmu.2018.00069

INTRODUCTION

Regulatory T cells (Tregs) play a key role in maintaining immune homeostasis and in preventing the onset of autoimmune diseases (1). Modulation of Treg suppressor function is being actively explored as a promising new approach to treat autoimmunity (2–4), promote transplant tolerance (5, 6), and enhance anti-tumor responses (7, 8). Although several subsets of Tregs have been described, the best characterized is defined by the expression of CD4, CD25, and the transcription factor Foxp3 (9). The majority of circulating Tregs originate from the thymus and are termed "tTregs." Naïve CD4$^+$ T cells may also be induced to express Foxp3 in the periphery, thereby constituting a minority "pTreg" population (10) which is required for fetal tolerance (11). Although reports do not always specify which of the two populations is examined, any findings concerning "Tregs" most likely apply primarily to tTregs since they constitute the majority of Tregs in blood and secondary lymphoid organs. The importance of Tregs in maintaining peripheral tolerance is illustrated by the fact that mice (12) or humans (13) lacking Foxp3 suffer severe systemic autoimmunity. Similar, albeit less severe, autoimmune phenotypes are observed in mice (14) or humans (15) lacking CD25. Most Tregs constitutively express CD25 in addition to Foxp3, and it is generally believed that Tregs require continuous IL-2 signals through CD25 for their survival, lineage maintenance, and suppressor function (16, 17).

It is now appreciated that cell-intrinsic metabolic pathways directly impact cellular fate and function (18). Broadly speaking, aerobic glycolysis tends to support the function of pro-inflammatory cells, while fatty acid oxidation (FAO) tends to be used by anti-inflammatory cells such as Tregs (19). However, increasing evidence shows that Tregs also utilize aerobic glycolysis to achieve full suppressor function (20, 21). These metabolic programs are controlled in large part by the PI(3)K/Akt/mTOR signaling axis (22), offering multiple pharmacologic avenues to differentially target immune subsets depending on their metabolic preferences. Given the importance of CD25 for initiating PI(3)K signaling (23), in this review, we will focus on how IL-2 may interact with metabolism and the mechanisms through which metabolism influences Treg function. We touch on the difficulty of directly evaluating the interplay between IL-2 signaling, metabolism, and Treg function using existing germline knockout models and propose a means by which this issue can be addressed.

IL-2 AND Tregs

IL-2 Signaling

The IL-2 receptor is composed of three subunits: CD25, CD122, and CD132, which are, respectively, referred to as the α, β, and γ_c (also termed the common gamma chain) subunits (24). CD122 and CD132 are the sole mediators of downstream signaling and may form a heterodimer capable of low-affinity binding to IL-2 (16) (**Figure 1**). The alpha subunit CD25 does not signal, but is needed for high-affinity binding to IL-2. Most Tregs constitutively express all three subunits, while conventional $CD4^+$ and $CD8^+$ T cells constitutively express the CD122/CD132 dimer and only express CD25 upon activation. Conventional T cells begin producing IL-2 1 h after activation (25) and constitute the primary source of IL-2 *in vivo*. IL-2 activates three major signaling axes: the STAT5, PI(3)K, and MAPK/ERK pathways. STAT5 is particularly important for Treg development, as it is necessary to initiate Foxp3 expression (26).

The receptors for two other cytokines, IL-15 and IL-7, share subunits with the IL-2 receptor and partially compensate for losses of IL-2 or CD25 (27). The IL-15 receptor is a trimer that is strikingly similar to the IL-2 receptor, sharing the CD122 and CD132 subunits used for downstream signaling. Its alpha subunit CD215, like CD25, does not signal but instead confers high ligand affinity (28). On the other hand, the IL-7 receptor is a dimer composed of CD132 and a unique alpha subunit, CD127, which is capable of activating STAT5 (29).

IL-2 Is (Partially) Needed for Treg Development

Germline knockouts of IL-2 or its receptor components yield similar autoimmune phenotypes due to Treg deficiency (14, 30). Mice develop hemolytic anemia and colitis accompanied by thymic involution, lymph node hyperplasia, and splenomegaly, with elevated numbers of activated effector $CD4^+$ and $CD8^+$ T cells. Analogous findings have been reported in three clinical cases of CD25 loss (15, 31, 32), indicating that human Tregs are similarly dependent on CD25 and IL-2 signaling. These phenotypes are less severe than the scurfy phenotype resulting from Foxp3 deletion (33), most likely because the loss of IL-2 signaling also impacts effector T cells.

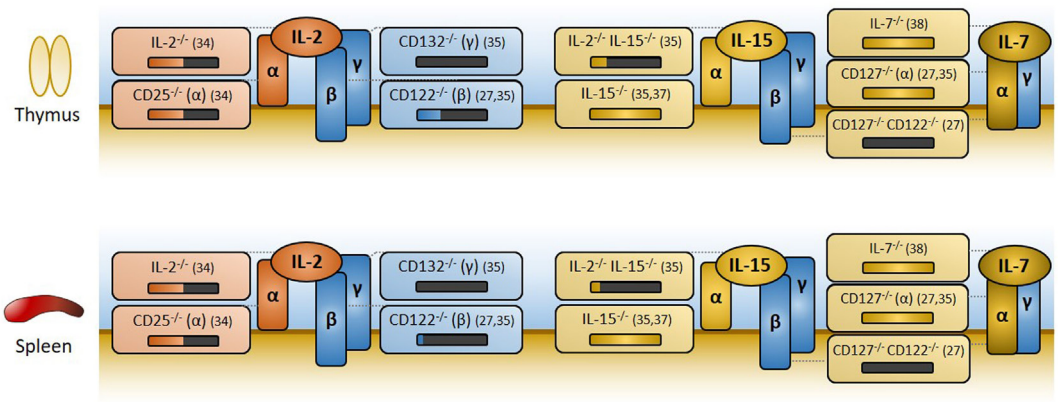


FIGURE 1 | Overview of IL-2, IL-15, and IL-7 receptor components, and effects of knockouts on regulatory T cell (Treg) generation. The IL-2 and IL-15 receptors are trimers with common β and γ_c subunits (CD122 and CD132, respectively) that mediate signaling. High ligand affinity is conferred by their α subunit (CD25 for IL-2, CD215 for IL-15) which does not signal. The IL-7 receptor is a dimer of CD127 (α) and CD132 (γ_c). Disruption of IL-2 signaling is detrimental to Treg development and subsequent Treg representation in the periphery, as measured by the percentage of $Foxp3^+$ cells among $CD4^+$ cells in the thymus and spleen, respectively. In the above figure, losses in Tregs are represented visually as black bars below knockout mouse genotypes, with relevant references for each knockout provided immediately to the right. Deletion of IL-2 or CD25 (IL-2Rα) leads to an approximate 50% reduction in $Foxp3^+$ cells. In the absence of IL-2 signaling, IL-15 or IL-7 appears to compensate, albeit imperfectly. Concomitant knockout of IL-2 and IL-15, or knockout of CD122 (the shared β subunit of both IL-2 and IL-15 receptors), exacerbates defects in Treg production. Removal of signaling through all three cytokines, whether through deletion of the common gamma chain CD132 (γ_c) or through the more targeted CD122/CD127 double knockout, virtually eliminates Treg development. Mice deficient in IL-15, IL-7, or CD127 (IL-7Rα) alone experience lymphopenia, but have normal percentages of $Foxp3^+$ cells among $CD4^+$ T cells and do not develop autoimmunity. Thus, IL-15 and IL-7 may partially compensate for Treg development in the absence of IL-2 signaling, but neither are required for Treg development when IL-2 signaling is fully functional.

The fact that IL-2 and CD25 knockout mice maintain appreciable numbers of Foxp3$^+$ cells in both thymus and spleen (34) indicates that IL-2 is not absolutely required for Treg development or subsequent survival, though it may be needed to achieve full suppressor function. The primary compensatory factor appears to be IL-15, as mice lacking both IL-2 and IL-15 are severely deficient in Foxp3$^+$ cells (as are mice lacking either of the shared CD122 or CD132 subunits) (35, 36). In the presence of IL-2, however, IL-15 and IL-7 are dispensable for Treg development and function: IL-15$^{-/-}$(37), IL-7$^{-/-}$ (38), and CD127$^{-/-}$ (27, 35) mice have normal percentages of Foxp3$^+$ cells and do not develop autoimmunity.

Post-Developmental Roles of IL-2 in Tregs

Although IL-2 signaling is an important component of Treg development (39), its roles following development are less thoroughly explored. It is generally believed that Tregs require constitutive IL-2 signals to survive and maintain Foxp3 expression, much in the same way these signals are needed during thymic development (40, 41). The role of IL-2 in Treg suppressor function has been difficult to address due to its roles in Treg survival during development. To date, the most prominent attempt to evaluate Treg function has been a Bim$^{-/-}$ IL-2$^{-/-}$ double knockout (42), in which targeting of the pro-apoptotic protein Bim was intended to decouple the roles of CD25 in Treg survival versus suppressor function. Although this study suggested that IL-2 is needed for full suppressor function, it should be noted that all germline knockout models of IL-2 signaling components are subject to a critical confounding factor: knockout mice develop lethal autoimmunity, which by its very nature is accompanied by immune activation and widespread inflammation. For this reason, it has been difficult to study how constitutive IL-2 signaling influences Treg lineage stability and function post development, much less study its effects on Treg metabolism.

Blocking antibody approaches can be dosed to avoid inducing autoimmunity. Although they are insufficient to address the issue of Treg function, due to off-target effects on effector T cells, these studies do not support IL-2 as a survival factor for Tregs. Anti-CD25 clone 7D4, widely used in commercial Treg magnetic isolation kits (43), induces loss of CD25 for up to 2 weeks following injection, yet Tregs persist and mice fail to develop autoimmunity (44, 45). It is critical to note that this antibody is distinct from anti-CD25 clone PC61, commonly as a tool to deplete Tregs *in vivo*, which is now recognized to act *via* opsonization for phagocytosis rather than through IL-2 deprivation (46–48).

PI(3)K SIGNALING IN Tregs

Because the role of IL-2-induced STAT5 signaling in Treg development has been reviewed extensively (16), here we focus on how lineage stability and suppressor function are influenced by metabolism in mature, post-developmental Tregs. PI(3)K catalyzes the conversion of PIP2 (PtdIns-4,5-P2) to PIP3 (PtdIns-3,4,5-P3) to permit activation of kinases with plextrin homology domains, most notably Akt. Targets of Akt include the protein translation regulator complex mTOR, which promotes cellular growth and survival (49). Thus, one major downstream effect of PI(3)K signaling is induction of aerobic glycolysis, which is increasingly emerging as a key control mechanism of Treg function (see below). The lipid phosphatase PTEN, which dephosphorylates PIP3 back into PIP2, and the protein phosphatase PHLPP, which dephosphorylates Akt, are the primary negative regulators of PI(3)K activity in T cells (50, 51). Excessive PI(3)K activity is detrimental to Tregs since loss of PTEN in mice (52, 53), loss of PHLPP in mice or in human cell culture (51), and induced Akt activation in human cell culture (54) all lead to Treg lineage instability and loss of suppressor function. Tregs may receive signals from three sources which would normally induce strong PI(3)K signaling: the TCR, CD28, and the IL-2 receptor (23). To prevent excessive PI(3)K signaling from these sources, Tregs express high levels of PTEN (55, 56) and PHLPP (51).

Treg METABOLISM

Glycolysis

Following immune cell activation by antigen or inflammatory signals, aerobic glycolysis and fatty acid synthesis are rapidly induced to support cell proliferation and cytokine secretion (57). This is reflected in the metabolic profiles of relevant immune subsets: effector T cells such as Th1, Th2, and Th17 cells show increased glycolytic rates following activation, as do effector CD8$^+$ T cells. Tregs, like memory CD8$^+$ T cells, rely on FAO for their basal metabolism but utilize some degree of aerobic glycolysis to properly execute their suppressor functions.

Beyond mere association with immune activation, several causal links have emerged between inflammatory stimuli, glycolysis, and Tregs (**Figure 2**). In T cells, signals through the TCR, CD28, or IL-2 activate the PI(3)K/Akt/mTOR cascade (58), which induces expression of the glucose transporter Glut1 to facilitate increased glycolysis (59). Akt also inhibits Foxo1 and Foxo3 transcription factors which are important for Foxp3 gene expression (60–62). mTOR engages Hif-1α, which may also be independently activated through toll-like receptor signaling, to promote the expression of key glycolytic genes (63). Hif-1α may also directly bind Foxp3 and target it for proteasomal degradation (64). Reciprocally, forced Foxp3 expression is sufficient to suppress glycolysis and promote FAO *in vitro* (20). Treg effector molecules such as CTLA4 and PD-1 suppress glycolysis in CD4$^+$ T cells by activating PTEN to antagonize PI(3)K signaling and subsequent glycolysis, with PD-1 also actively promoting FAO by increasing expression of CPT1A (65). These data suggest that elevated glycolysis is detrimental to Treg lineage stability and suppressor function.

However, most studies showing detrimental effects of glycolysis on Tregs were performed *in vitro*, where T cell activation and glycolysis were driven to their maximum extent. Under certain conditions, glycolysis actually supports Foxp3 expression, promotes Treg proliferation, and potentiates suppressor function. Among *in vitro* induced human Tregs, the glycolytic enzyme Enolase-1 binds the Foxp3 promoter and its CNS2 regions. This represses transcription of a splice isoform containing Exon 2 (Foxp3-E2), which is needed for optimal Treg suppressor function. Engaging glycolysis forces Enolase-1 into the cytoplasm,

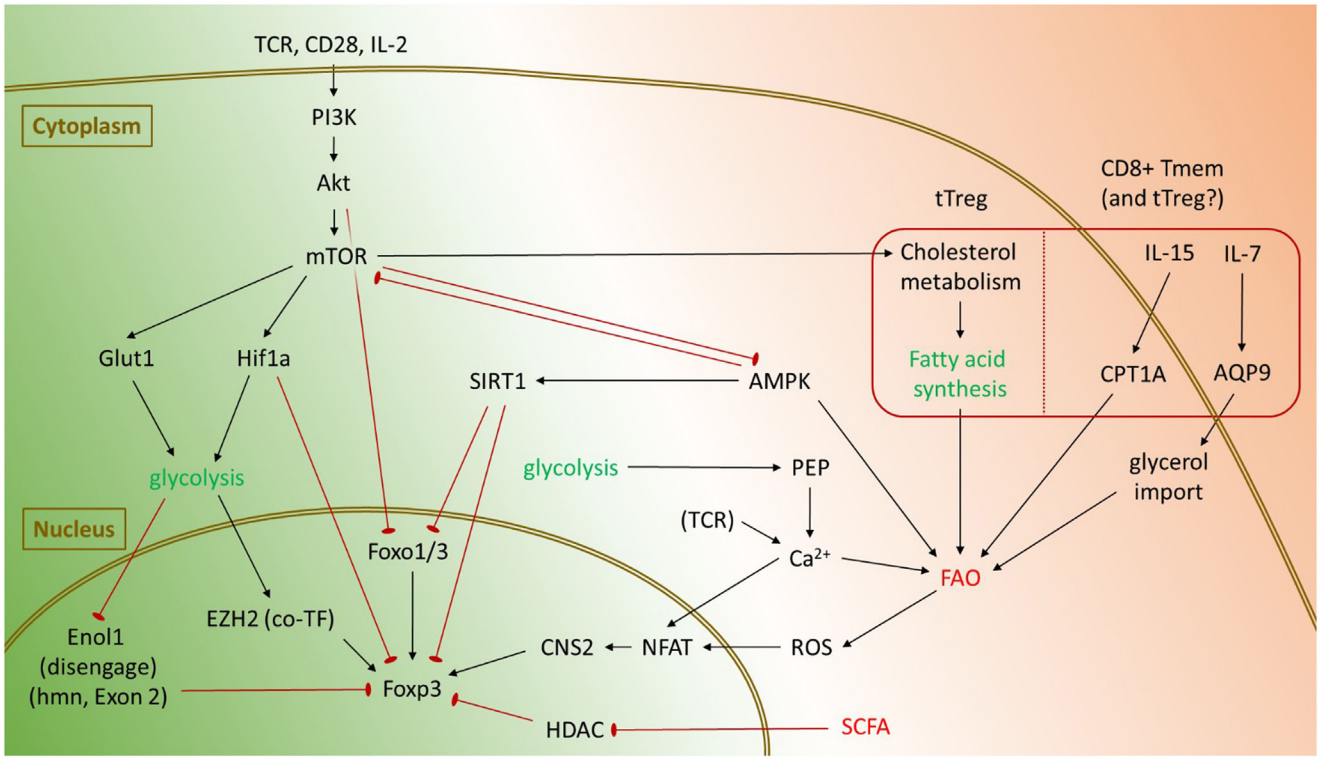


FIGURE 2 | Pathways promoting glycolysis and fatty acid oxidation (FAO) in regulatory T cells (Tregs), and known mechanisms affecting Foxp3. Glycolysis is primarily activated in Tregs through mTOR and tends to suppress Foxp3 expression and Treg lineage stability. Activation of the PI(3)K/Akt/mTOR signaling axis inhibits Foxo transcription factors and promotes activation of Hif-1α, which can directly target Foxp3 for degradation. However, under certain conditions, glycolysis also promotes Foxp3 expression. By disengaging Enolase 1 from its nuclear role, glycolysis enables expression of the Foxp3-E2 splice isoform in humans. Glycolysis also represses microRNAs such as miR-101 and miR-26a to enable expression of EZH2, which is a cotranscription factor for Foxp3. Tregs generally rely upon FAO for their metabolic needs. In the gut, short-chain fatty acids (SCFA) inhibit histone deacetylases (HDACs) to promote Foxp3 expression and conversion of naïve $CD4^+$ T cells into pTregs. Under certain conditions, FAO may also impinge upon Treg lineage stability. Sirt1 may repress Foxp3, either through direct deacetylation of Foxp3 or by targeting Foxo transcription factors. In $CD8^+$ memory T cells, cytokines such as IL-7 and IL-15 promote uptake of fatty acid precursors and increased FAO, respectively. It remains to be seen whether similar processes occur in Tregs as well. Both glycolysis and FAO can also promote Foxp3 expression through an NFAT-dependent mechanism.

thereby allowing transcription of Foxp3-E2 (66). Glycolysis also favors expression of the histone methyltransferase EZH2 by repressing inhibitory microRNAs such as miR-101 and miR-26a (67). EZH2 in turn binds Foxp3 to assist suppression of target genes (68), although no experiment has yet confirmed glycolysis-dependent EZH2 expression is essential for Treg lineage stability. The glycolytic metabolite phosphoenol pyruvate (PEP) can also increase Foxp3 expression through an NFAT-dependent mechanism. By inhibiting the calcium ATPase SERCA, PEP increases intracellular Ca^{2+} levels to promote nuclear translocation of NFAT, which facilitates interactions between the Foxp3 promoter and its CNS2 regions (69, 70).

A recent study suggests a possible resolution of these conflicting roles for glycolysis in Tregs. Using a Glut1 transgene to increase glucose uptake and glycolysis, the authors found that although elevated glycolysis boosts tTreg proliferation, it comes at the cost of their ability to execute suppressor functions (20). This suggests that for optimal Treg activity, a balance must be struck between the cell activating effects of glycolysis with its negative effects on the lineage.

Fatty Acid Oxidation

Fatty acid oxidation is generally associated with an anti-inflammatory phenotype and maintenance of Treg lineage stability. One mechanism is through simple antagonism of glycolysis: Tregs express high levels of AMPK, which simultaneously promotes FAO while inhibiting mTOR and subsequent glycolysis (71). In the gut, short-chain fatty acids are also known to inhibit mTOR (72). They have the added benefit of stabilizing pTregs by inhibiting histone deacetylases (HDACs) such as HDAC6 and HDAC9 which would otherwise inhibit Foxp3 expression (73, 74). Reactive oxygen species generated as a byproduct of oxidative phosphorylation have been shown to promote Foxp3 stability by increasing activity of the transcription factor NFAT, which binds the CNS2 enhancer of Foxp3 (70, 75). In addition, Foxp3 may experience post-transcriptional modifications such as acetylation, which prevents Foxp3 from being targeted for degradation thereby increasing its half-life (76). Foxp3 acetylation is dependent on nuclear availability of acetyl-CoA, whose supply is increased upon breakdown of fatty acids. As with glycolysis however, under certain conditions FAO may antagonize Treg

lineage stability. FAO promotes an increased NAD^+/NADH ratio, which elevates the activity of the deacetylase SIRT1 (77). By deacetylating Foxp3, SIRT1 promotes Foxp3 poly-ubiquitination and subsequent proteasomal degradation (78).

pTregs and tTregs diverge considerably in their execution of FAO: although pTregs generally rely upon exogenous fatty acids for their metabolic needs (79), it is uncertain whether tTregs can import exogenous fatty acids *in vivo* (18). While the coming years will likely clarify this issue, available literature suggests one peculiar metabolic feature among tTregs. One of the major roles for mTOR signaling in tTregs is to drive synthesis of endogenous fatty acid stores, primarily along cholesterol biosynthetic pathways (80). Whether these endogenously synthesized fatty acids are then used for energy is not known, although these specific pathways are needed for tTreg proliferation and optimal suppressor function. Memory $CD8^+$ T cells constitute the only major T cell subset known to synthesize endogenous fatty acids for subsequent FAO *in vivo* (18, 81) and rely in part on IL-7 and IL-15 to regulate these processes. IL-7 induces expression of the channel protein aquaporin 9, which facilitates glycerol import for fatty acid synthesis (82). IL-15 increases FAO by stimulating mitochondrial biogenesis and elevating expression of CPT1a, a key regulator of FAO (83). Given that Tregs appear to rely on IL-7 and/or IL-15 in the absence of IL-2, we speculate that tTregs from IL-2 or CD25 knockout mice may experience a shift from glycolysis to FAO, possibly with an associated loss of suppressor function. Whether similar events might occur in pTregs is unknown, although prior literature (11) suggests a loss of suppressor function in pTregs would result in increased fetal resorption among any IL-2 or CD25 knockout mothers which reach breeding age.

Therapeutic Interventions

One of the most exciting prospects of immunometabolism is developing therapeutic interventions which can selectively target T cell subsets. Since activated effector T cells are more reliant on glycolysis than Tregs, studies have examined whether inhibiting glycolysis might improve outcomes in mouse models of autoimmunity and transplant rejection. Blocking glycolysis with 2-DG (a competitive inhibitor of hexokinase), or with dichloroacetate (an inhibitor of PDHK isoforms) reduced the severity of experimental autoimmune encephalomyelitis with associated decreases in the percentage of Th17, but not Treg, cells (63, 84). Similar outcomes were reported following inhibition of another glycolytic enzyme, acetyl-CoA carboxylase 1 (ACC1), with sorafen A or with T cell specific genetic deletion of ACC1 (79). Furthermore, treatment with metformin (an agonist of AMPK, which increases fatty acid uptake and oxidation) reduced airway inflammation and fibrosis in a murine asthma model (85). In the transplant setting, treatment with 2-DG, metformin, and a glutamine uptake inhibitor DON prolonged allograft survival in heart and skin transplants, in part by suppressing the proliferation of antigen-specific T cells and by increasing the relative frequency of Tregs (86).

Conversely, interventions that promote glycolysis enhance immune function, presumably by increasing the proliferation and function of effector T cells while inhibiting Treg function. Pharmacological blockade or genetic loss of PTEN leads to Akt-dependent inhibition of Foxo3a and subsequent loss of Foxp3 and tumor regression (87). Furthermore, increasing glycolysis through forced expression of the glucose transporter Glut1 in Tregs exacerbated pathology in an adoptive transfer model of colitis (20). Tregs recovered from this system were also found to have lower levels of Foxp3 protein.

CONCLUSION AND FUTURE DIRECTIONS

The metabolic state of Tregs defies simple categorical explanations with regard to glycolysis and FAO. Although elevated glycolysis is generally associated with immune activation and can be detrimental to Treg lineage stability and function, controlled levels of glycolysis are necessary to sustain the same processes. The list of known links between metabolism and Treg function is far from complete, and the coming years will likely reveal other metabolic enzymes with moonlighting roles in Treg biology. In particular, the "futile cycle" approach of tTregs to FAO, and its preference for cholesterol synthesis may be a promising area of discovery.

Metabolic interventions offer a promising new approach to modulating Treg function and may be used to fine-tune therapies targeting other signaling pathways or used as a primary therapy in their own right. Of note, while there is clear potential for interplay between IL-2 signaling and immunometabolism through the PI(3)K/Akt/mTOR signaling cascade, to date no studies have specifically evaluated the effects of IL-2 signaling on Treg metabolism. In part, this is due to the inadequacies of germline knockout models to address this question. As mentioned before, such knockouts experience an autoimmune environment in which immune cells are already highly active and presumably glycolytic. It would be more appropriate to use a model in which Tregs can be inducibly made to lose IL-2 signaling while maintaining immune homeostasis. A tamoxifen-inducible CD25 knockout, with tamoxifen dosage adjusted to leave enough CD25-competent cells to prevent autoimmunity, would be well suited for this approach. Such studies would lay the framework for combination treatments in which metabolic interventions would be used with existing therapies such as CD25 blockade.

AUTHOR CONTRIBUTIONS

MF and LT conceived of and wrote the review.

ACKNOWLEDGMENTS

We thank B. Blazar, J. Bluestone, H. Chi, J. Rathmell, and members of the Turka laboratory for discussions.

FUNDING

The authors acknowledge financial support from the US National Institutes of Health (P01-HL018646 to LT, and T32-AI007529 to MF) and from the Herchel Smith Graduate Fellowship Program (MF).

REFERENCES

1. Sakaguchi S. Regulatory T cells: history and perspective. *Methods Mol Biol* (2011) 707:3–17. doi:10.1007/978-1-61737-979-6_1
2. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol Rev* (2008) 223:371–90. doi:10.1111/j.1600-065X.2008.00637.x
3. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol Rev* (2006) 212:8–27. doi:10.1111/j.0105-2896.2006.00427.x
4. Bluestone JA, Tang Q. How do CD4+CD25+ regulatory T cells control autoimmunity? *Curr Opin Immunol* (2005) 17(6):638–42. doi:10.1016/j.coi.2005.09.002
5. Di Ianni M, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* (2011) 117(14):3921–8. doi:10.1182/blood-2010-10-311894
6. Priyadharshini B, Turka LA. T-cell energy metabolism as a controller of cell fate in transplantation. *Curr Opin Organ Transplant* (2015) 20(1):21–8. doi:10.1097/MOT.0000000000000149
7. Nishikawa H, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Curr Opin Immunol* (2014) 27:1–7. doi:10.1016/j.coi.2013.12.005
8. Curiel TJ. Tregs and rethinking cancer immunotherapy. *J Clin Invest* (2007) 117(5):1167–74. doi:10.1172/JCI31202
9. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* (2003) 299(5609):1057–61. doi:10.1126/science.1079490
10. Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol* (2013) 14(4):307–8. doi:10.1038/ni.2554
11. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* (2012) 150(1):29–38. doi:10.1016/j.cell.2012.05.031
12. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat Immunol* (2003) 4(4):337–42. doi:10.1038/ni909
13. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* (2001) 27(1):20–1. doi:10.1038/83713
14. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* (1995) 3(4):521–30. doi:10.1016/1074-7613(95)90180-9
15. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J Allergy Clin Immunol* (2007) 119(2):482–7. doi:10.1016/j.jaci.2006.10.007
16. Malek TR. The biology of interleukin-2. *Annu Rev Immunol* (2008) 26:453–79. doi:10.1146/annurev.immunol.26.021607.090357
17. Benoist C, Mathis D. Treg cells, life history, and diversity. *Cold Spring Harb Perspect Biol* (2012) 4(9):a007021. doi:10.1101/cshperspect.a007021
18. Newton R, Priyadharshini B, Turka LA. Immunometabolism of regulatory T cells. *Nat Immunol* (2016) 17(6):618–25. doi:10.1038/ni.3466
19. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* (2016) 16(9):553–65. doi:10.1038/nri.2016.70
20. Gerriets VA, Kishton RJ, Johnson MO, Cohen S, Siska PJ, Nichols AG, et al. Foxp3 and toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat Immunol* (2016) 17(12):1459–66. doi:10.1038/ni.3577
21. Tanimine N, Turka LA, Priyadharshini B. Navigating T cell immunometabolism in transplantation. *Transplantation* (2017) Forthcoming. doi:10.1097/TP.0000000000001951
22. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* (2014) 15(3):155–62. doi:10.1038/nrm3757
23. Han JM, Patterson SJ, Levings MK. The role of the PI3K signaling pathway in CD4(+) T cell differentiation and function. *Front Immunol* (2012) 3:245. doi:10.3389/fimmu.2012.00245
24. Stauber DJ, Debler EW, Horton PA, Smith KA, Wilson IA. Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor. *Proc Natl Acad Sci U S A* (2006) 103(8):2788–93. doi:10.1073/pnas.0511161103
25. Sojka DK, Bruniquel D, Schwartz RH, Singh NJ. IL-2 secretion by CD4+ T cells in vivo is rapid, transient, and influenced by TCR-specific competition. *J Immunol* (2004) 172(10):6136–43. doi:10.4049/jimmunol.172.10.6136
26. Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, et al. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. *Blood* (2006) 108(5):1571–9. doi:10.1182/blood-2006-02-004747
27. Vang KB, Yang J, Mahmud SA, Burchill MA, Vegoe AL, Farrar MA. IL-2, -7, and -15, but not thymic stromal lymphopoeitin, redundantly govern CD4+ Foxp3+ regulatory T cell development. *J Immunol* (2008) 181(5):3285–90. doi:10.4049/jimmunol.181.5.3285
28. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat Rev Immunol* (2006) 6(8):595–601. doi:10.1038/nri1901
29. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu Rev Immunol* (2006) 24:657–79. doi:10.1146/annurev.immunol.24.021605.090727
30. Schorle H, Holtschke T, Hünig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* (1991) 352(6336):621–4. doi:10.1038/352621a0
31. Sharfe N, Dadi HK, Shahar M, Roifman CM. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc Natl Acad Sci U S A* (1997) 94(7):3168–71. doi:10.1073/pnas.94.7.3168
32. Goudy K, Aydin D, Barzaghi F, Gambineri E, Vignoli M, Mannurita SC, et al. Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity. *Clin Immunol* (2013) 146(3):248–61. doi:10.1016/j.clim.2013.01.004
33. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* (2001) 27(1):68–73. doi:10.1038/83784
34. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* (2005) 6(11):1142–51. doi:10.1038/ni1263
35. Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA. IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. *J Immunol* (2007) 178(1):280–90. doi:10.4049/jimmunol.178.1.280
36. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in Treg cell function. *Nat Immunol* (2016) 17(11):1322–33. doi:10.1038/ni.3540
37. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med* (2000) 191(5):771–80. doi:10.1084/jem.191.5.771
38. Peffault de latour R, Dujardin HC, Mishellany F, Burlen-Defranoux O, Zuber J, Marques R, et al. Ontogeny, function, and peripheral homeostasis of regulatory T cells in the absence of interleukin-7. *Blood* (2006) 108(7):2300–6. doi:10.1182/blood-2006-04-017947
39. Lio CW, Hsieh CS. A two-step process for thymic regulatory T cell development. *Immunity* (2008) 28(1):100–11. doi:10.1016/j.immuni.2007.11.021
40. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat Rev Immunol* (2011) 11(2):119–30. doi:10.1038/nri2916
41. Lin JX, Li P, Liu D, Jin HT, He J, Ur Rasheed MA, et al. Critical role of STAT5 transcription factor tetramerization for cytokine responses and normal immune function. *Immunity* (2012) 36(4):586–99. doi:10.1016/j.immuni.2012.02.017
42. Barron L, Dooms H, Hoyer KK, Kuswanto W, Hofmann J, O'Gorman WE, et al. Cutting edge: mechanisms of IL-2-dependent maintenance of functional regulatory T cells. *J Immunol* (2010) 185(11):6426–30. doi:10.4049/jimmunol.0903940

43. Miltenyi Biotec. *CD4+CD25+ Regulatory T Cell Isolation Kit Mouse*. (2006). Available from: http://www.miltenyibiotec.com/~/media/Images/Products/Import/0001100/IM0001159.ashx?force=1
44. Kohm AP, Mcmahon JS, Podojil JR, Begolka WS, DeGutes M, Kasprowicz DJ, et al. Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ T regulatory cells. *J Immunol* (2006) 176(6):3301–5. doi:10.4049/jimmunol.176.6.3301
45. Couper KN, Blount DG, De souza JB, Suffia I, Belkaid Y, Riley EM. Incomplete depletion and rapid regeneration of Foxp3+ regulatory T cells following anti-CD25 treatment in malaria-infected mice. *J Immunol* (2007) 178(7):4136–46. doi:10.4049/jimmunol.178.7.4136
46. Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. *Cancer Res* (1999) 59(13):3128–33.
47. Setiady YY, Coccia JA, Park PU. In vivo depletion of CD4+FOXP3+ Treg cells by the PC61 anti-CD25 monoclonal antibody is mediated by FcgammaRIII+phagocytes. *Eur J Immunol* (2010) 40(3):780–6. doi:10.1002/eji.200939613
48. Mohr F, Fischer JC, Nikolaus M, Stemberger C, Dreher S, Verschoor A, et al. Minimally manipulated murine regulatory T cells purified by reversible Fab multimers are potent suppressors for adoptive T-cell therapy. *Eur J Immunol* (2017) 47(12):2153–62. doi:10.1002/eji.201747137
49. Vanhaesebroeck B, Guillermet-guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* (2010) 11(5):329–41. doi:10.1038/nrm2882
50. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. *Oncogene* (2008) 27(41):5527–41. doi:10.1038/onc.2008.247
51. Patterson SJ, Han JM, Garcia R, Assi K, Gao T, O'Neill A, et al. Cutting edge: PHLPP regulates the development, function, and molecular signaling pathways of regulatory T cells. *J Immunol* (2011) 186(10):5533–7. doi:10.4049/jimmunol.1002126
52. Huynh A, Dupage M, Priyadharshini B, Sage PT, Quiros J, Borges CM, et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat Immunol* (2015) 16(2):188–96. doi:10.1038/ni.3077
53. Shrestha S, Yang K, Guy C, Vogel P, Neale G, Chi H. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. *Nat Immunol* (2015) 16(2):178–87. doi:10.1038/ni.3076
54. Crellin NK, Garcia RV, Levings MK. Altered activation of AKT is required for the suppressive function of human CD4+CD25+ T regulatory cells. *Blood* (2007) 109(5):2014–22. doi:10.1182/blood-2006-07-035279
55. Buckler JL, Walsh PT, Porrett PM, Choi Y, Turka LA. Cutting edge: T cell requirement for CD28 costimulation is due to negative regulation of TCR signals by PTEN. *J Immunol* (2006) 177(7):4262–6. doi:10.4049/jimmunol.177.7.4262
56. Bensinger SJ, Walsh PT, Zhang J, Carroll M, Parsons R, Rathmell JC, et al. Distinct IL-2 receptor signaling pattern in CD4+CD25+ regulatory T cells. *J Immunol* (2004) 172(9):5287–96. doi:10.4049/jimmunol.172.9.5287
57. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science* (2013) 342(6155):1242454. doi:10.1126/science.1242454
58. Newton RH, Turka LA. Regulation of T cell homeostasis and responses by pten. *Front Immunol* (2012) 3:151. doi:10.3389/fimmu.2012.00151
59. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20(1):61–72. doi:10.1016/j.cmet.2014.05.004
60. Kerdiles YM, Stone EL, Beisner DR, McGargill MA, Ch'en IL, Stockmann C, et al. Foxo transcription factors control regulatory T cell development and function. *Immunity* (2010) 33(6):890–904. doi:10.1016/j.immuni.2010.12.002
61. Ouyang W, Beckett O, Ma Q, Paik JH, Depinho RA, Li MO. Foxo proteins cooperatively control the differentiation of Foxp3+ regulatory T cells. *Nat Immunol* (2010) 11(7):618–27. doi:10.1038/ni.1884
62. Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV, et al. Novel Foxo1-dependent transcriptional programs control T(reg) cell function. *Nature* (2012) 491(7425):554–9. doi:10.1038/nature11581
63. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208(7):1367–76. doi:10.1084/jem.20110278
64. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* (2011) 146(5):772–84. doi:10.1016/j.cell.2011.07.033
65. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* (2015) 6:6692. doi:10.1038/ncomms7692
66. De Rosa V, Galgani M, Porcellini A, Colamatteo A, Santopaolo M, Zuchegna C, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat Immunol* (2015) 16(11):1174–84. doi:10.1038/ni.3269
67. Zhao E, Maj T, Kryczek I, Li W, Wu K, Zhao L, et al. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. *Nat Immunol* (2016) 17(1):95–103. doi:10.1038/ni.3313
68. DuPage M, Chopra G, Quiros J, Rosenthal WL, Morar MM, Holohan D, et al. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. *Immunity* (2015) 42(2):227–38. doi:10.1016/j.immuni.2015.01.007
69. Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* (2015) 162(6):1217–28. doi:10.1016/j.cell.2015.08.012
70. Li X, Liang Y, Leblanc M, Benner C, Zheng Y. Function of a Foxp3 cis-element in protecting regulatory T cell identity. *Cell* (2014) 158(4):734–48. doi:10.1016/j.cell.2014.07.030
71. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) 186(6):3299–303. doi:10.4049/jimmunol.1003613
72. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* (2015) 8(1):80–93. doi:10.1038/mi.2014.44
73. Beier UH, Wang L, Han R, Akimova T, Liu Y, Hancock WW. Histone deacetylases 6 and 9 and sirtuin-1 control Foxp3+ regulatory T cell function through shared and isoform-specific mechanisms. *Sci Signal* (2012) 5(229):ra45. doi:10.1126/scisignal.2002873
74. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341(6145):569–73. doi:10.1126/science.1241165
75. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* (2013) 38(2):225–36. doi:10.1016/j.immuni.2012.10.020
76. van Loosdregt J, Vercoulen Y, Guichelaar T, Gent YYJ, Beekman JM, van Beekum O, et al. Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood* (2010) 115(5):965–74. doi:10.1182/blood-2009-02-207118
77. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* (2009) 324(5930):1076–80. doi:10.1126/science.1164097
78. van Loosdregt J, Brunen D, Fleskens V, Pals CE, Lam EW, Coffer PJ. Rapid temporal control of Foxp3 protein degradation by sirtuin-1. *PLoS One* (2011) 6(4):e19047. doi:10.1371/journal.pone.0019047
79. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* (2014) 20(11):1327–33. doi:10.1038/nm.3704
80. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* (2013) 499(7459):485–90. doi:10.1038/nature12297
81. O'Sullivan D, Van der windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* (2014) 41(1):75–88. doi:10.1016/j.immuni.2014.06.005
82. Cui G, Staron MM, Gray SM, Ho PC, Amezquita RA, Wu J, et al. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. *Cell* (2015) 161(4):750–61. doi:10.1016/j.cell.2015.03.021
83. Van der windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+

T cell memory development. *Immunity* (2012) 36(1):68–78. doi:10.1016/j.immuni.2011.12.007

84. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, et al. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* (2015) 125(1):194–207. doi:10.1172/JCI76012
85. Park CS, Bang BR, Kwon HS, Moon KA, Kim TB, Lee KY, et al. Metformin reduces airway inflammation and remodeling via activation of AMP-activated protein kinase. *Biochem Pharmacol* (2012) 84(12):1660–70. doi:10.1016/j.bcp.2012.09.025
86. Lee CF, Lo YC, Cheng CH, Furtmuller GJ, Oh B, Andrade-Oliveira V, et al. Preventing allograft rejection by targeting immune metabolism. *Cell Rep* (2015) 13(4):760–70. doi:10.1016/j.celrep.2015.09.036
87. Sharma MD, Shinde R, Mcgaha TL, Huang L, Holmgaard RB, Wolchok JD, et al. The PTEN pathway in Tregs is a critical driver of the suppressive tumor microenvironment. *Sci Adv* (2015) 1(10):e1500845. doi:10.1126/sciadv.1500845

Conflict of Interest Statement: LT discloses personal or family financial interests with Third Rock Ventures, Tango Therapeutics, and Neon Therapeutics. LT has served as a consultant to Lycera, Solid Bio, UCB, and Intellia. M.F declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 23 February 2018
doi: 10.3389/fimmu.2018.00237

Herpesviruses in the Activated Phosphatidylinositol-3-Kinase-δ Syndrome

*Jeffrey I. Cohen**

Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

The phosphatidylinositol-3-kinase (PI3K)/Akt pathway is important for multiple stages of herpesvirus replication including virus entry, replication, latency, and reactivation. Recently, patients with gain-of-function mutations in the p110δ-catalytic subunit of PI3K or in the p85-regulatory subunit of PI3K have been reported. These patients have constitutively active PI3K with hyperactivation of Akt. They present with lymphoproliferation and often have infections, particularly recurrent respiratory infections and/or severe virus infections. The most frequent virus infections are due to Epstein–Barr virus (EBV) and cytomegalovirus (CMV); patients often present with persistent EBV and/or CMV viremia, EBV lymphoproliferative disease, or CMV lymphadenitis. No patients have been reported with CMV pneumonia, colitis, or retinitis. Other herpesvirus infections have included herpes simplex pneumonia, recurrent zoster, and varicella after vaccination with the varicella vaccine. Additional viral infections have included adenovirus viremia, severe warts, and extensive Molluscum contagiosum virus infection. The increased susceptibility to virus infections in these patients is likely due to a reduced number of long-lived memory CD8 T cells and an increased number of terminally differentiated effector CD8 T cells.

Keywords: phosphatidylinositol-3-kinase, Akt, PIK3CD, PIK3R1, APDS, PASLI, Epstein–Barr virus, cytomegalovirus

OPEN ACCESS

Edited by:
Carrie L. Lucas,
Yale University, United States

Reviewed by:
Silvia Clara Gilliani,
University of Brescia, Italy
Flore Rozenberg,
Université Paris Descartes, France

***Correspondence:**
Jeffrey I. Cohen
jcohen@niaid.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *24 November 2017*
Accepted: *26 January 2018*
Published: *23 February 2018*

Citation:
Cohen JI (2018) Herpesviruses in the Activated Phosphatidylinositol-3-Kinase-δ Syndrome. Front. Immunol. 9:237. doi: 10.3389/fimmu.2018.00237

INTRODUCTION

Viruses often exploit intracellular signaling pathways to facilitate entry, replication, latency, and reactivation. Among the many pathways that viruses manipulate is the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. This pathway has several important activities including inhibiting apoptosis, regulating the cell cycle, and enhancing protein synthesis, resulting in increased cell survival and control of cell growth (1). Activation of the pathway by the binding of viruses, growth factors, or cytokines to receptors on the plasma membrane results in the movement of the PI3K complex from the cytoplasm to the plasma membrane. Class I PI3K complexes are important for virus infection and consist of a regulatory subunit (p50, p55, or p85) and a catalytic subunit (p110α, β, γ, or δ). The interaction of phosphorylated tyrosines on receptors with the p85 subunit of PI3K reduces its inhibitory effect on the p110 subunit, resulting in the phosphorylation of phosphatidylinositol 4, 5-triphosphate (PIP_2) and the activation of downstream signaling molecules including PDK1, Akt, and mTOR. Mutations in the p85-regulatory subunit or the p110δ-catalytic subunit have been associated with immunodeficiencies often presenting with lymphoproliferative disease, recurrent respiratory infections, and severe herpesvirus infections (see below).

HERPESVIRUSES MODULATE THE PI3K PATHWAY

Eight herpesviruses infect humans: herpes simplex viruses (HSV) 1 and 2, varicella–zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesviruses (HHV) 6 and 7, and Kaposi's sarcoma-associated herpesvirus (KSHV). All herpesviruses infect and are shed from the epithelial cells, and all undergo latency and reactivation. HSV-1, HSV-2, and VZV establish latency in sensory neurons, CMV and HHV-6 in monocytes and CD34 cells, HHV-7 in CD4 cells, and EBV and KSHV in B cells. In healthy persons, HSV causes orolabial and genital herpes, VZV results in varicella and zoster, CMV, EBV, and HHV-6 cause mononucleosis, and HHV-6 and HHV-7 cause roseola. Each of the herpesviruses can result in a severe disease involving multiple organs in immunocompromised persons; CMV and EBV are frequently detected in the blood of immunocompromised persons. EBV is associated with lymphoproliferative disease in immunocompromised persons and B cell lymphoma, while KSHV is associated with Kaposi's sarcoma and primary effusion lymphoma. While antibody contributes to protection from initial infection with herpesviruses, T cells are critical for reducing the severity of infection and disease associated with reactivation. Thus, mutations in genes important for the function of T cells can impair the control of herpesviruses by the host.

The PI3K pathway has a critical role for herpesvirus infection as well as for the control of herpesviruses by the immune system (2–4). Accordingly, herpesviruses manipulate this pathway to enhance virus entry, replication, latency, and reactivation. The binding of HSV (5), CMV (6), EBV (7), and KSHV (8) to the cell results in the activation of PI3K/Akt. Several viral glycoproteins including HSV gD and gB (9), CMV gB (10), EBV gp350 (7), and KSHV gB (11), each of which is required for initial infection of cells, activate the PI3K/Akt pathway. Viral proteins expressed during infection of cells by HSV (12), VZV (13), CMV (14), EBV (15), and KSHV (16) activate PI3K/Akt. These include the first proteins expressed in infected cells, the immediate-early proteins, including CMV IE1 and IE2 (14), EBV Rta (15) and KSHV Rta (17) which activate the PI3K/Akt pathway.

The PI3K/Akt pathway is also important for maintaining latency in HSV (18), EBV (19), and KSHV (20). Proteins and RNAs expressed during latency including HSV LAT (21) and EBV LMP1 (22), LMP2 (23), and EBNA2 (24) all activate the PI3K/Akt pathway. In addition, this pathway is critical for the reactivation of HSV (18), EBV (25), and KSHV (26) from latency.

Several herpesvirus proteins including HSV VP11 (27), VZV ORF12 (13), EBV LMP1 (22), and KSHV K1 (28) directly interact with the p85-regulatory subunit of PI3K to activate the PI3K/Akt pathway. Additional herpesvirus proteins interact with other proteins in the PI3K/Akt pathway.

IMMUNODEFICIENCIES ASSOCIATED WITH MUTATIONS IN PIK3CD OR PIK3R1

Two laboratories (29, 30) independently reported a new immunodeficiency due to heterozygous gain-of-function mutations in the p110δ-catalytic subunit of PI3K, which is encoded by *PIK3CD*. Angulo et al. (29) reported a series of 17 patients with activated PI3K-δ syndrome (APDS) due to an E1012K mutation in *PIK3CD*. Lymphocytes from the patients had increased levels of activated Akt and phosphatidylinositol 3, 4, 5-triphosphate (PIP_3), and increased activation-induced cell death. The patients had lymphopenia, elevated levels of IgM and transitional B cells, and reduced levels of antibodies to *Streptococcus pneumoniae* and *Haemophilus influenzae* type B with a reduced number of circulating B cells and class-switched memory B cells. They also had repeated respiratory infections with damage to the lungs; some had severe virus infections. Lucas et al. (30) reported nine patients with p110δ-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI) with N334K, E525K, or E1012K mutations in *PIK3CD*. The patients' lymphocytes had an increased phosphorylation of Akt and mTOR, an increased number of senescent effector T cells and transitional B cells, and a reduced number of naïve T cells, CD4 cells, and class-switched memory B cells. The patients had lymphoid hyperplasia often with obstructive lymphoid nodules and recurrent sinopulmonary infections; several patients had autoimmune cytopenias. Two had EBV lymphomas and all had EBV and/or CMV viremia.

Two laboratories (31, 32) reported a new immunodeficiency due to heterozygous gain-of-function mutations in the p85-regulatory subunit of PI3K, which is encoded by *PIK3R1*. These patients' immune system and clinical phenotype were similar to those with *PIK3CD* mutations. Deau et al. (31) reported four patients with two different heterozygous splice mutations in *PIK3R1*, whose lymphocytes showed low numbers of memory B cells and naïve T cells, and increased levels of activated Akt, IgM, transitional B cells, senescent CD8 cells, and activation-induced cell death. One of the patients had CMV and EBV viremia and enterovirus enteritis. Lucas et al. (32) described four patients with heterozygous splice-site mutations in *PIK3R1*. Lymphocytes from the patients had an increased phosphorylation of Akt and an increased number of senescent effector T cells and CD8 cells; they had low numbers of CD4 cells and low levels of IgG. The patients had lymphoproliferative disease and frequent sinopulmonary infections.

With the recognition of similar phenotypes in patients with gain-of-function mutations in *PIK3CD* and *PIK3R1*, APDS and PASLI have now been divided into APDS1 and PASLI-CD when reporting patients with mutations in *PIK3CD*, and APDS2 and PASLI-R1 when reporting patients with mutations in *PIK3R1*. A different type of mutation was reported in *PIK3R1*. Conley et al. (33) reported a patient with a homozygous stop codon in the p85-regulatory subunit of PI3K. The patient had no B cells, normal numbers and activity of T cells, and no history of severe virus infection.

HERPESVIRUS INFECTIONS IN PATIENTS WITH GAIN-OF-FUNCTION PIK3CD MUTATIONS

The most frequent viral infectious complications associated with *PIK3CD* mutations have been EBV and CMV viremia and EBV lymphoproliferative disease or CMV lymphadenitis. In the first

report of gain-of-function *PIK3CD* mutations (29), patients were screened for frequent respiratory infections and family histories of increased susceptibility to infection; accordingly, all 17 had recurrent upper or lower respiratory tract infections. Four of the 17 patients had infections caused by EBV, CMV, VZV, or HSV, including one patient with HSV pneumonia. In the next report of *PIK3CD* gain-of-function mutations (30), patients were screened based on the persistence of CMV and EBV in the peripheral blood; all nine patients had EBV viremia, with peak viral loads ranging from <250 to 63,350 copies/μl in the blood (30). Two patients had EBV-positive B cell lymphomas; one had an EBV-diffuse B cell lymphoma and one had EBV-nodular sclerosis Hodgkin disease. In one patient without lymphoma, two other family members had EBV lymphoma. Interestingly, while patients had high EBV DNA levels in the blood and some developed EBV lymphomas, in the patients who had EBV-specific CD8 T cells quantified by staining with HLA tetramers specific for EBV lytic and latency proteins, normal or elevated numbers of EBV-specific CD8 T cells were noted. Two patients had CMV viremia and three had CMV lymphadenitis. While it is possible that chronic infection with CMV or EBV could have resulted in the observed senescence of CD8 T cells, there was no correlation with the EBV and CMV load in the blood and the number of senescent CD8 T cells.

Since these two reports were published, additional papers have reported persistent herpesvirus viremia or severe herpesvirus infections in patients with *PIK3CD* gain-of-function mutations (**Table 1**). Mutations at seven sites in *PIK3CD*—E81K, G124D, N334K, C416R, E525K, E1021K, and E1025G—have been reported in persons with herpesvirus viremia or severe virus infections. In the largest review to date, a total of 53 patients with *PIK3CD* gain-of-function mutations were reported, and 49% had persistent or recurrent herpesvirus infections (40). EBV viremia was detected in 26% (14/53) of the patients, and 6% were reported to have a disseminated infection. EBV was detected in multiple biopsies including lymph nodes, tonsils, and the gastrointestinal tract as well as in the cerebrospinal fluid. Seven patients had diffuse lymphadenopathy; EBV and/or CMV was detected by PCR in the blood of six of these patients. One case of EBV encephalitis was reported. Two patients had EBV lymphomas, one had Hodgkin lymphoma and one had diffuse large B cell lymphoma; both patients died. CMV viremia was reported in 15% (8/53) of patients, 4 of whom responded to ganciclovir. EBV and CMV coinfection was reported in four patients, one of whom had a lymph node biopsy that was positive for EBV, CMV, and HHV-6 by PCR. Severe or persistent HSV or VZV infections were detected in 21% (11/53) of patients. One patient had HSV pneumonitis and one had recurrent HSV keratitis. Varicella infections resulted in hospitalization of two patients, and two had recurrent zoster.

Two other patients have been reported with *PIK3CD* gain-of-function mutations and EBV lymphomas (30). One patient had fatal EBV lymphoproliferative disease (42), two had EBV lymphadenitis (42), and one had EBV encephalitis (40). Two developed varicella after receiving the varicella vaccine (39, 42) and one had varicella pneumonia (35). Interestingly, despite frequent reports of CMV viremia and lymphadenitis, severe complications of CMV including pneumonia, colitis, or retinitis have not been reported. The treatment of patients with CMV lymphadenitis is often unsatisfactory; while the disease responds to antiviral therapy, it often recurs when treatment is stopped.

HERPESVIRUS INFECTIONS IN PATIENTS WITH GAIN-OF-FUNCTION PIK3R1 MUTATIONS

In the first report of patients with gain-of-function *PIK3R1* mutations, Deau et al. (31) described four patients with recurrent respiratory bacterial infections, two of whom had EBV viremia and one of whom had both CMV and EBV viremia (9,300 and 1,500 copies/ml, respectively). In the next report of *PIK3R1* gain-of-function mutations, Lucas et al. (32) reported four patients, one of whom had CMV lymphadenitis. Since these two papers were published, additional papers have reported persistent EBV or CMV viremia or severe herpesvirus infections in patients with gain-of-function mutations in *PIK3R1* (**Table 2**). All patients with severe virus infections have had splice donor-site mutations resulting in loss of exon 11. In the largest series to date, Elkaim et al. (45) reported 36 patients with mutations in *PIK3R1*. EBV viremia was detected in 22% (8/36) of patients, 4 of whom were asymptomatic and 4 of whom had EBV lymphoproliferative disease. One of these patients had two episodes of EBV Hodgkin lymphoma. Asymptomatic CMV viremia was present in 17% (6/36) of patients, and two had CMV lymphadenitis. Two patients were hospitalized for severe VZV infections. In another report (47), a 15-year-old boy had CMV viremia and CMV lymphadenitis that was refractory to therapy. A lymph node biopsy showed 240,000 copies of CMV/mg of tissue and follicular hyperplasia. While he initially responded to ganciclovir and valganciclovir, his lymphadenopathy recurred associated with the obstruction of the upper airway. A repeat lymph node biopsy was CMV-positive, and he received additional valganciclovir and corticosteroids and had a good response, though he had intermittent low-grade CMV viremia. He later presented with recurrent massive lymphadenopathy, and a repeat lymph node biopsy was CMV-positive, and he was treated again with ganciclovir and valganciclovir. He relapsed once valganciclovir was stopped and when he became refractory to antivirals, hematopoietic stem cell transplantation was performed, and he responded well. Like patients with mutations in *PIK3CD*, no cases of severe CMV involving the lungs, colon, liver, or retina have been reported in patients with mutations in *PIK3R1*.

While most case reports of patients with EBV or CMV viremia did not indicate the level of viral DNA, in 13 patients with EBV viremia and 5 with CMV viremia and mutations in either *PIK3CD* or *PI3KR1*, the levels were quantified and expressed as copies of viral DNA/ml. In these cases, the mean and median EBV loads were 9,146 and 2,250 copies/ml, respectively, and the mean and median CMV loads were 2,749 and 1,211 copies/ml, respectively. Thus, the levels of EBV and CMV in the blood generally were not markedly elevated. EBV and CMV viremia were not reported as initiating with symptomatic primary infection; instead, they were associated with virus reactivation.

TABLE 1 | Viral infections in patients with germline gain-of-function mutations in *PIK3CD*.

Characteristic	Angulo et al. (29)	Lucas et al. (30)	Crank et al. (34)	Hartman et al. (35)	Kannan et al. (36)	Lawrence et al. (37)	Elgizouli et al. (38)	Dulau Florea et al. (39)[b]	Coulter et al. (40)[a]	Saerrini et al. (41)	Takeda et al. (42)	Chiriaco et al. (43)	Goto et al. (44)
Number of patients reported	17	9	3	5	1	1	5	10	53	1	3	1	1
Mutations	E1021K	N334K, E525K, E1021K	E1021K, C416R	E1021K	E1021K	NR	E1021K	E1021K, E525K, N334K, E1025G	E1021K, E525K	E1021K	G124D, E81K	E1021K	E1021K
EBV viremia	NR	9/9	1/3	NR	1/1	1/1	1/5	9/10	14/53	1/1	NR	1/1	1/1
EBV + lymphoma	NR	DLBCL in one, HL in one	NR	NR	NR	NR	NR	NR	DLBCL in one, HL in one	NR	NR	NR	NR
Other EBV disease	NR	NR	NR	NR	NR	NR	NR	NR	Encephalitis in one	NR	Two with lymphadenitis, one with fatal LPD	NR	lymphoid follicle in colon in one
CMV viremia	NR	2/8	NR	NR	0/1	1/1	1/5	4/10 viremia or lymphadenitis	8/53	NR	NR	NR	1/1
CMV lymphadenitis	NR	3/8	NR	NR	NR	1/1	NR	See above	NR	NR	NR	NR	NR
Other CMV diseases	NR	NR	NR	NR	NR	NR	One with systemic disease	NR	Four with systemic disease	NR	NR	NR	NR
Other severe herpesvirus infections	One with HSV pneumonia	NR	NR	One varicella pneumonia	NR	NR	NR	One with varicella after vaccine	Two with severe varicella, two with recurrent zoster, one with HSV keratitis, one with HSV pneumonitis	NR	Varicella after vaccine	NR	NR
Severe HPV infections	Severe warts in two patients	NR	NR	NR	NR	NR	NR	NR	Four with severe warts	NR	NR	NR	NR
Severe Molluscum contagiosum	NR	NR	NR	NR	NR	NR	NR	NR	Four with severe disease	NR	NR	NR	NR
Other viral infections	NR	NR	NR	NR	NR	NR	ADV viremia, norovirus GI disease for weeks	Two with ADV infection	Nine with ADV infection	NR	NR	NR	NR
CD4 cell numbers reduced	10/17	8/9	2/2	1/5	1/1	1/1	2/5	4/10	43/51	1/1	2/3	1/1	NR
CD8 cell numbers reduced	6/17	NR	0/1	NR	1/1	0/1	1/5	1/10	14/51	0/1	1/3	0/1	NR
NK cell numbers reduced	NR	2/9	0/1	NR	0/1	0/1	NR	NR	12/43	0/1	2/3	NR	NR

[a]*Twenty-five of the 53 patients were included in Angulo et al. (29).*

[b]*Five of the 10 patients were included in Lucas et al. (30).*

EBV, Epstein–Barr virus; DLBCL, diffuse large B cell lymphoma; HL, Hodgkin lymphoma; LPD, lymphoproliferative disease; EBER, EBV-encoded RNA; CMV, cytomegalovirus; HSV, herpes simplex virus; HPV, human papillomavirus; ADV, adenovirus.

TABLE 2 | Viral infections in patients with germline gain-of-function mutations in PIK3R1.

Characteristic	Deau et al. (31)	Lucas et al. (32)	Elkaim et al. (45)	Olbrich et al. (46)	Kuhlen et al. (47)	Bravo Garcia-Morato et al. (48)	Hauck et al. (49)
Number of patients reported	4	4	36[a]	2	1	2	3
Mutation	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11	Splice donor-site mutations resulting in loss of exon 11
EBV viremia	1/4	0/3	8/36	0/2	0/1	1/2	0/3
EBV + lymphoma	NR	0/3	One with HL	NR	NR	NR	1 DBCL
Other EBV diseases	NR	0/3	Four with EBV LPD	NR	NR	NR	NR
CMV viremia	1/4	0/3	6/35	2/2	1/1	0/2	NR
CMV lymphadenitis	NR	1/3	2	NR	1/1	NR	NR
Other severe herpesviruses	NR	NR	Two hospitalized for varicella	NR	NR	NR	NR
Other severe viral infections	Enterovirus enteritis	NR	One with measles encephalitis, two with chronic HBV, one with chronic HCV	ICU hospitalization for RSV	NR	NR	NR
CD4 cell numbers reduced	1/4	NR	8/23	0/2	NR	1/2	1/3
CD8 cell numbers reduced	0/4	NR	1/23	0/2	NR	0/2	0/3
NK cell numbers reduced	1/4	NR	NR	NR	NR	0/2	0/3

[a]*Eight of the 36 were reported in Deau et al. (31) or Lucas et al. (32).*

EBV, Epstein–Barr virus; HL, Hodgkin lymphoma; DBCL, diffuse large B cell lymphoma; LPD, lymphoproliferative disease; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; ICU, intensive care unit; RSV, respiratory syncytial virus.

OTHER VIRUS INFECTIONS IN PATIENTS WITH GAIN-OF-FUNCTION PIK3CD MUTATIONS

In the first report of gain-of-function *PIK3CD* mutations, two patients were described with severe warts (29); subsequently, two additional patients with severe warts have been reported (40). Four patients have been reported with severe Molluscum contagiosum infections (40), one with adenovirus viremia (38), and 11 others reported with adenovirus infections with virus isolated from the blood, bronchoalveolar lavage fluid, and/or stool (39, 40). One patient was reported with norovirus infection that lasted for several weeks and was associated with persistent diarrhea (38). CD4 T cell numbers were reduced in 72% of patients, and CD8 T cell and NK cell numbers were reduced in 27% of patients with severe virus infections and *PIK3CD* mutations (**Table 1**).

OTHER VIRUS INFECTIONS IN PATIENTS WITH GAIN-OF-FUNCTION PIK3R1 MUTATIONS

In the first report of gain-of-function *PIK3R1* mutations, one patient was reported with enterovirus gastroenteritis (31). In the largest report of *PIK3R1* mutations to date, Elkaim et al. (45) reported one patient with measles encephalitis and hydrocephalus and other patients with chronic hepatitis B and hepatitis C virus infections. In another report, one patient was hospitalized in the intensive care unit for bronchiolitis due to respiratory syncytial virus infection (46). CD4 T cell numbers were reduced in 32% of patients and NK cell numbers were reduced in 11% of patients with severe virus infections and *PIK3R1* mutations (**Table 2**).

MECHANISM FOR IMPAIRED CONTROL OF HERPESVIRUS INFECTIONS IN PATIENTS WITH PI3K MUTATIONS

Lucas et al. (30) found that patients with *PIK3CD* gain-of-function mutations had normal or high levels of EBV-specific CD8 T cells in the blood by tetramer staining, but that EBV-specific CD8 T cells were predominantly $CCR7^{-}CD45RA^{-}$ indicative of terminal effector memory cells and had more CD38 than control cells indicative of an increased activity. They postulated that the persistent hyperactivation of Akt results in an increase in the proliferation of CD8 T cells and an increase in the number of terminal differentiated effector CD8 T cells with a corresponding increase in senescent CD8 T cells and a decrease in long-lived memory CD8 T cells. Together, this may result in impaired control of EBV- and CMV-infected cells. The increased proliferation of EBV-infected B cells could also increase the risk for additional chromosomal mutations and result in an increased risk of EBV lymphomas. Interestingly, while older persons have a similar number of T cells than

younger persons, they have a higher frequency of senescent T cells (50, 51). Older persons also have higher levels of EBV and CMV in the blood than younger persons (52, 53), and older persons may develop EBV lymphoproliferative disorders in the absence of an immune deficiency disease (54). Thus, an increased number of senescent T cells has been associated with impaired control of EBV and CMV infections. Coulter et al. (40) also noted that a reduced number of CD4, CD8, or NK cells was not associated with herpesvirus infections in patients with *PIK3CD* mutations, indicating that a functional rather than a quantitative abnormality in lymphocytes was responsible for the infections.

Cytomegalovirus and EBV persist predominantly in the blood, lymph nodes, and spleen, and the disease is often associated with lymphadenopathy, lymphadenitis, or lymphomas involving the lymphoid tissues. By contrast, HSV and VZV are latent in the nervous system and most often result in disease involving the skin. CD8-naïve and central or effector memory T cells are the predominant T cell types in the blood, spleen, and lymph nodes, while tissue-resident memory T cells and terminally differentiated effector CD8 T cells are the predominant CD8 T cell subsets in the peripheral tissues including the skin (55, 56). In addition, the persistence of EBV and CMV in the blood allows for clonal expansions of T cells, with the persistence of memory T cells after initial infection. Thus, the increase in terminal differentiated effector CD8 T cells and the reduction in memory CD8 T cells in patients with mutations in *PIK3CD* or *PIK3R1* may allow EBV and CMV to proliferate in the blood and lymphoid organs while having less of an effect on HSV and VZV in the skin. Furthermore, the reduction in memory CD8 T cells in the blood and lymphoid tissues may allow EBV and CMV to proliferate to higher levels resulting in viremia, lymphadenitis, and EBV lymphoma. In addition, the increased numbers of terminal differentiated effector CD8 T cells in patients with *PIK3CD* or *PIK3R1*, which are generally more often present in the peripheral tissues than in the blood, may have a protective effect from CMV involvement of the lungs, colon, and liver in these patients.

SCREENING AND TREATMENT

Since EBV viremia was detected in 46 and 22% of patients with mutations in *PIK3CD* and *PIK3R1*, respectively, and CMV viremia was detected in 20 and 21% of patients with mutations in *PIK3CD* and *PIK3R1*, respectively, testing for mutations in these two genes should be considered in patients with unexplained EBV or CMV viremia. The frequency of other virus infections in these patients is much lower, and therefore screening for mutations in *PIK3CD* or *PIK3R1* would be less likely to be useful.

The treatment of patients with *PIK3CD* mutations with a PI3K inhibitor, leniolisib (57), or with an mTOR inhibitor, rapamycin (30, 40) reduced lymphoproliferation and the number of senescent T cells and increased the number of naïve T cells. Despite its immunosuppressive effects, patients with *PIK3CD* mutations treated with leniolisib did not have an increase in EBV or CMV viremia while on therapy (57). Similarly, complications associated with EBV or CMV have not been reported in patients treated with rapamycin (30, 40). Rapamycin has been shown to inhibit the development of EBV-positive B cell lymphomas in a mouse model (58) and has been associated with the resolution of EBV-positive lymphoproliferative disease in patients (59). Similarly, rapamycin modestly reduces CMV replication *in vitro* (60), and the use of rapamycin instead of other immunosuppressant drugs has resulted in the reduction in CMV infection and disease (61). Thus, despite its immunosuppressive activities, rapamycin or PI3K inhibitors do not appear to increase the risk of EBV or CMV disease in patients with *PIK3CD* mutations.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

FUNDING

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell* (2017) 170:605–35. doi:10.1016/j.cell.2017.07.029
2. Liu X, Cohen JI. The role of PI3K/Akt in human herpesvirus infection: from the bench to the bedside. *Virology* (2015) 479-480:568–77. doi:10.1016/j.virol.2015.02.040
3. Diehl N, Schaal H. Make yourself at home: viral hijacking of the PI3K/Akt signaling pathway. *Viruses* (2013) 5:3192–212. doi:10.3390/v5123192
4. Dunn EF, Connor JH. HijAkt: the PI3K/Akt pathway in virus replication and pathogenesis. *Prog Mol Biol Transl Sci* (2012) 106:223–50. doi:10.1016/B978-0-12-396456-4.00002-X
5. MacLeod IJ, Minson T. Binding of herpes simplex virus type-1 virions leads to the induction of intracellular signalling in the absence of virus entry. *PLoS One* (2010) 5:e9560. doi:10.1371/journal.pone.0009560
6. Johnson RA, Wang X, Ma XL, Huong SM, Huang ES. Human cytomegalovirus up-regulates the phosphatidylinositol 3-kinase (PI3-K) pathway: inhibition of PI3-K activity inhibits viral replication and virus-induced signaling. *J Virol* (2001) 75:6022–32. doi:10.1128/JVI.75.13.6022-6032.2001
7. Barel M, Balbo M, Le Romancer M, Frade R. Activation of Epstein–Barr virus/C3d receptor (gp140, CR2, CD21) on human cell surface triggers pp60src and Akt–GSK3 activities upstream and downstream to PI 3-kinase, respectively. *Eur J Immunol* (2003) 33:2557–66. doi:10.1002/eji.200324059
8. Naranatt PP, Akula SM, Zien CA, Krishnan HH, Chandran B. Kaposi's sarcoma-associated herpesvirus induces the phosphatidylinositol 3-kinase–PKC-zeta–MEK–ERK signaling pathway in target cells early during infection: implications for infectivity. *J Virol* (2003) 77:1524–39. doi:10.1128/JVI.77.2.1524-1539.2003
9. Cheshenko N, Trepanier JB, Stefanidou M, Buckley N, Gonzalez P, Jacobs W, et al. HSV activates Akt to trigger calcium release and promote viral entry: novel candidate target for treatment and suppression. *FASEB J* (2013) 27:2584–99. doi:10.1096/fj.12-220285
10. Cobbs C, Khan S, Matlaf L, McAllister S, Zider A, Yount G, et al. HCMV glycoprotein B is expressed in primary glioblastomas and enhances growth and invasiveness *via* PDGFR-alpha activation. *Oncotarget* (2014) 5:1091–100. doi:10.18632/oncotarget.1787
11. Sharma-Walia N, Naranatt PP, Krishnan HH, Zeng L, Chandran B. Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 envelope glycoprotein gB induces the integrin-dependent focal adhesion

kinase–Src–phosphatidylinositol 3-kinase–rho GTPase signal pathways and cytoskeletal rearrangements. *J Virol* (2004) 78:4207–23. doi:10.1128/JVI.78.8.4207-4223.2004
12. Laing JM, Smith CC, Aurelian L. Multi-targeted neuroprotection by the HSV-2 gene ICP10PK includes robust bystander activity through PI3-K/Akt and/or MEK/ERK-dependent neuronal release of vascular endothelial growth factor and fractalkine. *J Neurochem* (2010) 112:662–76. doi:10.1111/j.1471-4159.2009.06475.x
13. Liu X, Cohen JI. Varicella–zoster virus ORF12 protein activates the phosphatidylinositol 3-kinase/Akt pathway to regulate cell cycle progression. *J Virol* (2013) 87:1842–8. doi:10.1128/JVI.02395-12
14. Yu Y, Alwine JC. Human cytomegalovirus major immediate-early proteins and simian virus 40 large T antigen can inhibit apoptosis through activation of the phosphatidylinositide 3′-OH kinase pathway and the cellular kinase Akt. *J Virol* (2002) 76:3731–8. doi:10.1128/JVI.76.8.3731-3738.2002
15. Darr CD, Mauser A, Kenney S. Epstein–Barr virus immediate-early protein BRLF1 induces the lytic form of viral replication through a mechanism involving phosphatidylinositol-3 kinase activation. *J Virol* (2001) 75:6135–42. doi:10.1128/JVI.75.13.6135-6142.2001
16. Montaner S, Sodhi A, Pece S, Mesri EA, Gutkind JS. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor promotes endothelial cell survival through the activation of Akt/protein kinase B. *Cancer Res* (2001) 61:2641–8.
17. Li X, Chen S, Sun R. Cdk1 inhibition induces mutually inhibitory apoptosis and reactivation of Kaposi's sarcoma-associated herpesvirus. *J Virol* (2012) 86:6668–76. doi:10.1128/JVI.06240-11
18. Camarena V, Kobayashi M, Kim JY, Roehm P, Perez R, Gardner J, et al. Nature and duration of growth factor signaling through receptor tyrosine kinases regulates HSV-1 latency in neurons. *Cell Host Microbe* (2010) 8:320–30. doi:10.1016/j.chom.2010.09.007
19. Lam N, Sugden B. CD40 and its viral mimic, LMP1: similar means to different ends. *Cell Signal* (2003) 15:9–16. doi:10.1016/S0898-6568(02)00083-9
20. Sharma-Walia N, Patel K, Chandran K, Marginean A, Bottero V, Kerur N, et al. COX-2/PGE2: molecular ambassadors of Kaposi's sarcoma-associated herpes virus oncoprotein-v-FLIP. *Oncogenesis* (2012) 1:e5. doi:10.1038/oncsis.2012.5
21. Li S, Carpenter D, Hsiang C, Wechsler SL, Jones C. Herpes simplex virus type 1 latency-associated transcript inhibits apoptosis and promotes neurite sprouting in neuroblastoma cells following serum starvation by maintaining protein kinase B (Akt) levels. *J Gen Virol* (2010) 91:858–66. doi:10.1099/vir.0.015719-0
22. Dawson CW, Tramountanis G, Eliopoulos AG, Young LS. Epstein–Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. *J Biol Chem* (2003) 278:3694–704. doi:10.1074/jbc.M209840200
23. Fukuda M, Longnecker R. Epstein–Barr virus latent membrane protein 2A mediates transformation through constitutive activation of the Ras/PI3-K/Akt pathway. *J Virol* (2007) 81:9299–306. doi:10.1128/JVI.00537-07
24. Spender LC, Lucchesi W, Bodelon G, Bilancio A, Karstegl CE, Asano T, et al. Cell target genes of Epstein–Barr virus transcription factor EBNA-2: induction of the p55alpha regulatory subunit of PI3-kinase and its role in survival of EREB2.5 cells. *J Gen Virol* (2006) 87:2859–67. doi:10.1099/vir.0.82128-0
25. Goswami R, Gershburg S, Satorius A, Gershburg E. Protein kinase inhibitors that inhibit induction of lytic program and replication of Epstein–Barr virus. *Antiviral Res* (2012) 96:296–304. doi:10.1016/j.antiviral.2012.09.021
26. Peng L, Wu TT, Tchieu JH, Feng J, Brown HJ, Feng J, et al. Inhibition of the phosphatidylinositol 3-kinase–Akt pathway enhances gamma-2 herpesvirus lytic replication and facilitates reactivation from latency. *J Gen Virol* (2010) 91:463–9. doi:10.1099/vir.0.015073-0
27. Wagner MJ, Smiley JR. Herpes simplex virus requires VP11/12 to activate Src family kinase phosphoinositide 3-kinase–Akt signaling. *J Virol* (2011) 85:2803–12. doi:10.1128/JVI.01877-10
28. Lee BS, Lee SH, Feng P, Chang H, Cho NH, Jung JU. Characterization of the Kaposi's sarcoma-associated herpesvirus K1 signalosome. *J Virol* (2005) 79:12173–84. doi:10.1128/JVI.79.19.12173-12184.2005
29. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi:10.1126/science.1243292
30. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
31. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124:3923–8. doi:10.1172/JCI75746
32. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211:2537–47. doi:10.1084/jem.20141759
33. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang YD, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85α subunit of PI3K. *J Exp Med* (2012) 209:463–70. doi:10.1084/jem.20112533
34. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34:272–6. doi:10.1007/s10875-014-0012-9
35. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35:11–4. doi:10.1007/s10875-014-0109-1
36. Kannan JA, Dávila-Saldaña BJ, Zhang K, Filipovich AH, Kucuk ZY. Activated phosphoinositide 3-kinase δ syndrome in a patient with a former diagnosis of common variable immune deficiency, bronchiectasis, and lymphoproliferative disease. *Ann Allergy Asthma Immunol* (2015) 115:452–4. doi:10.1016/j.anai.2015.08.009
37. Lawrence MG, Uzel G. 6-year-old boy with recurrent sinopulmonary infections and lymphadenopathy. *J Allergy Clin Immunol Pract* (2015) 3:461.e–3.e. doi:10.1016/j.jaip.2014.10.017
38. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183:221–9. doi:10.1111/cei.12706
39. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Duab J, Goval RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2016) 139:1032–5. doi:10.1016/j.jaci.2016.08.028
40. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139:597–606. doi:10.1016/j.jaci.2016.06.021
41. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kδ syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
42. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110δ cause activated PI3Kδ syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140:1152.e10. doi:10.1016/j.jaci.2017.03.026
43. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased *in vitro* anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2017) 178:20–8. doi:10.1016/j.clim.2015.12.008
44. Goto F, Uchiyama T, Nakazawa Y, Imai K, Kawai T, Onodera M. Persistent impairment of T-cell regeneration in a patient with activated PI3K δ syndrome. *J Clin Immunol* (2017) 37:347–50. doi:10.1007/s10875-017-0393-7
45. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138:210.e–8.e. doi:10.1016/j.jaci.2016.03.022
46. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kδ syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27:640–4. doi:10.1111/pai.12585

47. Kuhlen M, Hönscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. *De novo* PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
48. Bravo García-Morato M, García-Miñaúr S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, López-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
49. Hauck F, Magg T, Krolo A, Bilic I, Hirschmugl T, Laass M, et al. Variant PIK3R1 hypermorphic mutation and clinical phenotypes in a family with short statures, mild immunodeficiency and lymphoma. *Klin Padiatr* (2017) 229:113–7. doi:10.1055/s-0043-104218
50. Johnstone J, Millar J, Lelic A, Verschoor CP, Walter SD, Devereaux PJ, et al. Immunosenescence in the nursing home elderly. *BMC Geriatr* (2014) 17:50. doi:10.1186/1471-2318-14-50
51. Wikby A, Johansson B, Olsson J, Löfgren S, Nilsson BO, Ferguson F. Expansions of peripheral blood CD8 T-lymphocyte subpopulations and an association with cytomegalovirus seropositivity in the elderly: the Swedish NONA immune study. *Exp Gerontol* (2002) 37:445–53. doi:10.1016/S0531-5565(01)00212-1
52. Thomasini RL, Pereira DS, Pereira FSM, Mateo EC, Mota TN, Guimarães GG, et al. Aged-associated cytomegalovirus and Epstein–Barr virus reactivation and cytomegalovirus relationship with the frailty syndrome in older women. *PLoS One* (2017) 12:e0180841. doi:10.1371/journal.pone.0180841
53. Parry HM, Zuo J, Frumento G, Mirajkar N, Inman C, Edwards E, et al. Cytomegalovirus viral load within blood increases markedly in healthy people over the age of 70 years. *Immun Ageing* (2016) 13:1. doi:10.1186/s12979-015-0056-6
54. Asano N, Yamamoto K, Tamaru J, Oyama T, Ishida F, Ohshima K, et al. Age-related Epstein–Barr virus (EBV)-associated B-cell lymphoproliferative disorders: comparison with EBV-positive classic Hodgkin lymphoma in elderly patients. *Blood* (2009) 113:2629–36. doi:10.1182/blood-2008-06-164806
55. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJ, et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity* (2013) 38:187–97. doi:10.1016/j.immuni.2012.09.020
56. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* (2014) 14:24–35. doi:10.1038/nri3567
57. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective 'activated PI3Kδ syndrome'-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130:2307–16. doi:10.1182/blood-2017-08-801191
58. Nepomuceno RR, Balatoni CE, Natkunam Y, Snow AL, Krams SM, Martinez OM. Rapamycin inhibits the interleukin 10 signal transduction pathway and the growth of Epstein–Barr virus B-cell lymphomas. *Cancer Res* (2003) 63:4472–80.
59. Boratynska M, Smolska D. Inhibition of mTOR by sirolimus induces remission of post-transplant lymphoproliferative disorders. *Transpl Int* (2008) 21:605–8. doi:10.1111/j.1432-2277.2008.00655.x
60. Kudchodkar SB, Yu Y, Maguire TG, Alwine JC. Human cytomegalovirus infection induces rapamycin-insensitive phosphorylation of downstream effectors of mTOR kinase. *J Virol* (2004) 78:11030–9. doi:10.1128/JVI.78.20.11030-11039.2004
61. Nashan B, Gaston R, Emery V, Säemann MD, Mueller NJ, Couzi L, et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. *Transplantation* (2012) 93:1075–85. doi:10.1097/TP.0b013e31824810e6

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 27 February 2018
doi: 10.3389/fimmu.2018.00369

Activated PI3 Kinase Delta Syndrome: From Genetics to Therapy

David Michalovich[1] *and Sergey Nejentsev*[2]*

[1] *Refractory Respiratory Inflammation Discovery Performance Unit, GlaxoSmithKline, Stevenage, United Kingdom,*
[2] *Department of Medicine, University of Cambridge, Cambridge, United Kingdom*

Activated PI3 kinase delta syndrome (APDS) is a primary immunodeficiency caused by dominant mutations that increase activity of phosphoinositide-3-kinase δ (PI3Kδ). APDS can be caused by mutations in the *PIK3CD* gene that encodes PI3Kδ catalytic subunit p110δ (APDS1) or mutations in the *PIK3R1* gene that encodes regulatory subunit p85α (APDS2). APDS research advanced rapidly after the initial discovery in 2013. More than 200 APDS patients have been identified around the world. Multiple novel APDS mutations were reported and molecular mechanisms leading to PI3Kδ activation have been elucidated. The finding of APDS significantly increased our understanding of the role of PI3Kδ in the human immune system. Perhaps most importantly, discovery of the molecular basis of this primary immunodeficiency suggested that APDS patients, who previously received only non-specific therapy, could be treated by a novel class of drugs that inhibits PI3Kδ activity. This led to the ongoing clinical trials of selective PI3Kδ inhibitors in APDS patients. Overall, the APDS story provides an excellent example of translational research, beginning with patients who had an unknown disease cause and leading to a novel specific knowledge-based treatment.

Keywords: activated PI3 kinase delta syndrome, primary immunodeficiency, phosphoinositide-3-kinase δ, mutation, inhibitor

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Yuval Itan, Icahn School of Medicine at Mount Sinai, United States
James E. Thaventhiran, MRC Toxicology Unit (MRC), United Kingdom

***Correspondence:**
Sergey Nejentsev
sn262@cam.ac.uk

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *14 December 2017*
Accepted: *09 February 2018*
Published: *27 February 2018*

Citation:
Michalovich D and Nejentsev S (2018) Activated PI3 Kinase Delta Syndrome: From Genetics to Therapy. Front. Immunol. 9:369. doi: 10.3389/fimmu.2018.00369

INTRODUCTION

Primary immunodeficiencies (PIDs) are a group of disorders that cause immune dysfunction and manifest with increased susceptibility to infections. Many PIDs are monogenic diseases. To date, mutations in more than 300 genes have been shown to cause various PIDs (1). Activated PI3 kinase delta syndrome (APDS) is a PID that results from gain-of-function mutations in genes encoding the phosphoinositide-3-kinase δ (PI3Kδ). This review will focus on the APDS mutations, phenotypes of the disease, and current therapeutic approaches.

Phosphoinositide-3-kinase δ is a class IA lipid kinase that phosphorylates phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2 or PIP_2] to produce phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3 or PIP_3]. There are three class IA PI3Ks in mammalian cells: α, β, and δ. Each class IA PI3K is composed of a catalytic subunit: p110α, p110β, or p110δ (encoded by genes *PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively), and one of the five regulatory subunits: p85α, p55α, p50α (all encoded by different transcripts of the *PIK3R1* gene), p85β (encoded by the *PIK3R2* gene), or p55γ (encoded by the *PIK3R3* gene). The regulatory subunit stabilizes the catalytic subunit to prevent its proteasomal degradation, inhibits activity of the catalytic subunit, and recruits it to the plasma membrane (2). Catalytic subunits p110α and p110β are broadly expressed, while p110δ is mainly expressed in cells of the hematopoietic system, primarily lymphocytes and myeloid cells (3). In immune cells, PI3Kδ is activated downstream of cytokine receptors, toll-like

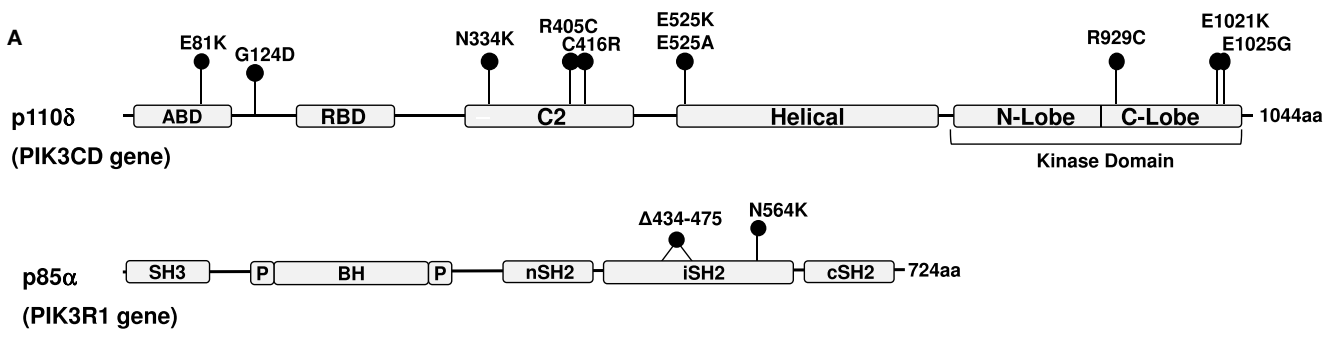
receptors, B-cell and T-cell receptors, and Ras superfamily of small GTPases (4). PIP_3 produced by PI3Ks activates kinases PDK1 and AKT, leading to the activation of mTOR complex 1 and inhibition of FOXO family of transcription factors. In lymphocytes, PIP_3 activates kinases BTK and ITK that mediate activation of phospholipase Cγ and other proteins (3). PIP_3 is dephosphorylated to PIP_2 by a phosphatase PTEN.

APDS MUTATIONS

In 2013, two groups, one in Cambridge (UK) and the other in Bethesda (USA), used whole-exome-sequencing analysis of PID patients with unknown etiology and reported a novel PID caused by rare heterozygous germline gain-of-function mutations in the *PIK3CD* gene (5, 6). The mutations led to the increased PI3Kδ activity and the disease was called APDS (5) or p110δ-activating mutation causing senescent T cell, lymphadenopathy, and immunodeficiency (PASLI) (6) (OMIM #615513). Subsequently, rare heterozygous germline mutations in the *PIK3R1* gene were described that also resulted in an increased PI3Kδ activity and immune deficiency, phenocopying patients with the *PIK3CD* mutations. This disorder has been termed APDS2 or PASLI-R1 (7, 8) (OMIM #616005). Now, a PID caused by activating mutations in the *PIK3CD* gene is referred to as APDS1 and both diseases together are known as APDS.

Since the initial publications, 10 activating missense mutations have been reported in the *PIK3CD* gene resulting in APDS1 (5, 6, 9–15) (**Figure 1**). The E1021K variant in the C-lobe of the p110δ kinase domain is by far the most frequently reported APDS mutation. In the p110δ protein, E1021K is positioned similar to the somatic mutation H1047R of another PI3K isoform, p110α. Both E1021K and H1047R increase PI3K activity by enhancing association of the catalytic subunits with membranes and facilitating more effective phosphorylation of PIP_2 (5, 16–18). The R929C mutation in the C-lobe of the p110δ kinase domain may also act in a similar manner (14). Other p110δ mutations located in the C2 domain (N334K, C416R) and the helical domain (E525K) likely interfere with inhibitory contacts between p110δ and p85α (18). Interestingly, activating somatic mutations of the homologous amino-acid residues of p110α (N345, C420, and E545) have been also found in tumors. The recently identified E81K and G124D mutations in the adapter-binding domain and the linker between the adapter-binding and the Ras-binding domains may affect the orientation of the adapter-binding domain and hence interaction between p110δ and p85α (11).

Several mutations causing APDS2 were identified in the *PIK3R1* gene (**Figure 1**). These include one missense mutation and seven mutations affecting the splice sites of exon 11 (coding exon 10), one affecting the splice acceptor site, and six affecting the splice donor site. All splice site mutations lead to the skipping of exon 11 and an in-frame deletion of 42 amino-acid residues in positions 434–475 within the inter-SH2 coiled-coil domain of p85α. The additional N564K variant in p85α is also found in the inter-SH2 domain (14). The inter-SH2 domain of p85α is



B

PIK3CD			
cDNA position[1]	**Mutation**	**Amino Acid[3]**	**Ref.**
c.241	G>A	E81K	[11]
c.371	G>A	G124D	[12]
c.1002	C>A	N334K	[6]
c.1213	C>T	R405C	[13]
c.1246	T>C	C416R	[9]
c.1573	G>A	E525K	[6]
c.1574	A>C	E525A	[10]
c.2784	C>T	R929C	[14]
c.3061	G>A	E1021K	[5, 6, 19]
c.3073	A>G	E1025G	[15]

PIK3R1			
cDNA position[2]	**Mutation**	**Amino Acid[4]**	**Ref.**
c.1300-1	G>C	434-475 del	[20]
c.1425+1	G>T	434-475 del	[7]
c.1425+1	G>C	434-475 del	[7, 8]
c.1425+1	G>A	434-475 del	[8]
c.1425+2	T>A	434-475 del	[20]
c.1425+2	T>G	434-475 del	[20]
c.1425+2	TG>del	434-475 del	[20]
c.1692	C>G	N564K	[14]

FIGURE 1 | **(A)** Domain structure of the p110δ and p85α proteins and positions of mutations. ABD, adaptor-binding domain; RBD, Ras-binding domain; BH, breakpoint cluster region homology domain; P, proline-rich regions. **(B)** Activated PI3 kinase delta syndrome mutations in the *PIK3CD* (5, 6, 9–15, 19) and *PIK3R1* (7, 8, 14, 20) genes. 1—NM_005026; 2—NM_181523 (RefSeq); 3—O00329; 4—P27986 (UniProt).

known to inhibit the catalytic p110 subunit by interacting with its C2 domain (2). Interestingly, the APDS2 mutations in p85α lead to the disease that phenocopy APDS1, despite that p85α is ubiquitously expressed and interacts not only with p110δ but also with p110α and p110β. However, it has been demonstrated that the 42 amino-acid deletion in p85α effectively disrupts inhibitory interactions between p85α and p110δ, leading to a strong basal activation of PI3Kδ, while it only weakly increases PI3Kα activity (18). This differential effect explains why the impact of this mutation is largely restricted to the immune system.

Mutations that cause APDS have been found in patients from different countries around the world. So far, more than 200 APDS patients carrying activating mutations in the *PIK3CD* and *PIK3R1* genes have been identified. None of these variants were found in large cohorts of healthy subjects, e.g., they are absent from the largest human exome and whole-genome database gnomAD that includes more than 138,000 subjects (21). In several families, APDS mutations were shown to appear *de novo* among children, while being absent in their parents (5, 6, 10, 20), and long-range haplotype analysis in families with the E1021K mutation showed no founder effect (5). These findings indicate that APDS mutations appear recurrently in human populations. It is possible that activation of PI3Kδ provides selective advantages to cells during gametogenesis. Current data show that APDS mutations have high penetrance, e.g., out of the 53 subjects from 30 APDS1 families only one adult carrier of the E1021K mutation had no reported health issues (22). The true prevalence of APDS is not known. In the original study, heterogeneous cohorts comprising 184 PID patients were screened for the E1021K mutation and 17 APDS patients from seven unrelated families were identified (5). However, these cohorts included multiple patients with hyper-IgM syndrome and, therefore, were enriched for APDS mutations. Another study screened 669 patients with undefined PIDs for the N334K, C416R, E525K, and E1021K mutations in *PIK3CD* and the *PIK3R1* splice site mutations and found only *PIK3CD* mutations in three siblings diagnosed with common variable immune deficiency (CVID) and two sporadic cases with combined immunodeficiency (23). Thus, prevalence of APDS may vary considerably between different PID cohorts.

APDS PHENOTYPES

Two comprehensive studies of APDS cohorts have been carried out recently and characterized its clinical and immunological manifestations (**Table 1**). One study examined the phenotypes of 53 patients with APDS1 (50 subjects with E1021K and 3 subjects E525K mutations) (22). The other studied 36 patients with APDS2 (20). Almost all APDS1 and APDS2 patients suffered from recurrent respiratory infections caused by bacterial pathogens, mainly *Streptococcus pneumoniae* and *Haemophilus influenzae*. Bronchiectasis was a common complication of lung infections affecting up to 60% of APDS1 patients. Interestingly, the majority of bronchiectasis patients had normal IgG levels and diagnosing PID in such subjects may not have been straightforward. Therefore, screening bronchiectasis patients without a clear PID for APDS mutations can reveal unrecognized APDS cases. Severe, persistent, or recurrent herpes virus infections, including EBV, CMV, HSV, and VZV infections, were found in 49% APDS1 and 31% APDS2 patients and were associated with lymphadenopathy. Immunologically, increased frequency of transitional B cells was often observed in APDS patients (**Table 1**). Many patients also had increased serum IgM levels and, therefore, some of the patients previous were diagnosed with hyper-IgM syndrome (5). Approximately one third of APDS1 patients and 17% of APDS2 patients had autoimmune or autoinflammatory manifestations. High incidence of lymphomas was also recorded in APDS patients (20, 22, 24). Unexpectedly, neurodevelopmental delay was found to be a relatively frequent manifestation in both APDS cohorts (**Table 1**), which may suggest an important role of PI3Kδ in the development of central nervous system that was not recognized previously.

TABLE 1 | Characteristic clinical and immunological features of activated PI3 kinase delta syndrome (APDS).

Manifestations	APDS1 (22)	APDS2 (20)
Recurrent respiratory tract infections	96%	100%
Pneumonia	85%	71%
Bronchiectasis	60%	18%
Herpesvirus infections	49%	31%
Lymphadenopathy	64%	75%
Splenomegaly	58%	43%
Autoimmune or autoinflammatory disease	34%	17%
Neurodevelopmental delay	19%	31%
Lymphoma	13%	25%
Increased IgM	76%	58%
Increased transitional B cells[a]	75%	93%

[a]*If data were available and B cells were sufficient for analysis.*

Thus, APDS manifests as a PID with a high rate of recurrent respiratory tract infections, often leading to bronchiectasis, herpes virus infections, lymphadenopathy, splenomegaly, increased risk of lymphomas, frequent autoimmune manifestations, and, occasionally, developmental delay. In addition, APDS2 patients had a high frequency of growth retardation (45%), a feature that was not found in APDS1 patients. This difference may reflect impaired interactions of p85α with p110α and p110β catalytic subunits. Of note, a number of other dominant germline mutations, which reside within the nSH2 and iSH2 domains of p85α and reduce PI3K signaling, are known to cause the SHORT syndrome that includes short stature, hyperextensibility of joints, hernia, ocular depression, Rieger anomaly, and teething delay (25–28) (OMIM #269880). A single patient with a homozygous loss-of-function mutation in the *PIK3R1* gene was described that resulted in the absent p85α and reduced expression of p110δ. The patient had B lymphopenia and hypogammaglobulinemia and suffered from recurrent Campylobacter bacteremia and inflammatory bowel disease (29) (OMIM #615214). Also, a patient with biallelic loss-of-function mutations in the *PIK3CD* gene and reduced p110δ expression was reported to have B lymphopenia and hypogammaglobulinemia, sinopulmonary infections, septic arthritis, inflammatory bowel disease, and autoimmune hepatitis (30).

Therefore, although p110δ deficiency also leads to a PID, its phenotype is different from APDS.

THERAPIES FOR APDS PATIENTS—PRECISION MEDICINE FOR A RARE DISEASE

Treatment regimes for APDS patients include antibiotic prophylaxis and immunoglobulin replacement therapy. Hematopoietic stem cell transplantation (HSCT) has been successful in several APDS patients and can be a treatment option, especially in young patients (20, 22). Immunosuppressive therapies aimed at reducing lymphoproliferation have included treatment with rituximab (anti-CD20 monoclonal antibody) and rapamycin to target the activation of the mTOR pathway. Treatment with rapamycin led to the improvement of immunological markers and a reduction in splenomegaly and lymphadenopathy (6). Nevertheless, the discovery of the APDS etiology and the causative role of mutations that activate PI3Kδ opened an opportunity for a novel specific treatment using selective PI3Kδ inhibitors. This class of drugs has been developed for cancer treatment (31), as well as inflammatory disorders, such as rheumatoid arthritis, asthma, and chronic obstructive pulmonary disease (COPD) (32–34). One of the PI3Kδ inhibitors, idelalisib, has been approved for treatment of chronic lymphocytic leukemia and non-Hodgkin lymphoma (35, 36). Idelalisib (previously known as GS-1101) reduced the catalytic activity of mutant PI3Kδ as efficiently as the activity of the wild type PI3Kδ (5, 18). PI3Kδ inhibitors also normalized PI3Kδ hyperactivation in cells of APDS patients *in vitro* (5–8, 37). These results opened way for clinical trials of PI3Kδ inhibitors in APDS patients.

Two phase-II clinical trials are currently ongoing to study the safety, pharmacokinetics, pharmacodynamics, and efficacy of PI3Kδ inhibitors in APDS patients. Clinical trial NCT02435173 sponsored by Novartis uses an oral PI3Kδ inhibitor leniolisib (CDZ173) (38), while clinical trial NCT02593539 sponsored by GSK uses an inhaled PI3Kδ inhibitor nemiralisib (GSK2269557) (39) that had been originally developed for treatment of COPD (34). Recently, the clinical trial NCT02435173 has reported efficacy data from six APDS patients (37). The patients were part of a 12-week within subject dose-escalation study of oral leniolisib, administered twice daily. Leniolisib was well tolerated and the study reported normalization of circulating transitional and naïve B cells, reduction in senescent T cells, decrease in the elevated serum IgM levels, and inflammatory markers. After 12 weeks of treatment, lymph node and spleen sizes reduced by 39% and 40%, respectively (37). Normalization of immunophenotypes was most notable in the final 4-week dosing period. The study has now proceeded to a long-term treatment arm with patients receiving treatment for over 9 months (70-mg leniolisib, twice daily) and no significant adverse events have been detected (37). These exciting initial findings validate the focused approach to target the activated PI3Kδ in APDS patients. It will be of interest to see if the oral or inhaled inhibitors under development provide specific advantages for the APDS patients. Inhaled PI3Kδ inhibitors will have a different safety profile and may be appropriate for patients who are primarily affected by airway infections, potentially limiting progression of bronchiectasis.

FUTURE DIRECTIONS

Whole-exome and whole-genome sequencing of PID patients will likely identify novel variants in the *PIK3CD* and *PIK3R1* genes and it remains essential to distinguish pathogenic mutations from neutral variants. Given that APDS is a rare monogenic disorder with high penetrance, variants that cause it are unlikely to be found in healthy subjects outside of patients' families. Therefore, excluding variants detected in healthy cohorts, e.g., reported in the gnomAD database (21), will help initial screening of potential APDS-causing mutations. However, rare variants can still be neutral, so it will remain important to demonstrate that a novel candidate mutation leads to increased PI3K activity, e.g., by showing increased levels of PIP_3 or phosphorylated AKT. The growing list of known APDS mutations will facilitate genetic diagnosis in future patients. Early diagnosis of APDS will be essential, as it will allow early therapy, e.g., HSCT or treatment with PI3Kδ inhibitors, which should prevent many APDS complications.

As our understanding of APDS improves, new questions emerge. With more APDS patients carrying various mutations being identified, it will be interesting to understand if specific mutations are associated with disease severity and clinical or immunological subphenotypes. The varying degree of disease severity in APDS patients raises the question as to whether rare activating mutations in genes encoding PI3Kδ or other proteins that regulate PI3K activity may be responsible for causing similar disorders, perhaps resembling only some of the APDS manifestations. In support of this hypothesis, loss-of-function mutations have been described in PTEN resulting in an APDS-like phenotype (10). Larger exome- or genome-sequencing studies in patients with diseases that resemble aspects of the APDS phenotype will be interesting to explore in this regard. These studies may reveal monogenic etiology in some of the patients with disorders, such as bronchiectasis. Furthermore, it is plausible that combinations of common polymorphisms in genes regulating PI3Kδ signaling may lead to its increased activity. Such subjects may be predisposed to APDS-like manifestations, e.g., bacterial respiratory infections, herpes virus infections, or bronchiectasis. Future genetic, biochemical, and immunological studies should address these questions.

In conclusion, the story of APDS illustrates how modern biomedical approaches led to the discovery of disease etiology in a group of uncharacterized patients and then provided a novel knowledge-based therapeutic strategy. Promising data emerging from the ongoing clinical trials of PI3Kδ inhibitors (37) rises the hope that the success of this approach may translate into therapies for APDS and, possibly, for APDS-like diseases in future.

AUTHOR CONTRIBUTIONS

DM wrote the first draft of the manuscript and prepared **Figure 1**. SN edited the manuscript and prepared **Table 1**.

FUNDING

SN received support from the Wellcome Trust (095198/Z/10/Z), UK Medical Research Council grant MR/M012328/1 co-sponsored by GSK, and National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre.

REFERENCES

1. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2018) 38(1):129. doi:10.1007/s10875-017-0465-8
2. Burke JE, Williams RL. Synergy in activating class I PI3Ks. *Trends Biochem Sci* (2015) 40(2):88. doi:10.1016/j.tibs.2014.12.003
3. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kdelta and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16(11):702. doi:10.1038/nri.2016.93
4. Okkenhaug K. Signaling by the phosphoinositide 3-kinase family in immune cells. *Annu Rev Immunol* (2013) 31:675. doi:10.1146/annurev-immunol-032712-095946
5. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866. doi:10.1126/science.1243292
6. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88. doi:10.1038/ni.2771
7. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923. doi:10.1172/JCI75746
8. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537. doi:10.1084/jem.20141759
9. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34(3):272. doi:10.1007/s10875-014-0012-9
10. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138(6):1672. doi:10.1016/j.jaci.2016.03.055
11. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110delta cause activated PI3Kdelta syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140(4):1152. doi:10.1016/j.jaci.2017.03.026
12. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110delta cause activated PI3K-delta syndrome 1 (APDS1). *Haematologica* (2017) 102(7):e278. doi:10.3324/haematol.2017.167601
13. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29. doi:10.1016/j.clim.2017.05.020
14. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Scholvinck L, Kalina T, et al. Genetic defects in PI3Kdelta affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77. doi:10.1016/j.clim.2017.01.004
15. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2017) 139(3):1032. doi:10.1016/j.jaci.2016.08.028
16. Mandelker D, Gabelli SB, Schmidt-Kittler O, Zhu J, Cheong I, Huang CH, et al. A frequent kinase domain mutation that changes the interaction between PI3Kalpha and the membrane. *Proc Natl Acad Sci U S A* (2009) 106(40):16996. doi:10.1073/pnas.0908444106
17. Burke JE, Perisic O, Masson GR, Vadas O, Williams RL. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110alpha (PIK3CA). *Proc Natl Acad Sci U S A* (2012) 109(38):15259. doi:10.1073/pnas.1205508109
18. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kdelta regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114(8):1982. doi:10.1073/pnas.1617244114
19. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33(5):361. doi:10.1111/j.1744-313X.2006.00627.x
20. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210. doi:10.1016/j.jaci.2016.03.022
21. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* (2016) 536(7616):285. doi:10.1038/nature19057
22. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2016) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
23. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hulsdunker J, Eskandarian Z, et al. Activating PI3Kdelta mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2015) 183(2):221–9. doi:10.1111/cei.12706
24. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol* (2014) 134(1):233. doi:10.1016/j.jaci.2014.02.020
25. Thauvin-Robinet C, Auclair M, Duplomb L, Caron-Debarle M, Avila M, St-Onge J, et al. PIK3R1 mutations cause syndromic insulin resistance with lipoatrophy. *Am J Hum Genet* (2013) 93(1):141. doi:10.1016/j.ajhg.2013.05.019
26. Dyment DA, Smith AC, Alcantara D, Schwartzentruber JA, Basel-Vanagaite L, Curry CJ, et al. Mutations in PIK3R1 cause SHORT syndrome. *Am J Hum Genet* (2013) 93(1):158. doi:10.1016/j.ajhg.2013.06.005
27. Koenig R, Brendel L, Fuchs S. SHORT syndrome. *Clin Dysmorphol* (2003) 12(1):45. doi:10.1097/00019605-200301000-00008
28. Chudasama KK, Winnay J, Johansson S, Claudi T, Konig R, Haldorsen I, et al. SHORT syndrome with partial lipodystrophy due to impaired phosphatidylinositol 3 kinase signaling. *Am J Hum Genet* (2013) 93(1):150. doi:10.1016/j.ajhg.2013.05.023
29. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang YD, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85alpha subunit of PI3K. *J Exp Med* (2012) 209(3):463. doi:10.1084/jem.20112533
30. Zhang K, Husami A, Marsh RA, Jordan MB. Identification of phosphoinositide-3-kinase (PI-3K) p110delta (PIK3CD) deficient individual. *J Clin Immunol* (2013) 33:673–4.
31. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov* (2014) 13(2):140. doi:10.1038/nrd4204
32. Bartok B, Boyle DL, Liu Y, Ren P, Ball ST, Bugbee WD, et al. PI3 kinase delta is a key regulator of synoviocyte function in rheumatoid arthritis. *Am J Pathol* (2012) 180(5):1906. doi:10.1016/j.ajpath.2012.01.030
33. Sriskantharajah S, Hamblin N, Worsley S, Calver AR, Hessel EM, Amour A. Targeting phosphoinositide 3-kinase delta for the treatment of respiratory diseases. *Ann N Y Acad Sci* (2013) 1280:35–9. doi:10.1111/nyas.12039
34. Cahn A, Hamblin JN, Begg M, Wilson R, Dunsire L, Sriskantharajah S, et al. Safety, pharmacokinetics and dose-response characteristics of GSK2269557, an inhaled PI3Kdelta inhibitor under development for the treatment of COPD. *Pulm Pharmacol Ther* (2017) 46:69. doi:10.1016/j.pupt.2017.08.008
35. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med* (2014) 370(11):997. doi:10.1056/NEJMoa1315226
36. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kdelta inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* (2014) 370(11):1008. doi:10.1056/NEJMoa1314583
37. Rao VK, Webster S, Dalm V, Sediva A, van Hagen PM, Holland S, et al. Effective "activated PI3Kdelta syndrome"-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130(21):2307. doi:10.1182/blood-2017-08-801191
38. Hoegenauer K, Soldermann N, Zecri F, Strang RS, Graveleau N, Wolf RM, et al. Discovery of CDZ173 (leniolisib), Representing a structurally novel class of PI3K delta-selective inhibitors. *ACS Med Chem Lett* (2017) 8(9):975. doi:10.1021/acsmedchemlett.7b00293
39. Down K, Amour A, Baldwin IR, Cooper AW, Deakin AM, Felton LM, et al. Optimization of novel indazoles as highly potent and selective

inhibitors of phosphoinositide 3-kinase delta for the treatment of respiratory disease. *J Med Chem* (2015) 58(18):7381. doi:10.1021/acs.jmedchem.5b00767

Conflict of Interest Statement: DM is an employee of GSK. SN is a recipient of a grant from the UK Medical Research Council and GSK to study APDS.

frontiers in Immunology

REVIEW
published: 05 March 2018
doi: 10.3389/fimmu.2018.00338

Respiratory Manifestations of the Activated Phosphoinositide 3-Kinase Delta Syndrome

Alison M. Condliffe[1]* *and Anita Chandra*[2,3]

[1] Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom, [2] Department of Medicine, University of Cambridge, Cambridge, United Kingdom, [3] Laboratory of Lymphocyte Signalling and Development, Babraham Institute, Cambridge, United Kingdom

The activated phosphoinositide 3-kinase δ syndrome (APDS), also known as p110δ-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency (PASLI), is a combined immunodeficiency syndrome caused by gain-of-function mutations in the phosphoinositide 3-kinase (PI3K) genes *PIK3CD* (encoding p110δ: APDS1 or PASLI-CD) and *PIK3R1* (encoding p85α: APDS2 or PASLI-R1). While the disease is clinically heterogeneous, respiratory symptoms and complications are near universal and often severe. Infections of the ears, sinuses, and upper and lower respiratory tracts are the earliest and most frequent manifestation of APDS, secondary to both respiratory viruses and to bacterial pathogens typical of defective B cell function. End organ damage in the form of small airways disease and bronchiectasis frequently complicates APDS, but despite documented T cell defects, opportunistic infections have rarely been observed. Antimicrobial (principally antibiotic) prophylaxis and/or immunoglobulin replacement have been widely used to reduce the frequency and severity of respiratory infection in APDS, but outcome data to confirm the efficacy of these interventions are limited. Despite these measures, APDS patients are often afflicted by benign lymphoproliferative disease, which may present in the respiratory system as tonsillar/adenoidal enlargement, mediastinal lymphadenopathy, or mucosal nodular lymphoid hyperplasia, potentially causing airways obstruction and compounding the infection phenotype. Treatment with rapamycin and PI3Kδ inhibitors has been reported to be of benefit in benign lymphoproliferation, but hematopoietic stem cell transplantation (ideally undertaken before permanent airway damage is established) remains the only curative treatment for APDS.

Keywords: activated phosphoinositide 3-kinase delta syndrome, respiratory infection, pneumonia, bronchiectasis, antibody deficiency, lymphoproliferation

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Marina Cavazzana, Necker-Enfants Malades Hospital, France
Kenneth Neil Olivier, National Heart Lung and Blood Institute (NIH), United States

***Correspondence:**
Alison M. Condliffe
a.m.condliffe@sheffield.ac.uk

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *28 November 2017*
Accepted: *06 February 2018*
Published: *05 March 2018*

Citation:
Condliffe AM and Chandra A (2018) Respiratory Manifestations of the Activated Phosphoinositide 3-Kinase Delta Syndrome. Front. Immunol. 9:338. doi: 10.3389/fimmu.2018.00338

INTRODUCTION

Following the initial description in 2013 of gain-of-function (GOF) mutations resulting in enhanced phosphoinositide 3-kinase (PI3K) δ signaling as the cause of a combined immune deficiency syndrome [the activated phosphoinositide 3-kinase δ *s*yndrome (APDS), also known as p110δ-*a*ctivating mutation causing *s*enescent T cells, *l*ymphadenopathy, and *i*mmunodeficiency (PASLI)], multiple case reports and several case series have highlighted the protean clinical feature of this newly recognized disease. The first reports (1–3) identified mutations in the gene (*PIK3CD*) encoding the p110δ catalytic subunit of PI3Kδ, and several additional GOF mutations have since been described [e.g., Ref. (4–9)]. Subsequently, patients with a highly reminiscent clinical phenotype who did not harbor APDS-associated *PIK3CD* mutations were found instead to have exon-skipping mutations in

the Class 1A regulatory PI3K subunit p85α encoded by *PIK3R1* [e.g., Ref. (10–16)]; these mutations disrupt the inhibitory interactions with the catalytic subunit of PI3Kδ (17), increasing both basal and stimulated activation. The resulting clinical syndrome, termed APDS2 (or PASLI-R1), phenocopies many of the APDS1 disease manifestations but with a higher incidence of growth retardation and in some cases, overlap with SHORT syndrome [short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay (14, 18)]. More recently, four patients with mutations leading to haploinsufficiency of PTEN (a lipid phosphatase that opposes PI3K activation) have been found to have immunodeficiency with an APDS-like syndrome (9, 19). Despite the different genetic underpinnings, the clinical features have marked similarities; a recurring theme is that respiratory manifestation (predominantly infections but also non-infectious complications) affect the majority of patients, occur early in the course of the disease, and are challenging to manage clinically.

RESPIRATORY INFECTIONS IN APDS

Incidence and Age of Onset

While a few isolated cases have been identified who are completely asymptomatic (20) or who have severe extrapulmonary manifestations but minimal or no respiratory symptomatology (21), recurrent respiratory tract infections are reported near universally in APDS; indeed, they may be the sole manifestation of the disease (16), and they may be both very frequent and severe (5). Unfortunately, however, differences in definitions and nomenclature make direct comparisons between published studies challenging at times. For example, Coulter et al. (20) reported that 51 (98%) of a cohort of 53 patients with APDS1 suffered recurrent respiratory infections, subdividing these episodes further into radiologically confirmed pneumonia (85%), recurrent otitis media (49%, severe enough to cause permanent hearing loss in 8% of the total), chronic rhinosinusitis (45%), and tonsillitis (28%). By contrast, in their description of 36 patients with APDS2, Elkaim et al. (22) noted recurrent upper respiratory tract infections (including both otitis media and sinusitis in this definition) in 100% of cases, and lower respiratory infections (defined as either bronchitis or pneumonitis) in 70% of their cohort, without further breakdown. A recently published Dutch cohort (8) reporting 13 newly identified patients (11 with APDS1 and 2 with APDS2) stated that all had both upper and lower respiratory tract infections but did not supply further clinical details as the focus of the manuscript was B cell differentiation and maturation. A Chinese case series of 15 APDS1 patients (23) reported pneumonia had been diagnosed in 12 of the cases (80%).

In addition to the high frequency of such infections, their onset is early in life [10 months–10 years (22) and <1–7 years (20)] and is the commonest reason for presentation to medical/immunological services. Even in patients whose presentation is precipitated by other acute manifestations [e.g., intussusception (24) or gut-associated T cell lymphoproliferation (25)], a retrospective history of recurrent respiratory infections is usually present. Thus, although precise definitions vary between studies, it is possible to conclude that APDS patients suffer early, frequent, and severe respiratory infections. This concurs with the accompanying article presenting initial data collected by the ESID APDS registry (Maccari et al., personal communication[1]).

Despite these broad similarities, the severity and pattern of infections (as well as other manifestations) varies considerably between individual patients, even when grouped according to genotype and even within affected family members. In one E1021K APDS1 kindred (26) in which three individual affected family members exhibited a mild, intermediate, and severe spectrum respiratory infections, there seemed to be a broad association of severer phenotype with more suppressed IgG and lower class-switched memory B cells. However, this correlation was not observed in other affected families (2, 27) and does not seem to be recapitulated in larger cohort studies. To date, no circulating biomarker has been reliably linked to respiratory phenotypes, but larger longitudinal studies may enable such correlation to be identified in future.

Microbiology of Infections

While some microbiological data have been published, milder infections are generally self-reported and not supported by identification of a causal pathogen. It is therefore likely that most of the reported isolates are derived from infections at the severer end of the spectrum, in particular those requiring consultation with health-care professionals; this could skew the available data.

Bacterial Infections

There is concordance that the commonest respiratory bacterial isolates are *Haemophilus influenzae* and *Streptococcus pneumoniae* (20, 22), *Staphylococcus aureus*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, and *Klebsiella* species have also been reported (20, 28). This spectrum of pathogens is highly reminiscent of other primary antibody deficiency syndromes such as common variable immune deficiency. Defective antibody production (**Figure 1**) results in failure of antibody-mediated killing mechanisms such as opsonophagocytosis. However, abnormalities in immunoglobulin levels are heterogeneous in all of the published case series of APDS; Coulter et al. (20) reported that total IgG was reduced in just 43% of their APDS1 patient group, although defective class switch recombination and (when measured) specific antibody formation were more frequent; similarly, 50% of the Dutch APDS cohort had low IgG and high IgM levels (8). Hypogammaglobulinemia was more frequent (87%) in the APDS2 patients reported by Elkaim et al. (22). Interestingly, low IgG/IgA levels do not seem to reliably predict a more severe respiratory phenotype or correlate with the presence of bronchiectasis [for example, Coulter et al. (20) noted that 63% of patients with CT proven bronchiectasis had normal total IgG levels]. It is uncertain whether this lack of correlation of end organ damage with IgG reflects the widespread prevalence of more subtle antibody defects, additional aberrant B cell functions (e.g., abnormal cytokine production), the additive impact of the well-established abnormalities in T cell function (3) or other,

[1] Maccari ME, Abolhassani H, Aghamohammadi A, Aiuti A, Aleinikova O, Bangs C, et al. Disease evolution and response to rapamycin in Activated PI3Kδ Syndrome: the ESID-APDS registry. (submitted to this Research Topic).

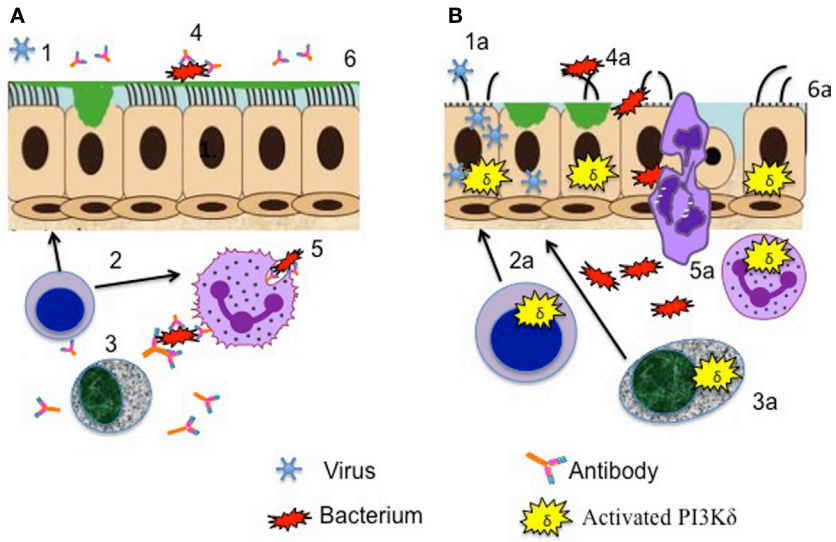


FIGURE 1 | Aberrant cellular functions contributing to respiratory infection in activated phosphoinositide 3-kinase δ syndrome. **(A)** Healthy lung defenses. (1) Epithelial defenses counteract viral pathogens, aided by (2) effective T cells cytokine production. (3) Antibody production by B cells promotes (4) bacterial killing and (5) opsonophagocytosis. (6) Respiratory epithelial surfaces are preserved intact and continue to function to repulse invading pathogens. **(B)** Lung defenses compromised by activating mutations leading to enhanced phosphoinositide 3-kinase (PI3K) δ signaling. (1a) Viral entry and replication in airway epithelial cells are promoted, reducing barrier integrity. (2a) Aberrant cytokine production by T cells and (3a) failure of antibody production promote (4a) bacterial invasion with (5a) inadequate handling of pathogens by phagocytes. (6a) Repeated cycles of infection lead to long-term airway damage.

as yet undetermined mechanisms. Of note, PI3Kδ inhibition reduced airway epithelial oxidative and endoplasmic reticulum stress in response to *Aspergillus fumigatus* exposure, both in cultured cells and in mouse lungs (29), suggesting that excessive PI3Kδ activity may be detrimental to local respiratory defenses as well as impairing adaptive immunity (**Figure 1**).

Viral Infections

The susceptibility of APDS patients to systemic infection with herpes viruses is well documented; however, they also seem to experience an excessive burden of respiratory viral infections. Coulter et al. (20) noted that significant adenovirus infections occurred in 17% of their APDS1 cohort, with adenovirus isolated from various sites including bronchoalveolar lavage fluid; other common viruses identified during respiratory exacerbations included respiratory syncytial virus (RSV), parainfluenza virus, and echovirus and coxsackie viruses (20). Significant RSV infections have also been noted by others [e.g., Ref. (14, 15)], and additionally a patient with pericarditis caused by echovirus infection has also been reported (30). While T cell-mediated antiviral mechanisms are undoubtedly compromised in APDS patients, it is worth reflecting that many viral pathogens subvert local host cell PI3K signaling (**Figure 1**). Herpesviruses in particular express multiple proteins that target PI3K/Akt to facilitate viral infection, replication, latency, and reactivation (31). Increased PI3Kα, rather than PI3Kδ expression and activity in primary bronchial epithelial cells isolated from patients with COPD, was found to underpin increased susceptibility to H3N2 and H1N1 influenza viral infection (32); inhibition of PI3K signaling restored protective antiviral responses and suppressed infection in this setting. It is plausible to extrapolate from these findings that excessive airway cell PI3K activity (whatever the isoform responsible) might predispose to airway viral invasion (**Figure 1**). With regard to APDS-relevant respiratory viral pathogens, the adenovirus E4-ORF1 (early region 4 open reading frame 1) protein enhances viral replication by activating PI3K (33). Likewise, infection with coxsackie virus activates PI3K/AKT signaling and suppression of these pathways diminished viral capsid protein expression and viral release (34), and PI3Kδ mediates dsRNA-induced upregulation of airway epithelial PD-L1, a co-inhibitory molecule associated with the escape of viruses from the mucosal immunity (35).

Mycobacterial and Fungal Infections

Although pulmonary mycobacterial infections have not been reported in APDS, local infection with Bacillus Calmette–Guérin (BCG) have been documented following vaccination (20), and in a separate study, a failure of patient-derived monocyte-derived macrophages to kill internalized BCG, restored by a PI3Kδ inhibitor, was demonstrated (36). It would therefore seem prudent to ensure patients with APDS have sputum samples screened for mycobacteria as well as standard pathogens. To date, despite the marked T cell senescence that characterizes APDS, no patients with pulmonary pneumocystis pneumonia (PCP) or invasive aspergillosis have been reported, but interestingly one of two patients reported with a PTEN mutations causing an "APDS-like" syndrome contracted PCP at the age of 4 months and the other was reported to have suffered from "pulmonary aspergillosis," although further details were not supplied (9). PI3Kδ activity supports neutrophil-mediated killing of *A. fumigatus* hyphae (37), and normal neutrophil PIP_3 levels and oxidative burst were seen in response to soluble stimuli (2), hence increased susceptibility to this organism would not be predicted.

Complications of Respiratory Infections in APDS

Bronchiectasis

Bronchiectasis (abnormal widening of the bronchi or their branches; **Figure 2**) is one of the commonest and most debilitating consequences of recurrent respiratory infection, and compounds the problem, increasing the host susceptibility to further lower respiratory tract infections and facilitating airway colonization with pathogenic bacteria (38, 39). A number of mechanisms may lead to the development of bronchiectasis in APDS. First, the frequent respiratory infections noted above may lead directly to airway damage, weakening the airway wall. Second, focal nodular lymphoid hyperplasia may be of sufficient magnitude to obstruct segmental or even lobar airways, potentially leading to post-obstructive bronchiectasis. Third, compromise of the adaptive immune response may predispose to bronchiectasis. Aberrant neutrophil function has been linked to bronchiectasis and correlates with disease severity and exacerbations (40, 41). Excessive (and perhaps dysregulated) neutrophil PI3K activation has been linked to airway damage in COPD (42), and inhibition of PI3K (using pan-PI3K inhibitors or inhibitors selective for PI3Kδ or PI3Kγ) was able to restore neutrophil migratory accuracy in both COPD and in the elderly (42, 43). Neutrophil function has been little studied in APDS: Angulo et al. (2) presented data from just $n = 1$–2 patients but did show an apparent reduction in neutrophil chemotaxis to IL-8 in cells derived from a patient with APDS; however, directionality and accuracy or migration were not assessed in this limited study. Further assessment of neutrophil function in APDS patients or in animal models of APDS would be of interest.

Recurrent respiratory infections precede a diagnosis of bronchiectasis by several years in most reported cases of APDS (see text footnote 1), but this apparent temporal progression may be confounded by delays in undertaking CT scans, and uptake of this investigation may vary between institutions and on a wider scale between countries. Earlier identification of patients and establishing treatment regimens including immunoglobulin replacement and antibiotic prophylaxis, or HSCT, might delay or prevent this complication, but to confirm this will require longitudinal observation. An early review (15) of 49 APDS1 and 15 APDS2 patients (all that had been published at the time of their review) suggested a higher incidence of bronchiectasis in APDS1 versus APDS2. In the most detailed study of bronchiectasis in APDS to date (20), CT chest scans from 31 patients with APDS1 were independently reviewed by 2 specialist thoracic radiologists; bronchiectasis was felt by both radiologists to be present in 21 of the 31 available scans (60%), with an average of three lobes affected. In one case, lobar consolidation was observed to progress to focal bronchiectasis, supporting a causal link between airway infection and airway wall damage. In contrast in a study of APDS2 (22), an incidence of just 18% bronchiectasis was found, but this study relied on the attending physician's response to a questionnaire, and central review of scans was not undertaken. Could this reflect a true difference between APDS1 and APDS2? A lower incidence of bronchiectasis (only 2 of 10 APDS1 patients in whom CT scans were available were diagnosed with this condition) was noted in a smaller study (8) although bronchial wall thickening was highlighted in an additional four patients; neither of the APDS2 patients in this cohort had bronchiectasis. In a further case series (23), the reported incidence of bronchiectasis in APDS1 was just 5/15 (33%). Given the variability in chest CT uptake and reporting, it seems reasonable to conclude that bronchiectasis is a frequent complication of APDS, whatever the causal mutation; indeed initial data from the ESID APDS both APDS1 and APDS2 patients suggest an overall incidence of bronchiectasis of

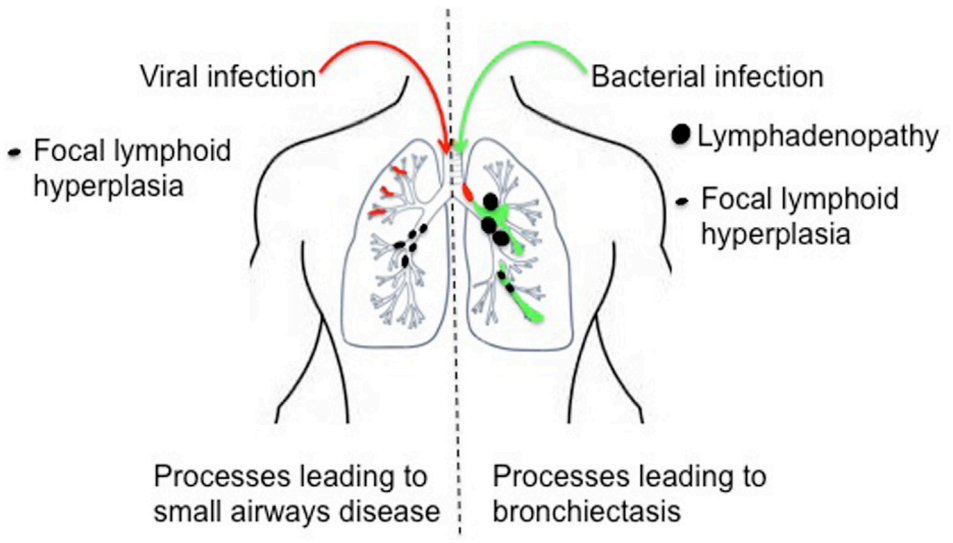


FIGURE 2 | Processes leading to airway damage in activated phosphoinositide 3-kinase δ syndrome. Repeated episodes of viral bronchiolitis may lead to small airway damage and mosaic attenuation, compounded by local obstruction secondary to focal lymphoid hyperplasia. Recurrent bacterial infection leads to chronic inflammatory damage of the larger airways and the development of bronchiectasis; post-obstructive bronchiectasis may also occur secondary airway obstruction, which may be extra-luminal (intrathoracic lymphadenopathy) or intra-luminal (focal lymphoid hyperplasia).

approximately 60%. Apparent differences between studies may reflect small sample sizes, geographical differences in CT uptake, and interindividual variation in CT reporting; however, larger cohort studies and longitudinal observation may be required to clarify this and exclude a genuine difference between APDS1 and APDS2.

Small Airways Disease

Bronchiectasis is an expected complication of recurrent bacterial respiratory infection and is well known to be associated with primary antibody deficiency. Unexpectedly, the commonest radiological abnormality (in 88%) flagged by specialist radiologists in the APDS1 cohort described by Coulter et al. (20) was not bronchiectasis or inflammatory change but mosaic attenuation, indicative of reduced perfusion of poorly ventilated lung regions. Air trapping (a related finding, secondary to airway obstruction) was also noted in 2/9 APDS1 patients in a separate study (8), and mosaic attenuation was flagged as a radiological feature of APDS1 but not enumerated by Angulo et al. (2). These more subtle CT abnormalities are likely to reflect the impact of recurrent episodes of viral bronchiolitis but could also be secondary to focal lymphoid hyperplasia (**Figure 2** and see below). Further assessment of APDS patients for small airways disease using specialist pulmonary function methodologies (e.g., multi-breathe washout and forced oscillometry) or imaging modalities such as MRI with hyperpolarized helium or xenon might more accurately delineate this unexpectedly common radiological abnormality (44).

NON-INFECTIOUS RESPIRATORY MANIFESTATIONS OF APDS

Benign Lymphoproliferation

Tonsillar and adenoidal hypertrophy is a frequent manifestation of APDS. A detailed analysis of this complication in APDS2 (22) revealed ear, nose, and throat chronic lymphoid hyperplasia without the need for surgical interventions in three (11%) patients, adenoidectomies, tonsillectomy, or both in seven (26%) patients and multiple surgical resections in three patients; one afflicted patient developed postoperative pharyngeal stenosis ultimately requiring tracheotomy. Coulter et al. (20) noted recurrent tonsillitis in 15/53 APDS1 patients (28%) with a need for tonsillectomy in 5/53 (13%) but listed this as an infectious rather than a lymphoproliferative complication. While occasional case reports have highlighted significant tonsillar hypertrophy in APDS1 (6, 24), it seems to be noted more frequently, and to be more severe in APDS2 [e.g., Ref. (12, 14, 27, 45)]. Tonsillar biopsies from two APDS2 patients demonstrated small B cell follicles rather than the atypical follicular hyperplasia reported in biopsies of lymph nodes/mucosal follicular hyperplasia from APDS1 (7, 20) and APDS2 (14), but other features such as reduced mantle layers and infiltration with PD1 +ve T cells were concordant, suggesting a related immunopathogenesis.

Benign lymphoproliferation has been widely reported in both APDS1 and 2, but in most cases mediastinal lymphadenopathy (which requires CT for ascertainment) is not separately reported. However, 16/31 APDS patients (20) were noted to have mediastinal lymphadenopathy, which was in a regional draining station to concurrent lobar consolidation in four instances, compatible with an infection-driven etiology. In the same study, 8 of 10 patients with persistent intrathoracic lymphadenopathy had bronchiectasis and recurrent consolidation, again suggesting a possible role for infection driving lymphoproliferation in this setting. In this study, 5/53 patients had mucosal nodular lymphoid hyperplasia identified bronchoscopically; the same phenomenon was observed in 6/9 of the APDS1 patients reported by Lucas et al. (3), all of whom underwent bronchoscopy, suggesting that milder cases will go undetected unless this invasive test is undertaken. As noted above, it is possible that this process contributes to the mosaic attenuation/air trapping noted on CT (**Figure 2**), and larger nodules might also lead to partial or total airway occlusion, segmental collapse, and post-obstructive bronchiectasis (**Figure 2**).

Of interest, although APDS can present with a CVID-like picture, it has not been associated with interstitial lymphoid or granulomatous infiltrates (granulomatous lymphocytic interstitial lung disease).

Malignant Lymphoproliferation

Lymphoma has been reported to be a frequent complication of both APDS1 and APDS2 (20, 22, 30). The metabolic reprogramming that occurs during malignant transformation through the upregulation of aerobic glycolysis has been used to distinguish benign lymphoproliferation from malignant disease; this can be probed on positron emission tomography by the increased uptake of the glucose analog, ^{18}F-fluorodeoxyglucose; biopsy is required where clinical or radiological suspicion is high. Lymphoma may involve mediastinal lymph nodes, or bronchus-associated lymphoid tissue, but this would normally be as part of a systemic process, and mediastinal nodes are more challenging to sample for histology than more peripheral nodes. While many lymph node stations in the chest are accessible *via* endobronchial ultrasound, and this technique has been used to diagnose lymphoma in immunocompetent patients (46), whole nodes cannot be removed in their entirety by this route; given the challenges in distinguishing between benign or malignant disease in immunodeficiency in general and APDS in particular, a larger pathological sample may be required. In this setting, if other nodes are not readily biopsied, a mediastinoscopy or video-assisted thoracoscopy might be required.

Other Non-Infectious Complications

Although congenital abnormalities have been reported, most are extra-thoracic. One patient with APDS1 was diagnosed with a pulmonary sequestration requiring lobectomy (47). A patient with SHORT syndrome associated APDS2 was found to have pulmonary hypertension, but this was likely secondary to the presence of mitral stenosis, although significant respiratory infections were also present (18). A single patient with a PIK3R1 mutation was found to have tracheomegaly as well as megancephaly and a double aortic arch in the context of megalencephaly capillary malformation syndrome (8). Common airway diseases such as asthma have seemingly been observed only at low frequency [e.g., Ref. (26)], and it is difficult to draw conclusions from these occasional reports.

MANAGEMENT OF THE RESPIRATORY MANIFESTATIONS OF APDS

The majority of patients with APDS1 [87% (20) and 73% (8)] and APDS2 [89% (22)] are reported as receiving immunoglobulin replacement, often from an early age; this high proportion exceeds the numbers reported to have low IgG levels, suggesting that the drivers for commencing therapy include recurrent infections in the setting of specific antibody deficiency or subclass deficiencies. More patients in the APDS1 cohorts (62 or 63%) than the APDS2 cohort (17%) noted above received additional prophylactic antibiotics (most commonly co-trimoxazole or azithromycin); the reason for this difference is unclear. There are little available data on the efficacy of these interventions; Coulter et al. (20) stated that there was "reported benefit in most cases," with none of the other case series specifically addressing this issue. Case reports have suggested that some patients exhibit marked (14, 23, 30, 36) or partial improvements (27), but others have flagged patients who had significant ongoing respiratory sepsis in the face of these treatments (15, 18). Of note, 42/68 patients currently listed on the APDS registry are currently receiving immunoglobulin replacement (see text footnote 1), with an overall reported decrease in respiratory infection and no withdrawals from therapy.

Rapamycin has been used to treat benign lymphoproliferative disease in APDS with some reported success (3), but respiratory-specific outcomes have not been published to date. A 12-week experimental medicine study (48) of the selective PI3Kδ inhibitor Leniolisib in six patients with APDS1 (three of whom had bronchiectasis) again did not report respiratory outcomes, but the observed improvements in B cell abnormalities characteristic of this disease (e.g., a reduction in circulating transitional B cells) suggest the potential for restoration of B cell function and hence a pulmonary protective role. Longer treatment regimens will be required to fully evaluate the benefits (and potential risks) of such interventions. Concerns have been raised that long-term PI3Kδ blockade increases genomic instability in B cells (49); however, these experiments were undertaken in mouse cells, and it is not clear that the same issues would complicate a therapeutic strategy aimed at normalizing, rather than abolishing PI3Kδ activity (48). Improvements in sinopulmonary infection have been reported following hematopoietic stem cell transplantation, with the majority of surviving patients no longer requiring immunoglobulin therapy (50); however, this procedure carries a significant mortality and will not alleviate established structural lung damage such as bronchiectasis. Early identification of patients with APDS (16) may allow transplantation before the development of such complications; however, the clinical heterogeneity makes prediction of future disease severity challenging.

CONCLUSION AND OUTLOOK

Despite the varied clinical manifestations of APDS, respiratory infections are a near-universal feature and often predominate in the early phase of the disease. A number of mechanisms may lead to this enhanced respiratory susceptibility (**Figure 1**). Viral pathogens subvert host PI3K signaling, and this may contribute to recurrent upper respiratory infections and impaired airway epithelial defensive function. Compromised antibody production, perhaps combined with aberrant cytokine production and the viral-induced airway damage, contributes to increased susceptibility to bacterial pathogens and recurrent lower respiratory infections. Cycles of infection lead to permanent damage to the lower airways, with the development of bronchiectasis, and may further drive the benign lymphoproliferation that is a prominent feature of APDS. In addition to supportive treatment (with immunoglobulin replacement and prophylactic antibiotics), the use of PI3Kδ inhibitors has the potential for a highly personalized treatment strategy. The identification of biomarkers to predict specific complications and disease severity would be of value in selecting patients for potentially curative bone marrow transplantation.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to writing this review.

FUNDING

AMC is supported by research funding from the Medical Research Council (MR/M012328) and the British Lung Foundation (RG14-1 and PRG16-13). AC is the recipient of a Wellcome Trust Postdoctoral Training Fellowship for Clinicians (103413/Z/13/Z).

REFERENCES

1. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33(5):361–9. doi:10.1111/j.1744-313X.2006.00627.x
2. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
3. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
4. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34(3):272–6. doi:10.1007/s10875-014-0012-9
5. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110δ cause activated PI3Kδ syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140(4):1152.e–6.e. doi:10.1016/j.jaci.2017.03.026
6. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110δ cause activated PI3K-δ syndrome 1 (APDS1). *Haematologica* (2017) 102(7):e278–81. doi:10.3324/haematol.2017.167601
7. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29–31. doi:10.1016/j.clim.2017.05.020
8. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Schölvinck L, Kalina T, et al. Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
9. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated

phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138(6):1672.e–80.e. doi:10.1016/j.jaci.2016.03.055
10. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923–8. doi:10.1172/JCI75746
11. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
12. Kuhlen M, Hönscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
13. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159(1):33–6. doi:10.1016/j.clim.2015.04.014
14. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36(5):462–71. doi:10.1007/s10875-016-0281-6
15. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kδ syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27(6):640–4. doi:10.1111/pai.12585
16. Martínez-Saavedra MT, García-Gomez S, Domínguez Acosta A, Mendoza Quintana JJ, Páez JP, García-Reino EJ, et al. Gain-of-function mutation in PIK3R1 in a patient with a narrow clinical phenotype of respiratory infections. *Clin Immunol* (2016) 173:117–20. doi:10.1016/j.clim.2016.09.011
17. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kδ regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114(8):1982–7. doi:10.1073/pnas.1617244114
18. Bravo García-Morato M, García-Miñaúr S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, López-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
19. Browning MJ, Chandra A, Carbonaro V, Okkenhaug K, Barwell J. Cowden's syndrome with immunodeficiency. *J Med Genet* (2015) 52(12):856–9. doi:10.1136/jmedgenet-2015-103266
20. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597.e–606.e. doi:10.1016/j.jaci.2016.06.021
21. Goto F, Uchiyama T, Nakazawa Y, Imai K, Kawai T, Onodera M. Persistent impairment of T-cell regeneration in a patient with activated PI3K δ syndrome. *J Clin Immunol* (2017) 37(4):347–50. doi:10.1007/s10875-017-0393-7
22. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210.e–8.e. doi:10.1016/j.jaci.2016.03.022
23. Tang WJ, Wang W, Luo Y, Wang YP, Li L, An YF, et al. [Clinical and immunological analysis of patients with activated phosphoinositide 3-kinase δ syndrome resulting from PIK3CD mutation]. *Zhonghua Er Ke Za Zhi* (2017) 55(1):19–24. doi:10.3760/cma.j.issn.0578-1310.2017.01.004
24. Mettman D, Thiffault I, Dinakar C, Saunders C. Immunodeficiency-associated lymphoid hyperplasia as a cause of intussusception in a case of activated PI3K-δ syndrome. *Front Pediatr* (2017) 5:71. doi:10.3389/fped.2017.00071
25. Teranishi H, Ishimura M, Koga Y, Eguchi K, Sonoda M, Kobayashi T, et al. Activated phosphoinositide 3-kinase δ syndrome presenting with gut-associated T-cell lymphoproliferative disease. *Rinsho Ketsueki* (2017) 58(1):20–5. doi:10.11406/rinketsu.58.20
26. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183(2):221–9. doi:10.1111/cei.12706
27. Hauck F, Magg T, Krolo A, Bilic I, Hirschmugl T, Laass M, et al. Variant PIK3R1 hypermorphic mutation and clinical phenotypes in a family with short statures, mild immunodeficiency and lymphoma. *Klin Padiatr* (2017) 229(3):113–7. doi:10.1055/s-0043-104218
28. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kδ syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
29. Lee KS, Jeong JS, Kim SR, Cho SH, Kolliputi N, Ko YH, et al. Phosphoinositide 3-kinase-δ regulates fungus-induced allergic lung inflammation through endoplasmic reticulum stress. *Thorax* (2016) 71(1):52–63. doi:10.1136/thoraxjnl-2015-207096
30. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase δ syndrome. *J Allergy Clin Immunol* (2014) 134(1):233–6. doi:10.1016/j.jaci.2014.02.020
31. Liu X, Cohen JI. The role of PI3K/Akt in human herpesvirus infection: from the bench to the bedside. *Virology* (2015) 47(9–480):568–77. doi:10.1016/j.virol.2015.02.040
32. Hsu AC, Starkey MR, Hanish I, Parsons K, Haw TJ, Howland LJ, et al. Targeting PI3K-p110α suppresses influenza virus infection in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* (2015) 191(9):1012–23. doi:10.1164/rccm.201501-0188OC
33. Frese KK, Lee SS, Thomas DL, Latorre IJ, Weiss RS, Glaunsinger BA, et al. Selective PDZ protein-dependent stimulation of phosphatidylinositol 3-kinase by the adenovirus E4-ORF1 oncoprotein. *Oncogene* (2003) 22(5):710–21. doi:10.1038/sj.onc.1206151
34. Esfandiarei M, Luo H, Yanagawa B, Suarez A, Dabiri D, Zhang J, et al. Protein kinase B/Akt regulates coxsackievirus B3 replication through a mechanism which is not caspase dependent. *J Virol* (2004) 78(8):4289–98. doi:10.1128/JVI.78.8.4289-4298.2004
35. Kan-o K, Matsumoto K, Asai-Tajiri Y, Fukuyama S, Hamano S, Seki N, et al. PI3K-delta mediates double-stranded RNA-induced upregulation of B7-H1 in BEAS-2B airway epithelial cells. *Biochem Biophys Res Commun* (2013) 435(2):195–201. doi:10.1016/j.bbrc.2013.04.082
36. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased in vitro anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2017) 178:20–8. doi:10.1016/j.clim.2015.12.008
37. Boyle KB, Gyori D, Sindrilaru A, Scharffetter-Kochanek K, Taylor PR, Mócsai A, et al. Class IA phosphoinositide 3-kinase β and δ regulate neutrophil oxidase activation in response to *Aspergillus fumigatus* hyphae. *J Immunol* (2011) 186(5):2978–89. doi:10.4049/jimmunol.1002268
38. Boyton RJ, Altmann DM. Bronchiectasis: current concepts in pathogenesis, immunology, and microbiology. *Annu Rev Pathol* (2016) 11:523–54. doi:10.1146/annurev-pathol-012615-044344
39. Chalmers JD, Hill AT. Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis. *Mol Immunol* (2013) 55(1):27–34. doi:10.1016/j.molimm.2012.09.011
40. Gifford AM, Chalmers JD. The role of neutrophils in cystic fibrosis. *Curr Opin Hematol* (2014) 21(1):16–22. doi:10.1097/MOH.0000000000000009
41. Chalmers JD, Moffitt KL, Suarez-Cuartin G, Sibila O, Finch S, Furrie E, et al. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. *Am J Respir Crit Care Med* (2017) 195(10):1384–93. doi:10.1164/rccm.201605-1027OC
42. Sapey E, Stockley JA, Greenwood H, Ahmad A, Bayley D, Lord JM, et al. Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* (2011) 183(9):1176–86. doi:10.1164/rccm.201008-285OC
43. Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, et al. Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood* (2014) 123(2):239–48. doi:10.1182/blood-2013-08-519520
44. Thien F. Measuring and imaging small airways dysfunction in asthma. *Asia Pac Allergy* (2013) 3(4):224–30. doi:10.5415/apallergy.2013.3.4.224
45. Sugiyama M, Iguchi A, Yamada M, Terashita Y, Ohshima J, Cho Y, et al. Successful bone marrow transplantation in two sisters with activated phosphoinositide 3-kinase δ syndrome 2. *Bone Marrow Transplant* (2017) 52(12):1678–80. doi:10.1038/bmt.2017.189
46. Nason KS, Kirchner A, Schuchert MJ, Luketich JD, Christie NA, Pantanowitz L, et al. Endobronchial ultrasound-transbronchial needle aspiration for lymphoma in patients with low suspicion for lung cancer and mediastinal lymphadenopathy. *Ann Thorac Surg* (2016) 101(5):1856–63. doi:10.1016/j.athoracsur.2015.12.019

47. Kannan JA, Dávila-Saldaña BJ, Zhang K, Filipovich AH, Kucuk ZY. Activated phosphoinositide 3-kinase δ syndrome in a patient with a former diagnosis of common variable immune deficiency, bronchiectasis, and lymphoproliferative disease. *Ann Allergy Asthma Immunol* (2015) 115(5):452–4. doi:10.1016/j.anai.2015.08.009
48. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective 'activated PI3Kδ syndrome'-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
49. Compagno M, Wang Q, Pighi C, Cheong TC, Meng FL, Poggio T, et al. Phosphatidylinositol 3-kinase δ blockade increases genomic instability in B cells. *Nature* (2017) 542(7642):489–93. doi:10.1038/nature21406
50. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol* (2017) 139(3):1046–9. doi:10.1016/j.jaci.2016.09.040

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 07 March 2018
doi: 10.3389/fimmu.2018.00446

Exhaustion of the CD8+ T Cell Compartment in Patients with Mutations in Phosphoinositide 3-Kinase Delta

*Marjolein W. J. Wentink[1†], Yvonne M. Mueller[1†], Virgil A. S. H. Dalm[1,2], Gertjan J. Driessen[3,4], P. Martin van Hagen[1,2], Joris M. van Montfrans[5], Mirjam van der Burg[1] and Peter D. Katsikis[1]**

[1] Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, Netherlands, [2] Department of Internal Medicine – Division of Clinical Immunology, Erasmus MC, University Medical Center, Rotterdam, Netherlands, [3] Division of Pediatrics, Juliana Children's Hospital, Haga Teaching Hospital, The Hague, Netherlands, [4] Division of Pediatric Infectious Disease and Immunology, Erasmus MC, University Medical Center, Rotterdam, Netherlands, [5] Division of Pediatrics, Pediatric Immunology and Infectious Disease, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, Netherlands

OPEN ACCESS

Edited by:
Stuart G. Tangye,
Garvan Institute of Medical Research, Australia

Reviewed by:
Emily Mace,
Baylor College of Medicine, United States
Toru Uchiyama,
National Center for Child Health and Development (NCCHD), Japan

***Correspondence:**
Peter D. Katsikis
p.katsikis@erasmusmc.nl

[†]These authors have contributed equally to this work.

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: 15 November 2017
Accepted: 19 February 2018
Published: 07 March 2018

Citation:
Wentink MWJ, Mueller YM, Dalm VASH, Driessen GJ, van Hagen PM, van Montfrans JM, van der Burg M and Katsikis PD (2018) Exhaustion of the CD8+ T Cell Compartment in Patients with Mutations in Phosphoinositide 3-Kinase Delta. Front. Immunol. 9:446. doi: 10.3389/fimmu.2018.00446

Pathogenic gain-of-function mutations in the gene encoding phosphoinositide 3-kinase delta (PI3Kδ) cause activated PI3Kδ syndrome (APDS), a disease characterized by humoral immunodeficiency, lymphadenopathy, and an inability to control persistent viral infections including Epstein–Barr virus (EBV) and cytomegalovirus (CMV) infections. Understanding the mechanisms leading to impaired immune response is important to optimally treat APDS patients. Immunosenescence of CD8+ T cells was suggested to contribute to APDS pathogenesis. However, the constitutive activation of T cells in APDS may also result in T cell exhaustion. Therefore, we studied exhaustion of the CD8+ T cell compartment in APDS patients and compared them with healthy controls and HIV patients, as a control for exhaustion. The subset distribution of the T cell compartment of APDS patients was comparable with HIV patients with decreased naive CD4+ and CD8+ T cells and increased effector CD8+ T cells. Like in HIV+ patients, expression of activation markers and inhibitory receptors CD160, CD244, and programmed death receptor (PD)-1 on CD8+ T cells was increased in APDS patients, indicating exhaustion. EBV-specific CD8+ T cells from APDS patients exhibited an exhausted phenotype that resembled HIV-specific CD8+ T cells in terms of inhibitory receptor expression. Inhibition of PD-1 on EBV-specific CD8+ T cells from APDS patients enhanced *in vitro* proliferation and effector cytokine production. Based on these results, we conclude that total and EBV-specific CD8+ T cells from APDS patients are characterized by T cell exhaustion. Furthermore, PD-1 checkpoint inhibition may provide a possible therapeutic approach to support the immune system of APDS patients to control EBV and CMV.

Keywords: activated phosphoinositide 3-kinase delta syndrome, p110δ, PI3K, CD8+ T cells, exhaustion, programmed death receptor-1, checkpoint inhibition

INTRODUCTION

The phosphoinositide 3-kinase–AKT (PI3K–AKT) signaling pathway is involved in many crucial cellular processes including regulation of metabolism, proliferation, apoptosis, cell cycle regulation, and protein synthesis (1–3). In human lymphocytes, the phosphoinositide 3-kinase delta (PI3Kδ) isoform, a heterodimer consisting of the catalytic subunit p110δ (encoded by *PIK3CD*) and

regulatory subunit p85α (encoded by *PIK3R1*), is essential for both B cell and T cell development and maturation (4–7). For $CD8^+$ T cells, PI3Kδ has been shown to be essential for optimal immune responses to pathogens (8, 9).

Over the past years, patients with gain-of-function (GOF) mutations in PI3Kδ have been described (10–12). These patients suffer from a specific form of primary immune deficiency called activated PI3Kδ syndrome (APDS) (13, 14). This disease is characterized by disturbed humoral immunity resulting in hypogammaglobulinemia, recurrent respiratory tract infections an absent response to polysaccharide vaccination, pulmonary damage, lymphadenopathy, hepatosplenomegaly, an increased risk for hematological malignancies, and an inability to control persistent viral infections such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV) infections (13, 14). Immunophenotypically, these patients have decreased numbers of total $CD4^+$ and especially naive $CD4^+$ T cells together with increased $CD8^+$ effector T cells. Furthermore, they have a relative increase in their transitional B cells accompanied by reduced memory B cells (15). Several studies indicated that the effector function of their T cells is defective, causing an inability to control chronic viral infections including CMV and EBV infections (13, 14).

Impaired T cell effector function can be caused by different mechanisms, one of which is senescence (16, 17). Hallmarks of senescence are permanent cell cycle arrest (18, 19) and resistance to apoptosis (20, 21). Importantly, senescent T cells are metabolically and functionally active and retain their cytotoxic functions and ability to produce and secrete cytokines (22, 23). Reduced telomere length and surface-expression of CD57 were used to define senescent T cells. However, to reliably distinguish senescence from other causes of T cell impairment additional markers like senescence-associated β-galactosidase and cyclin-dependent kinase inhibitor 2A (p16Ink4A) can be used (24, 25). Although senescence is age dependent, other factors such as CMV infection can contribute to senescence (26).

Exhaustion of T cells due to chronic antigenic stimulation is another mechanism leading to impaired T cell effector functions. T cell exhaustion was first described in chronic viral infections such as lymphocytic choriomeningitis virus infection in mice (27–29) but is also recognized as an underlying mechanism in immunological failure in human viral infections including HIV infection (30–33) and tumors (34–36). Exhaustion is a hierarchical process (37, 38) by which $CD8^+$ T cells first lose their proliferative capacity and IL-2 secretion, followed by diminished secretion of effector cytokines such as tumor necrosis factor (TNF) α and interferon (IFN) γ and eventually they become sensitive to apoptosis, which leads to the loss of these cells (29, 38, 39). Simultaneously, these cells upregulate several inhibitory receptors including programmed death receptor (PD)-1, CD160, and CD244 which, when co-expressed, indicate later stages of exhaustion (40–43). These inhibitory receptors are considered to play a central role in exhaustion. Blocking these inhibitory receptors on exhausted $CD8^+$ T cells can restore or improve their function in chronic viral infections as shown *in vitro* (41, 42, 44–46) and *in vivo* (40, 47–51). In addition, inhibitory receptor blockade was introduced into the clinic to re-activate exhausted T cells in cancer (52, 53).

Previously, total and virus-specific $CD8^+$ T cells in APDS patients were shown to have upregulated CD57 expression and reduced proliferative capacity. These findings were interpreted as T cell senescence (12, 54–56). However, patients' lymphocytes also exhibited an increased rate of apoptosis compared with healthy controls (11, 15, 54), which is not in line with the resistance to apoptosis that has been ascribed to T cell senescence (21). Increased apoptosis sensitivity is associated with exhaustion rather than senescence (39, 57). In addition, APDS patient T cells have been reported to express more PD-1, a receptor associated with T cell activation and exhaustion (12, 54, 55). The PI3Kδ pathway is critical for TCR signaling in $CD8^+$ T cells (58) and chronic antigen stimulation alone is sufficient to lead to $CD8^+$ T cell exhaustion (59). This raises the question whether GOF mutations in PI3Kδ lead to changes in the activation of T cells which might predispose for T cell exhaustion rather than or in addition to immune senescence. Understanding the mechanisms leading to impaired immune response in APDS patients is a requirement to define the best treatment options for these patients that can support the control of viral infections, which could in turn reduce virus-related morbidities in these patients.

To elucidate the role of exhaustion in APDS patients, total $CD8^+$ T cells and $CD4^+$ T cells from APDS patients were phenotypically characterized and compared with T cells from healthy individuals and HIV-infected patients (HIV^+ patients). We have included peripheral blood mononuclear cells (PBMC) from HIV-infected patients since it is well established that HIV infection leads to exhaustion of HIV-specific $CD8^+$ T cells but not CMV-specific $CD8^+$ T cells in HIV-infected patients (39, 41) and can therefore serve as a positive control for exhaustion. Furthermore, virus-specific $CD8^+$ T cells in all three groups were characterized, and the effect of PD-1 blockade on proliferation and effector functions was investigated. Our findings indicate that indeed $CD8^+$ T cells from APDS patients are more similar to the ones from HIV^+ patients and exhibit characteristics of exhaustion. Importantly, we show that blocking PD-1 signaling can increase virus-specific $CD8^+$ T cell proliferation and cytokine production. Our findings suggest that $CD8^+$ T cells in APDS patients undergo exhaustion, and that this may contribute to the impaired control of persistent viral infections such as EBV and CMV. These findings raise the possibility of checkpoint inhibition as a treatment strategy to support APDS patients to control recurrent or chronic viral infections.

MATERIALS AND METHODS

Cell Samples and Ethical Approval

This study was carried out in accordance with the recommendations of Erasmus MC Medical Ethics Committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Erasmus MC Medical Ethics Committee.

Ten APDS patients were included with a median age of 27 years (range 6–44 years), and a gender ratio of six males to four females. Nine of the patients have a mutation in the PIK3CD gene (seven

patients: E1021K mutation, one patient: S312R mutation, and one patient: R929C mutation) (15), and one patient has a mutation in the PIK3R1 gene (N564K). From the patients including in this study, 9/10 received immunoglobulin substitution therapy, and 4/10 received prophylactic antibiotics. None of the patients received steroids or immune modulating drugs at the time of sampling. None of the APDS patients had active EBV or CMV infection at the time of sampling. Three of the APDS patients are EBV-antibody positive, and two are EBV-antibody negative, for the other patients the EBV status is not known. CMV status is not known from these APDS patients. From the healthy controls, nine are EBV positive. The five HIV-infected patients included have a median age of 42 years (range 35–46), gender ratio is one female to four males, three patients have undetectable viral loads (below 20 HIV copies/ml), and two have detectable viral loads (295 and 4,900 copies/ml, respectively). The median CD4 count is 300 cells/μl (range 50–630 cells/μl), and four out of the five patients are on antiretroviral therapy. The 10 healthy control individuals included have a median age of 27 years (range 18–51) and a gender ratio of five males to five females.

Peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque (GE Healthcare Life Sciences) density centrifugation, frozen in freezing media [90% fetal bovine serum (FBS)/10% DMSO], and stored in liquid nitrogen until used. Clinical data were provided by treating physicians. Due to availability of material, not all tests could be performed on all samples.

Flow Cytometric Immunophenotyping

Peripheral blood mononuclear cells were thawed, rested for 30–60 min at 37°C, and stained with previously determined optimal amounts of tetramers and antibodies. For phenotyping of surface antigens, $0.8–1 \times 10^6$ cells were washed with Facs wash [FW, Hanks' buffered saline solution (Corning), 3% fetal bovine serum (Gibco), and 0.02% NaN_3], stained with tetramer/antibody mix for 30 min at 4°C, washed two times with FW, and fixed with 1% paraformaldehyde. Anti-HLA-A2-PE antibodies (clone BB7.2) were used to identify HLA-$A2^+$ donors. Virus-specific $CD8^+$ T cells were identified by using APC- or PE-conjugated HLA class I A*0201-β2-microglobulin tetramers loaded with HIV Gag p17 77–85 (SLYNTVATL) peptide, HIV Pol 476–484 (ILKEPVHGV) peptide, EBV peptide (GLCTLVAML), and CMV peptide (NLVPMVATV) (all tetramers were prepared in the lab). The following directly conjugated monoclonal antihuman antibodies were used: CD3-BV421 (clone UCHT1), CD4-BV650 (SK3), CD8-BV786 (RPA-T8), CD45RA-APC-H7 (HI100), CCR7-PE-CF594 and Alexa Fluor 700 (CD197, 150503), PD-1-BV711 (CD279, EH12.1), CD160-Alexa Fluor 488 (BY55), CD244-PE (eBioC1.7, eBioscience), HLA-DR-BV605 (G46-6), CD38-PE-Cy7 (HIT2), CD57-BV605 (NK-1), TNFα-FITC (MAb11, eBioscience), and IFNγ-PECy7 (4S.B3, eBioscience). All antibodies were purchased from BD Biosciences unless otherwise indicated. When AnnexinV-PerCP–Cy5.5 was used to exclude dead cells, 2.5 mM $CaCl_2$ was added to all solutions.

Between 1 and 4×10^5 events were collected per sample within 24 h after staining on an LSRFortessa (BD Biosciences, 4 lasers, 18 parameters) and analyzed using FlowJo software (version 9.9.4, Tree Star). Data are represented as frequency within a defined population.

In Vitro Proliferation

To determine proliferative capacity of proliferation-dye-labeled virus-specific $CD8^+$ T cells, thawed PBMC were incubated with 0.1 μM of CellTrace Far Red Cell stain (Invitrogen) in PBS for 20 min at 37°C, and free dye was removed by adding RPMI-10% FBS and incubating for 5 min at 37°C. Cells were spun down and resuspended in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate and added to 24-well plates in a concentration of 1×10^6 PBMC/ml. To inhibit PD-1/PDL-1 interaction, 10 μg/ml of anti-PD-L1 antibody (CD274, MIH1, eBioscience) or isotype control (Mouse IgG1, eBioscience) was added, and cells were incubated for 30 min at 37°C. Virus-specific peptide in a concentration of 1 μg/ml was added then in appropriate wells: Gag peptide (SL9, SLYNTVATL, ANASPEC), Pol peptide (IV9, ILKEPVHGV, ANASPEC), CMV peptide (pp65, NLVPMVATV, ANASPEC), EBV peptide (BMLF1, GLCTLVAML, ANASPEC), EBV peptide pool (PepMix EBV BMLF1, JPT), or media alone (no peptide stimulation). Purified anti-CD28 (1 μg/ml, clone CD28.2, BD Biosciences) and anti-CD49d (1 μg/ml, clone 9F10, BD Biosciences) were added to all wells. Cells were incubated for 5 days at 37°C in a 5% CO_2 incubator. Cells were harvested on day 5, counted and resuspended in RPMI-10% FBS/1 μg/ml Brefeldin A (GolgiPlug, BD Biosciences)/anti-CD28 (1 μg/ml)/anti-CD49d (1 μg/ml), and incubated for 6 h to determine cytokine production of these cells. For intracellular staining for cytokines, PBMC were first stained for surface antigens, fixed and permeabilized (Cytofix/Cytoperm, BD Bioscience), incubated with the antibodies (see above) for 60 min, washed two times with Perm/Wash Buffer (BD Biosciences), and fixed with 1% paraformaldehyde.

Statistical Analysis

All data sets were tested for normal distribution using the D'Agostino–Pearson omnibus test. Relative distributions of T cell subsets and comparisons of population frequencies data were analyzed using either the non-parametric Mann–Whitney *U* test or a *T*-test, dependent on whether or not the data were normally distributed ($p < 0.05$ was considered statistically significant). When more than two data sets were analyzed in the same test, the Kruskal–Wallis test was performed, combined with a Dunn's multiple comparisons test. Correlations were calculated using a Spearman model for correlation, since in the majority of groups at least one population was not normally distributed. Statistics were performed using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). Populations' frequencies per group are represented as mean with SEM.

RESULTS

Patient Characteristics

We included 10 patients with APDS. From one patient (Pt1), two samples were included, one taken at age 7 years, and one collected at age 25 years. For analysis of total $CD4^+$ and $CD8^+$ T cells, we

only included the adult sample. For analysis of virus-specific CD8$^+$ T cells, we included samples from both time-points. Most of the patients have been described before (15). The majority of patients ($n = 7$) carried the previously described E1021K mutation in *PIK3CD* (11, 12), one carried an R929C mutation in *PIK3CD*, and one carried an N564K (15) GOF mutation in *PIK3R1*. One patient carried a missense variant c.935C>G (NM_005026.3) resulting in an amino acid change p.S312C (NP_005017.3) in *PIK3CD*. This latter variant is also found in the general population with a minor allele frequency of around 2% (SNP reference: rs61755420) (60) and can therefore not be classified as disease causing. However, this patient does suffer from antibody deficiency and autoimmunity and increased phosphorylation of AKT in lymphocyte subsets was found (Wentink, unpublished data). Therefore, we decided to study the effect of this variant together with patients with known disease causing mutations. Two of the patients were HLA-A2-positive (including the patient with samples available as a child and an adult) and EBV-specific CD8$^+$ T cells were analyzed.

We compared the PBMC from APDS patients to PBMC from 10 healthy controls and 5 HIV$^+$ patients.

The Phenotype of T Cells in APDS Patients Is Distinct from Healthy Controls and Resembles HIV-Infected Patients

Previous studies on the T cell compartment of APDS patients showed a reduction of the naive CD4$^+$ and CD8$^+$ T cells and an increase in the CD8$^+$ T cell effector memory (EM) population (11–13). In our patient cohort, we observed a comparable skewing of the T cell populations. The frequencies of CD45RA$^+$CCR7$^+$ naive CD4$^+$ T cells were significantly decreased in APDS patients compared with healthy controls and comparable with the frequencies in HIV$^+$ patients (**Figures 1A,B**). CD45RA$^+$CCR7$^+$ naive CD8$^+$ T cells were significantly reduced in our APDS patient cohort compared with the healthy controls although not as profound as observed in HIV$^+$ patients (**Figure 1C**). Within memory CD4$^+$ T cells, an increase in CD45RA$^-$CCR7$^+$ central memory (CM) cells was found for the APDS and the HIV$^+$ patients compared with healthy controls (**Figure 1D**). The CD45RA$^-$CCR7$^-$ EM CD4$^+$ T cell frequencies were not significantly different when healthy controls, APDS and HIV$^+$ patients were compared (**Figure 1D**). Frequencies of CM CD8$^+$ T cells were comparable between healthy controls and APDS patients as were the CD45RA$^+$CCR7$^-$ EM re-expressing CD45RA (EMRA) CD8$^+$ T cell populations (**Figure 1E**). A significant increase was found for the CD45RA$^-$CCR7$^-$ EM CD8$^+$ T cell population for APDS patients and HIV$^+$ patients when compared with healthy controls. These findings indicate that both patient populations show comparable reduction of naive T cells and increased EM CD8$^+$ T cells (**Figure 1E**).

To examine the effect of the GOF mutations on chronic activation we determined the expression of several chronic activation markers on T cells from healthy controls, APDS patients and HIV$^+$ patients. The frequency of CD38^{bright}CD8$^+$ T cells was increased in APDS patients compared with healthy controls; however, HIV$^+$ patients had an even higher percentage of CD38^{bright}CD8$^+$ T cells (**Figure 2A**). Although the frequency of CD38^{bright}CD4$^+$ T cells was also higher in APDS patients compared with healthy controls this was not significant (**Figure 2D**). HLA-DR was examined as a second marker of chronic activation, and indeed a significant increase was found for HIV$^+$ patients within the CD8$^+$ T cell population. HLA-DR expression was significantly increased on CD8$^+$ T cells (**Figure 2B**) and CD4$^+$ T cells (**Figure 2E**) from the APDS patients compared with healthy controls.

We studied the expression of CD57 on CD8$^+$ and CD4$^+$ T cells, since this was reported to be increased in a subset of APDS patients. We found that in APDS patients 34.9 ± 5.0% (mean ± SEM) of CD8$^+$ T cells express CD57, compared with 25.2 ± 4.0% in healthy controls and 56.4 ± 5.2% in HIV$^+$ patients (**Figure 2C**). This indicates that although CD57$^+$CD8$^+$ T cells are increased in APDS patients, this is not significantly different from the frequency in healthy controls and lower than the frequency in HIV$^+$ patients. A small but non-significant increase of CD57$^+$ cells was also found within the CD4$^+$ T cell population of APDS patients compared with healthy controls (**Figure 2F**). Overall, the expression of activation markers and CD57 indicate that T cells from APDS patients tend to be more activated than healthy controls and are therefore more alike T cells from HIV$^+$ patients.

APDS Patients Have Increased Inhibitory Receptor Expression on CD8$^+$ T Cells

Since exhaustion is a gradual process in which cells over time co-express multiple inhibitory receptors, we studied both the single expression of PD-1, CD160, and CD244 and co-expression of these three receptors on CD8$^+$ T cells (**Figures 3A–E**). As we observed with the activation markers, the expression profile of inhibitory receptors within the APDS patients is very heterogeneous, with some patients in the range of controls and others in the range of HIV$^+$ patients. We observed a significantly higher percentage of CD8$^+$ T cells from APDS patients expressing CD160 compared with controls (mean 38 ± 6.0 and 21 ± 4.5%, respectively). This frequency in APDS patients was closer to HIV$^+$ patients (mean 50 ± 6.8%) (**Figure 3B**). The frequency of CD244-expressing CD8$^+$ T cells within APDS patients and HIV$^+$ patients was significantly increased compared with healthy controls (**Figure 3C**). PD-1 was also found increased on CD8$^+$ T cells in APDS patients compared with controls (mean 38.8 ± 6.5 and 23.6 ± 6.9%, respectively) (**Figure 3D**). Most importantly, we observed a significant increase in the frequency of CD8$^+$ T cells expressing all three inhibitory receptors, PD-1, CD244, and CD160 (PD-1$^+$CD160$^+$CD244$^+$), in the APDS patients which was 20 ± 5.6% compared with 6 ± 2.3% PD-1$^+$ CD160$^+$CD244$^+$CD8$^+$ T cells in healthy controls. This frequency of PD-1$^+$CD160$^+$CD244$^+$CD8$^+$ T cells in APDS patients is comparable with the one observed in HIV$^+$ patients (21 ± 7.6%).

We examined whether a history of EBV infection influences the expression of inhibitory receptors on CD8$^+$ T cells. From the APDS patients, EBV status information was available from five individuals, with three being EBV-antibody positive and two being EBV-antibody negative. We compared the expression of inhibitory receptors on CD8$^+$ T cells from these two groups to the expression of the inhibitory receptors on the CD8$^+$ T cells in the nine EBV-positive healthy controls. We found that the frequency

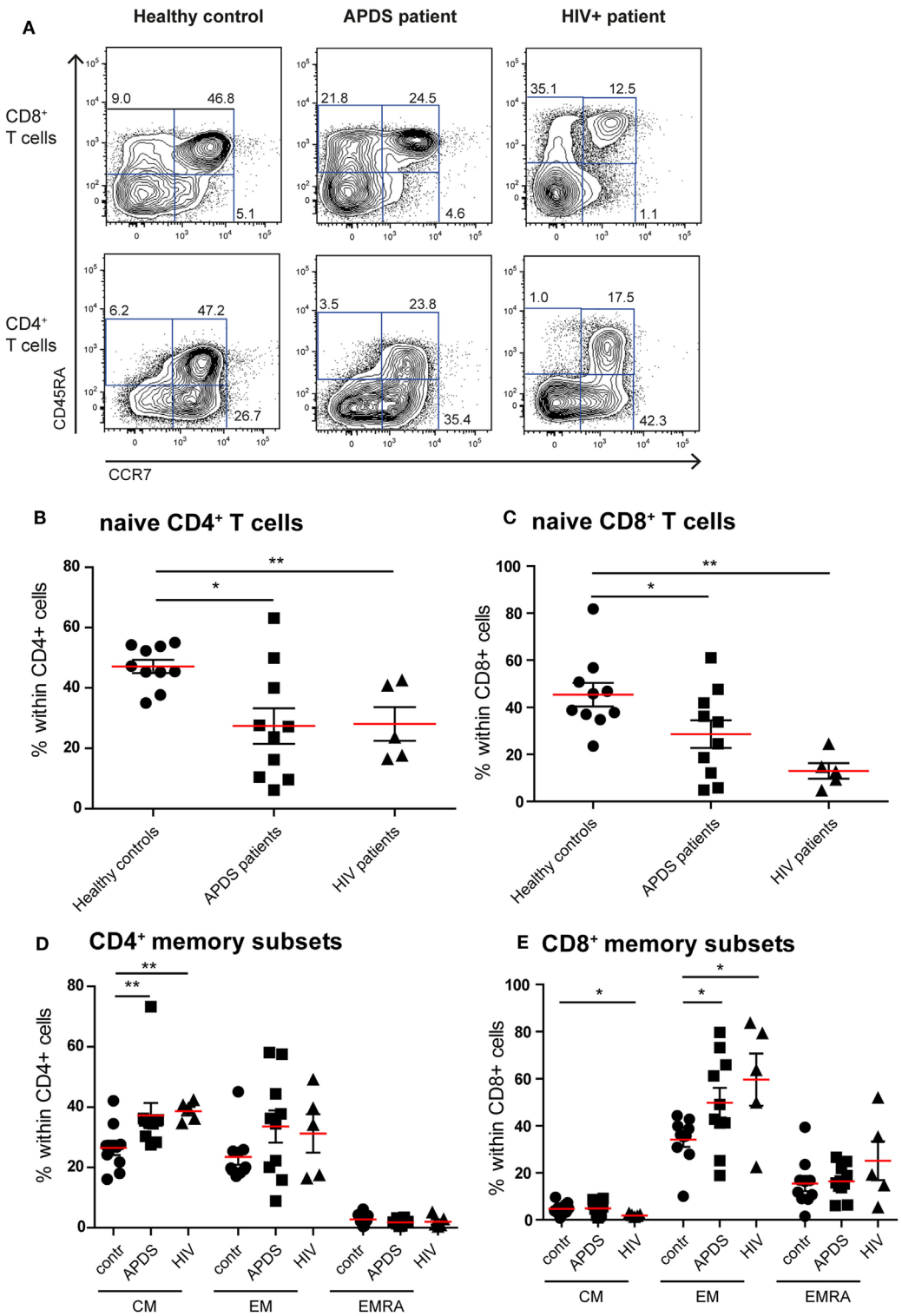


FIGURE 1 | Immunophenotyping of the T-cell compartment of controls (black dots), activated PI3Kδ syndrome (APDS) patients (black squares), and HIV+ patients (black triangles). Red lines and brackets indicate the mean and SEM of each group (*$p < 0.05$ and **$p < 0{,}005$). **(A)** Representative dot plots of controls and patients indicating naive (CD45RA+CCR7+), central memory (CM) (CD45RA−CCR7+), effector memory (EM) (CD45RA−CCR7−), and EMRA (CD45RA+CCR7−) CD8+ and CD4+ T cell subsets. Numbers depict frequency of cell populations. **(B,C)** The frequency of naive CD4+ T cells **(B)** and CD8+ T cells **(C)** is reduced in APDS patients and HIV+ patients compared with controls. **(D)** The frequency of CM CD4+ T cells is increased in APDS patients and HIV+ patients. The frequency of the different memory CD4+ T cells in controls, APDS patients, and HIV+ patients is shown. **(E)** The frequency of EM CD8+ T cells is increased in APDS patients and HIV+ patients. The different memory CD8+ T cell subpopulations are shown for controls, APDS patients and HIV+ patients.

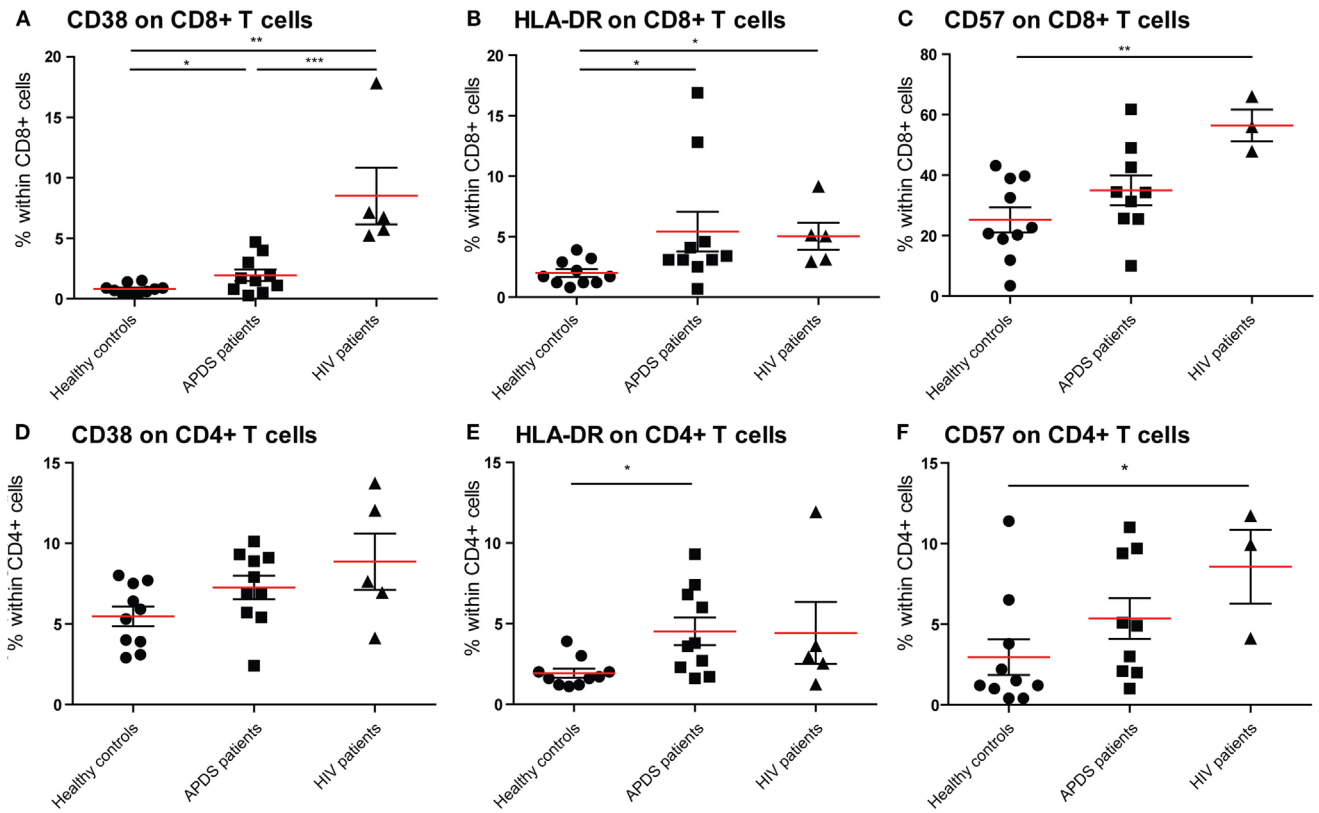


FIGURE 2 | Expression of CD38, HLA-DR, and CD57 on CD8⁺ and CD4⁺ T cells from controls (black dots), activated PI3Kδ syndrome (APDS) patients (black squares), and HIV⁺ patients (black triangles). Red lines and brackets indicate the mean and SEM of each group (*$p < 0.05$ and **$p < 0,005$). **(A)** The frequency of $CD38^{bright}$ cells is increased in APDS patients and HIV⁺ patients compared with controls. The frequencies of $CD38^{bright}$ cells within CD8⁺ cells are shown for healthy controls, APDS and HIV⁺ patients. **(B)** The frequency of HLA-DR⁺ cells is increased in APDS patients and HIV⁺ patients compared with controls. Frequencies of HLA-DR⁺ cells within CD8⁺ T cells are shown. **(C)** Frequency of CD57⁺CD8⁺ T cells is not increase in APDS patients. Frequencies of CD57⁺ cells within CD8⁺ cells shown for healthy controls, APDS patients, and HIV⁺ patients. **(D)** Frequency of CD38⁺CD4⁺ T cells is comparable in healthy controls, APDS patients, and HIV⁺ patients. **(E)**, Frequency of HLA-DR⁺CD4⁺ T cells is increased in APDS patients. Frequency of HLA-DR⁺ cells within CD4⁺ T cells shown for healthy controls, APDS patients, and HIV⁺ patients. **(F)** Frequency of CD57⁺CD4⁺ T cells is not increased in APDS patients compared with healthy controls. Frequency of CD57-expressing cells within CD4⁺ T cells shown.

of $CD160^+$, $CD244^+$, PD-1^+, and $CD160^+CD244^+$PD-1^+CD8^+ T cells is highest in the EBV⁺ APDS patients ($CD160^+CD8^+$: 57 ± 4.9%; $CD244^+CD8^+$: 87 ± 3.4%; PD-1^+CD8^+: 61 ± 8.2%; $CD160^+CD244^+$PD-1^+CD8^+: 36 ± 5.6%), but lower in the EBV⁻ APDS patients and the EBV⁺ healthy controls ($CD160^+CD8^+$: 26 ± 3.8 and 22 ± 4.7%; $CD244^+CD8^+$: 43 ± 5.3 and 48 ± 5.9%; PD-1^+CD8^+: 18 ± 3.0 and 25 ± 7.6%; $CD160^+CD244^+$PD-1^+CD8^+: 5.6 ± 1.5 and 7.0 ± 2.5% for EBV⁻ APDS patients and EBV⁺ healthy control, respectively). Thus, EBV-antibody positivity is accompanied by increased inhibitory receptor expression on CD8⁺ T cells.

We next analyzed whether reduced naive CD8⁺ T cell frequency and PD-$1^+CD160^+CD244^+CD8^+$ T cells correlate in healthy controls and APDS patients. Although a negative correlation was already observed for healthy controls (**Figure 3F**), the correlation between the frequencies of PD-$1^+CD160^+CD244^+CD8^+$ T cells and naive CD8⁺ T cells was highly significant in APDS patients (**Figure 3F**). For the HIV⁺ patients, this relationship was not significantly correlated. These results indicate that total CD8⁺ T cells from APDS patients have increased co-expression of inhibitory receptors similar to what is observed in HIV⁺ patients. Furthermore, the negative correlation of PD-$1^+CD160^+CD244^+CD8^+$ T cells and naive CD8⁺ T cells indicates that exhaustion in this compartment is associated with the skewed subset distribution.

Virus-Specific CD8⁺ T Cells from APDS Patients Exhibit an Exhaustion Phenotype

To further compare exhaustion in APDS patients to exhaustion due to HIV infection, we analyzed virus-specific CD8⁺ T cells in both patient groups and healthy controls. HIV-specific CD8⁺ T cells from HIV⁺ patients are highly sensitive to apoptosis, present with a skewed memory phenotype, have proliferative defects, and show increased expression of inhibitory receptors (29, 38, 39). However, in the same HIV⁺ patients, CMV-specific CD8⁺ T cells are not impaired. Using peptide-loaded HLA-A2 tetramers, virus-specific CD8⁺ T cells were analyzed from three APDS

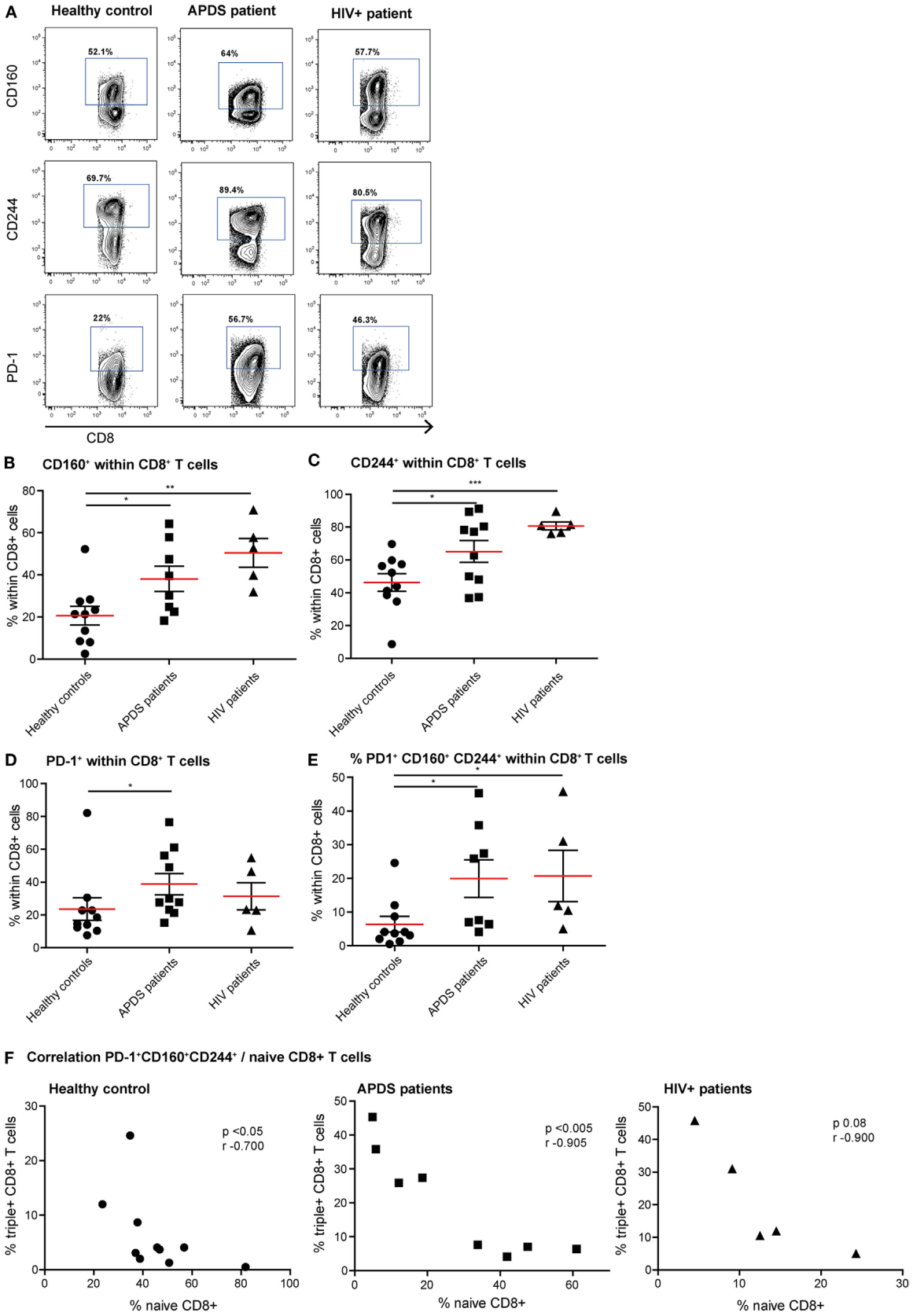


FIGURE 3 | Continued

FIGURE 3 | Expression of inhibitory receptors on CD8+ cells from controls (black dots), activated PI3Kδ syndrome (APDS) patients (black squares), and HIV+ patients (black triangles). Red lines and brackets indicate the mean and SEM of each group (*$p < 0.05$ and **$p < 0{,}005$). **(A)** Representative dot plots of controls and patients indicating populations that were considered positive for inhibitory receptors. **(B)** CD160 expression is increased on CD8+ T cells from APDS patients and HIV+ patients compared with controls. **(C)** CD244 expression is increased on CD8+ T cells from APDS patients and HIV+ patients compared with controls. **(D)** Programmed death receptor (PD)-1 expression is increased on CD8+ T cells from APDS patients but not HIV+ patients compared with controls. **(E)** The frequency of PD-1+CD160+CD244+CD8+ T cells is increased in APDS patients and HIV+ patients compared with controls. **(F)** The frequency of PD-1+CD160+CD244+CD8+ T cells is negatively correlated with the frequency of naive CD8+ T cells in controls and APDS patients.

patients (EBV-specific CD8+ T cells), five HIV+ patients (HIV Gag- or Pol-specific CD8+ T cells, CMV-specific CD8+ T cells), and four healthy controls (EBV-specific CD8+ T cells). Within the three APDS samples are two different donors with one donor being represented as a child (7 years, red square in **Figure 4**) and as an adult (25 years, open square).

The virus-specific CD8+ T cells in all four groups have a predominantly CD45RA−CCR7− EM phenotype (**Figure 4A**) which is lowest in CMV-specific CD8+ T cells from HIV+ patients since these cells have the highest frequency of more differentiated EMRA (**Figure 4A**). No clear difference was observed for the frequencies of the chronic activation markers CD38 and HLA-DR when the four virus-specific CD8+ T cell groups were compared (**Figure 4B**). All virus-specific cells have higher frequencies of CD38+ and HLA-DR+ cells than total CD8+ T cells (**Figures 2A,B**) from the same groups. CD57-expressing cells seem to be more abundant within EBV-specific CD8+ T cells from APDS patients and CMV-specific CD8+ T cells from HIV patients than in EBV-specific CD8+ T cells from healthy controls and HIV-specific CD8+ T cells from HIV patients.

To assess exhaustion in the virus-specific CD8+ T cell population, the expression of PD-1, CD160, and CD244 was analyzed (**Figure 5**). The mean percentage of CD244+ CD8+ T cells was above 90% in all virus-specific CD8+ T cell groups. Frequency of CD160 expression slightly increased on HIV-specific CD8+ T cells compared with the other groups. The frequency of PD-1 expressing CD8+ T cells was lowest in CMV-specific CD8+ T cells from HIV+ patients and EBV-specific CD8+ T cells from healthy controls but higher within EBV-specific CD8+ T cells from APDS patients and HIV-specific CD8+ T cells from HIV+ patients. We found that within the HIV-specific CD8+ T cells 71 ± 4.0% express all three inhibitory receptors, and within the EBV-specific CD8+ T cells from APDS patients 47 ± 10.6% express all three inhibitory receptors. EBV-specific cells from controls and CMV-specific cells from HIV+ patients have a lower frequency of PD-1+CD160+CD244+ populations (32 ± 3.2 and 23 ± 5.2%, respectively). Compared with overall CD8+ T cells, all virus-specific CD8+ T cells show increased expression of inhibitory receptors.

PD-1 Blockade Can Enhance *In Vitro* Proliferation of Virus-Specific Cells in APDS and HIV+ Patients

Although both senescent and exhausted cells do not proliferate, the mechanism leading to the replicative impairment is different. In senescence, a cell cycle arrest causes the inability of cells to replicate whereas in exhaustion receptor-ligand interaction (such as between the inhibitory receptor PD-1 and its ligand PD-L1) leads to inhibition of TCR-signaling and therefore inhibition of proliferation. Thus, blocking the interaction between receptor and ligand through checkpoint inhibitors could lead to an increase in proliferation of exhausted cells, as shown for HIV-specific CD8+ T cells from HIV+ patients (44–46) but not in senescent cells. We stimulated PBMC from HIV+ patients and APDS patients with virus-specific peptide in the presence of blocking anti-PD-L1 or isotype control antibodies for 5 days before analysis of proliferation and effector function. As reported previously (44–46), inhibition of PD-1/PD-L1 interaction increased proliferation of HIV-specific CD8+ T cells in two out of the four tested PBMC samples from HIV+ patients by ~2-fold (**Figure 6**). When PBMC from APDS patients were stimulated with EBV-peptides in the presence of anti-PD-L1 antibodies, proliferation was increased in all three samples compared with samples stimulated with peptide in the presence of an isotype control. Not only did we observe improved proliferation of these cells but also increased effector function as demonstrated by the frequency of cytokine-producing cells (**Figure 6**). The patient with the highest proliferative and effector cytokine response was also the patient with the highest frequency of PD-1+ and PD-1+CD160+CD244+ EBV-specific CD8+ T cells. These findings indicate that PD-1 signal inhibition not only increased proliferation but also enabled these cells to release effector cytokines including IFNγ and TNFα. These results suggest that exhausted EBV-specific CD8+ T cells from APDS patients can be functionally restored through checkpoint inhibitors, resulting in increased proliferation and effector functions.

DISCUSSION

How T cell defects due to GOF mutations of PI3Kδ contribute to morbidity in APDS patients is not fully understood. The goal of this study was to determine whether the GOF mutations in PI3Kδ (causing APDS) lead to exhaustion of CD8+ T cells. Furthermore, we determined whether virus-specific CD8+ T cells against recurrent or persistent infections such as EBV are exhausted in APDS patients and if immune-checkpoint blockade can rejuvenate exhausted CD8+ T cells in APDS patients. We compared CD8+ T cells from APDS patients to healthy controls and HIV+ patients, which served as a control for antigen induced exhaustion of CD8+ T cells. Understanding the effect these mutations have on the immune system is central to further treatment strategies to support these patients in controlling chronic infections like EBV and CMV to reduce morbidity and mortality.

In line with previous reports (13, 14), we found significantly reduced frequencies of naive CD4+ and CD8+ T cells in APDS patients. Loss of naive T cells is thought to be caused by hyperactivation of the mTOR pathway induced by PI3Kδ mutations. This

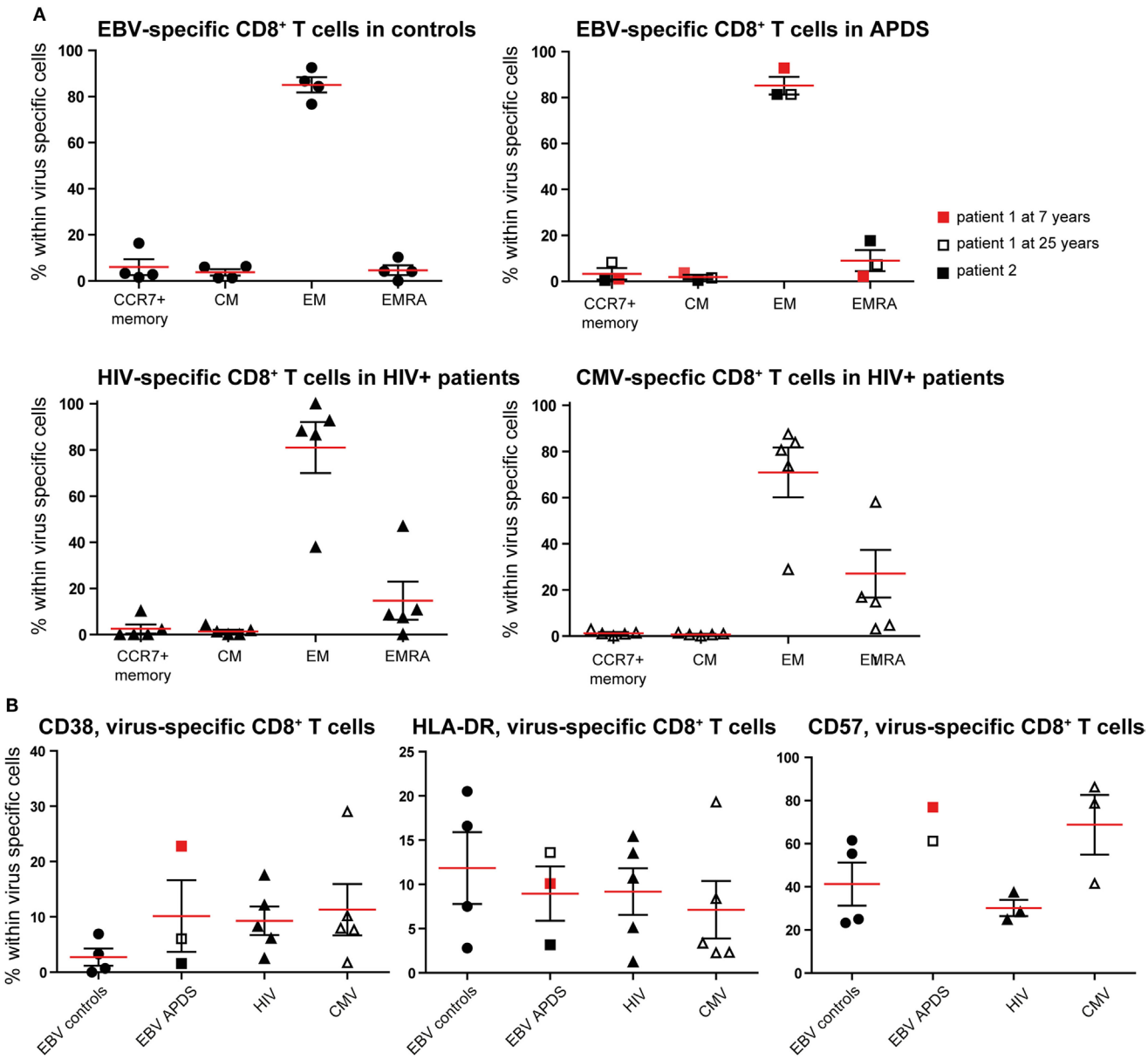


FIGURE 4 | Phenotyping and activation marker expression on Epstein–Barr virus (EBV)-specific CD8+ T cells from controls (black dots), EBV-specific CD8+ T cells from activated PI3Kδ syndrome (APDS) patient 1 at the age of 7 years (red square), at the age of 25 years (open square), APDS patient 2 (black square), HIV-specific CD8+ T cells from HIV+ patients (black triangles), and cytomegalovirus (CMV)-specific CD8+ T cells from HIV+ patients (open triangles). Red lines and brackets indicate the mean and SEM of each group (*$p < 0.05$ and **$p < 0,005$). **(A)** Frequency of memory subpopulations [central memory (CM), effector memory (EM), and EMRA, CCR7+ memory (CD45RA+CCR7+)] within virus-specific CD8+ T cells shown for healthy controls (EBV-specific CD8+ T cells), APDS patients (EBV-specific CD8+ T cells), and HIV+ patients (HIV- and CMV-specific CD8+ T cells). **(B)** Frequency of CD38+ (left), HLA-DR+ (center), and CD57+ (right) cells shown within virus-specific CD8+ T cells.

leads to increased glycolysis, proliferation, and differentiation into short-lived effector cells (11, 12). The APDS patients showed a skewed CD8+ T cell subset distribution, with increased EM CD8+ T cells, which we also found in HIV+ patients. Impaired control of viral infection could be an underlying mechanism causing the increased EM pool in HIV+ patients. In APDS patients, chronic and/or recurrent viral infections such as EBV and CMV on top of the GOF mutation are likely to contribute to this phenotype. Interestingly, although the EM population is also slightly increased in CD4+ T cells, it is the CM population which is significantly higher in both patient groups.

To further examine the effect of PI3Kδ GOF mutations on the total CD4+ and CD8+ T cell compartments, we analyzed the expression of the chronic activation markers CD38 and HLA-DR. Especially HLA-DR was shown before to correlate with immune activation and disease progression in HIV infection (61). This is the first time that these chronic activation markers are analyzed on T cells from APDS patients. HLA-DR expression

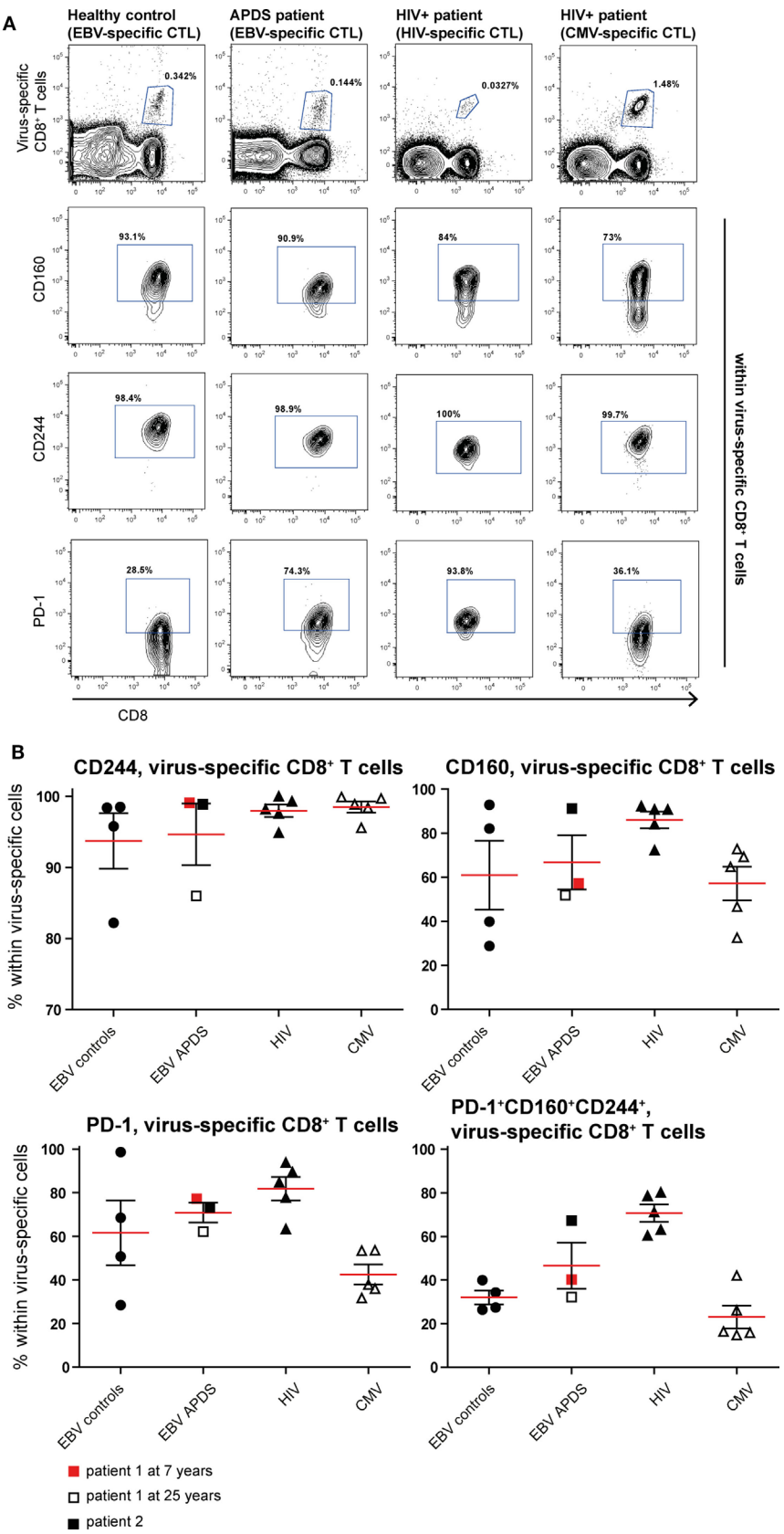


FIGURE 5 | Continued

FIGURE 5 | Inhibitory receptor expression on virus-specific CD8+ T cells from controls and patients. **(A)** Representative dot plots of healthy controls and patients indicating identification of virus-specific cells and inhibitory receptors positive cells within virus-specific populations. **(B)** Inhibitory receptor expression on Epstein–Barr virus (EBV)-specific cells from controls (black dots), EBV-specific cells from activated PI3Kδ syndrome (APDS) patient 1 at the age of 7 years (red square), at the age of 25 years (open square), APDS patient 2 (black square), HIV-specific cells from HIV+ patients (black triangles), and cytomegalovirus (CMV)-specific cells from HIV+ patients (open triangles). Red lines and brackets indicate the mean and SEM of each group.

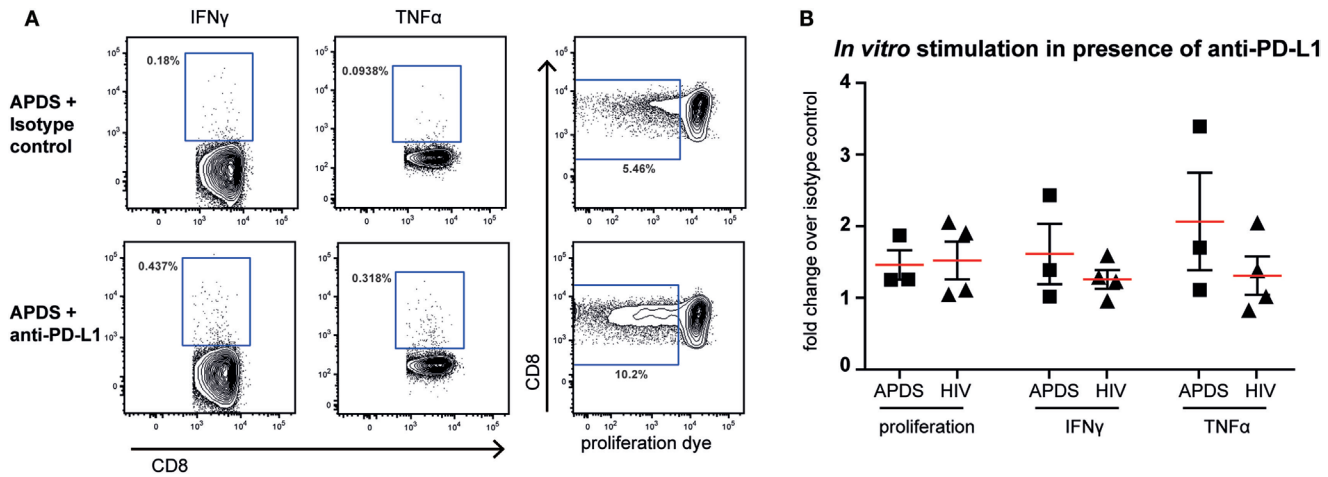


FIGURE 6 | Proliferation and effector cytokine production *in vitro* of Epstein–Barr virus-specific CD8+ T cells from activated PI3Kδ syndrome (APDS) patients and HIV-specific CD8+ T cells from HIV+ patients after peptide stimulation in the presence of anti-programmed death receptor (PD)-L1 antibodies or isotype controls. **(A)** Representative dot plots showing cytokine-producing and proliferated CD8+ T cells after 5-day peptide stimulation of peripheral blood mononuclear cells from an APDS patient in the presence of isotype control (upper panel) or anti-PD-L1 monoclonal antibodies (lower panel). Gates indicate cells positive for interferon (IFN) γ, tumor necrosis factor (TNF) α, or cells with diluted cell-trace proliferation dye. **(B)** Anti-PD-L1 antibody treatment resulted in increased proliferation and production of TNFα and IFNγ upon peptide stimulation in both APDS and HIV+ patients. Pooled data showing fold change of the frequency of either cells undergoing proliferation or cytokine-producing cells in the presence of anti-PD-L1 antibody over isotype control. Cells were stimulated with viral peptide for 5 days in the presence of monoclonal antibodies. Red lines and brackets indicate the mean and SEM of each group.

was significantly increased on CD8+ and CD4+ T cells in APDS patients compared with healthy controls, and it is comparable with what is observed in HIV+ patients. Our findings indicate that a subpopulation of CD4+ and CD8+ T cells in APDS patients are chronically activated. However, APDS patients show great heterogeneity, with only a small part of T cells expressing the chronic activation markers, our findings thus suggest that the mutation alone does not lead to a chronic activation of T cells. One exhaustion-inducing factor could perhaps be infection with a persistent virus; we therefore analyzed the expression of CD38 and HLA-DR on CD8+ T cells specific for persistent viruses. We did not find significant differences in the expression of activation markers on virus-specific cells between controls, APDS patients, and HIV+ patients. Thus, we cannot at this moment conclude that infections with persistent viruses contribute to the higher frequency of HLA-DR+ T cells in APDS patients.

Some APDS patients have increased CD57 expression on CD8+ T cells, and previously this was interpreted as increased senescence (12, 54, 55). The surface marker CD57 is commonly used as a senescence marker, but T cells in HIV+ patients also express increased CD57, indicating that exhaustion and senescence can co-exist in patients (62–64). In our study cohort, the mean CD57+ T cell frequency in APDS patients is only slightly increased compared with healthy controls and much lower than that of HIV+ patients. However, the CD57 expression in the APDS cohort is very heterogeneous, with some patients comparable with controls and others in the range of HIV+ patients. CD57 expression levels were not correlated with age, gender, or mutation in our patient cohort. Neither was this correlated with clinical characteristics such as lymphadenopathy, autoimmunity, malignancies, the expression of other markers we studied here or with B-cell phenotypes. Our results suggest that senescence and exhaustion might not be totally separate processes, but rather two intertwined cellular states that can occur together in specific types of diseases such as APDS.

To further assess exhaustion in APDS patients, we analyzed the expression of the inhibitory receptors CD160, CD244, and PD-1. We have included PBMC from HIV-infected patients since it is well established that HIV infection leads to exhaustion of HIV-specific CD8+ T cells but not CMV-specific CD8+ T cells in HIV-infected patients (39, 41) and can therefore serve as a positive control for exhaustion. We found that CD244+, CD160+, and PD-1+ CD8+ T cells are significantly increased in APDS patients. CD8+ T cells expressing all three inhibitory receptors (PD-1+CD160+CD244+), which would indicate the most exhausted state, were also significantly increased in APDS patients and to a similar degree as in HIV-infected individuals. We did not find any correlates for the PD-1+CD160+CD244+CD8+

T cell population in APDS patients when age, gender, type of mutations, B cell phenotype, or any other marker we have described were analyzed. When we separated the APDS patients in EBV-antibody positive and EBV-antibody negative, we found that the highest expression of the inhibitory receptors including the concurrent expression of all three inhibitory receptors was observed in EBV$^+$ APDS patients. However, EBV$^-$ APDS patients had a frequency of inhibitory receptor expressing CD8$^+$ T cells which was similar to the one from EBV$^+$ healthy controls. These findings suggest that chronic EBV infection or antigen stimulation in APDS patients contributes to the exhaustion of CD8$^+$ T cells.

Within virus-specific CD8$^+$ T cells, we found increased expression of inhibitory receptors in all subpopulations. Overall, the expression of inhibitory receptors on CD8$^+$ T cells from APDS patients has more similarities with HIV$^+$ patients, supporting the idea that the PI3Kδ GOF mutations may contribute to exhaustion. This was supported by the highly significant negative correlation between the frequency of naive and PD-1$^+$CD160$^+$CD244$^+$CD8$^+$ T cells, indicating that the hyperactivation leading to reduced naive T cells, may also be responsible for the increased expression of inhibitory receptors on T cells. Our findings do not imply that signaling mechanisms leading to exhaustion is identical in APDS and HIV infection although chronic antigen exposure may play an important role in both APDS and HIV patients as has been suggested in mouse studies (59).

Commonly, exhaustion is studied in the context of chronic antigen stimulation due to chronic viral infections and cancer. In APDS, we see a similar increase of exhausted CD8$^+$ T cells especially in patients that are EBV-antibody positive implying that chronic antigen stimulation contributes to T cell exhaustion. Chronic TCR stimulation is sufficient to lead to CD8$^+$ T cell exhaustion (59). Since PI3Kδ participates in TCR signaling in T cells (58), a cell-intrinsic chronic activation due to the hyperactivity of the PI3K–AKT signaling pathway could be a factor promoting the exhausted phenotype in the absence of specific chronic antigen in APDS. Since not all APDS patients' T cells show a significant increase in inhibitory receptor expression, the PI3Kδ mutations may be necessary but not sufficient for exhaustion. One mechanism to lower the threshold for exhaustion in APDS could be PI3K-induced epigenetic modifications. Several studies have shown epigenetic alterations in exhausted T cells (65, 66). The altered gene expression patterns induced by epigenetic modifications are rather stable resulting in permanent exhaustion independent of the level of remaining antigen (67, 68). Demethylation of the PD-1 promotor region in exhausted CD8$^+$ T cells allows sustained expression of PD-1. That PI3K can indeed alter epigenetic modifications was indicated in a study in mouse embryonic stem cells, suggesting that *de novo* DNA methyltransferases are downregulated due to PI3K-induced AKT, leading to reduced DNA methylation of imprinted loci (69). Therefore PI3Kδ GOF mutations could promote the demethylation of inhibitory receptors or reduce the threshold for such demethylation, thus facilitating the exhaustion in CD8$^+$ T cells.

Over the past years, PD-1 blockade has been a subject of research in both HIV and cancer treatment. By blocking inhibitory receptors, exhausted CD8$^+$ T cells can regain effectors functions and provide antiviral or anti-tumor immunity. However, upregulation of inhibitory receptors on activated T cells has also a physiological function: they function as negative regulators, downregulating the immune response after successful control of infections (70, 71). They are important to prevent autoimmunity and pathological responses leading to tissue damage. In the absence of inhibitory receptors an increased risk for autoimmunity and immunopathology was reported (72–74). Upregulation of inhibitory receptors in APDS could be a protective mechanism to prevent damage through an overly activated immune system. This hypothesis is supported by a study, which showed how PD-1 signaling can prevent activation of PI3K and AKT phosphorylation, thereby preventing proliferation (75). This benefit of preventing autoimmunity or inflammation in APDS comes at a cost of impairing immunity to viruses. Indeed, we have seen an increase in proliferation and effector cytokine secretion in virus-specific CD8$^+$ T cells from APDS patients *in vitro* in the presence of PD-L1 blocking antibodies. The increase of proliferation and cytokine production we have observed in the presence of a PD-L1 blocking antibody is moderate, and this raises the question of biological significance. Since we have observed that the patient with the highest expression of PD-1 showed the highest increase in proliferation and cytokine-producing EBV-specific CD8$^+$ T cells this suggests that the more responsive patients to PD-1 blockade will be the highest expressers. To more significantly improve the effector functions in all patients a combination of blocking several checkpoint inhibitors may be required, as indicated by PD-1/CTLA-4 blocking in cancer studies (76, 77) Furthermore, the increase of effector function is similar when EBV-specific CD8$^+$ T cells from APDS patients are compared with HIV-specific T cells from HIV-infected patients and within the range reported previously for HIV-specific CD8$^+$ T cells (41, 44, 45). Thus, short-term treatment of patients with checkpoint inhibitors during a recurrent EBV and/or CMV infection could be a means to augment efficacy of exhausted virus-specific CD8$^+$ T cells and thus reduce EBV and CMV related morbidity. Because inhibitory receptor upregulation may possibly be beneficial in APDS by preventing excessive inflammation or autoimmunity, manipulating this pathway has to be done with caution, and ideally it should be combined with long-term treatment that can reduce the hyperactivation of the pathway, for example selective PI3K inhibition (78).

In summary, we have shown that CD8$^+$ T cells from APDS patients have an exhausted phenotype and upregulated expression of inhibitory receptors. This exhaustion of the CD8$^+$ T cells contributes to the deficient antiviral capacities of the CD8$^+$ T cell compartment. Blocking the PD-1–PD1L interaction could be a target for treatment in these patients during recurrent or persistent viral infections.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Erasmus MC Medical Ethics Committee with written

informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Erasmus MC Medical Ethics Committee.

AUTHOR CONTRIBUTIONS

YM and MW performed experiments and analyzed data. VD, GD, PH, and JM contributed to study design and data analysis. Study was conceived and designed by PK, MB, YM, and MW. Manuscript was written by PK, YM, and MW. All the authors have read and approved the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank Elisabeth Schölvinck (Department of Pediatrics, University Medical Center Groningen) for providing patient material.

FUNDING

This work was supported by the department of Immunology (PK and YM) and ZonMw (Vidi grant 91712323 to MB). The research for this manuscript was (in part) performed within the framework of the Erasmus Postgraduate School Molecular Medicine.

REFERENCES

1. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* (2003) 3(4):317–30. doi:10.1038/nri1056
2. Okkenhaug K, Vanhaesebroeck B. PI3K-signalling in B- and T-cells: insights from gene-targeted mice. *Biochem Soc Trans* (2003) 31(Pt 1):270–4. doi:10.1042/bst0310270
3. Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. *Biochim Biophys Acta* (1998) 1436(1–2):127–50. doi:10.1016/S0005-2760(98)00139-8
4. Ramadani F, Bolland DJ, Garcon F, Emery JL, Vanhaesebroeck B, Corcoran AE, et al. The PI3K isoforms p110alpha and p110delta are essential for pre-B cell receptor signaling and B cell development. *Sci Signal* (2010) 3(134):ra60. doi:10.1126/scisignal.2001104
5. Srinivasan L, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA, et al. PI3 kinase signals BCR-dependent mature B cell survival. *Cell* (2009) 139(3):573–86. doi:10.1016/j.cell.2009.08.041
6. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* (2010) 11(5):329–41. doi:10.1038/nrm2882
7. Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, et al. P110delta, a novel phosphoinositide 3-kinase in leukocytes. *Proc Natl Acad Sci U S A* (1997) 94(9):4330–5. doi:10.1073/pnas.94.9.4330
8. Pearce VQ, Bouabe H, MacQueen AR, Carbonaro V, Okkenhaug K. PI3Kdelta regulates the magnitude of CD8+ T cell responses after challenge with *Listeria monocytogenes*. *J Immunol* (2015) 195(7):3206–17. doi:10.4049/jimmunol.1501227
9. Gracias DT, Boesteanu AC, Fraietta JA, Hope JL, Carey AJ, Mueller YM, et al. Phosphatidylinositol 3-kinase p110delta isoform regulates CD8+ T cell responses during acute viral and intracellular bacterial infections. *J Immunol* (2016) 196(3):1186–98. doi:10.4049/jimmunol.1501890
10. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33(5):361–9. doi:10.1111/j.1744-313X.2006.00627.x
11. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
12. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
13. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
14. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210–8e9. doi:10.1016/j.jaci.2016.03.022
15. Wentink MWJ. Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infection. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
16. Miller RA. The aging immune system: primer and prospectus. *Science* (1996) 273(5271):70–4. doi:10.1126/science.273.5271.70
17. Cambier J. Immunosenescence: a problem of lymphopoiesis, homeostasis, microenvironment, and signaling. *Immunol Rev* (2005) 205:5–6. doi:10.1111/j.0105-2896.2005.00276.x
18. Rodier F, Campisi J. Four faces of cellular senescence. *J Cell Biol* (2011) 192(4):547–56. doi:10.1083/jcb.201009094
19. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* (2007) 8(9):729–40. doi:10.1038/nrm2233
20. Spaulding C, Guo W, Effros RB. Resistance to apoptosis in human CD8+ T cells that reach replicative senescence after multiple rounds of antigen-specific proliferation. *Exp Gerontol* (1999) 34(5):633–44. doi:10.1016/S0531-5565(99)00033-9
21. Salminen A, Ojala J, Kaarniranta K. Apoptosis and aging: increased resistance to apoptosis enhances the aging process. *Cell Mol Life Sci* (2011) 68(6):1021–31. doi:10.1007/s00018-010-0597-y
22. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nat Rev Immunol* (2011) 11(4):289–95. doi:10.1038/nri2959
23. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity* (2007) 27(3):393–405. doi:10.1016/j.immuni.2007.08.007
24. Hall BM, Balan V, Gleiberman AS, Strom E, Krasnov P, Virtuoso LP, et al. Aging of mice is associated with p16(Ink4a)- and beta-galactosidase-positive macrophage accumulation that can be induced in young mice by senescent cells. *Aging (Albany NY)* (2016) 8(7):1294–315. doi:10.18632/aging.100991
25. Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol* (2013) 75:685–705. doi:10.1146/annurev-physiol-030212-183653
26. Koch S, Larbi A, Ozcelik D, Solana R, Gouttefangeas C, Attig S, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. *Ann N Y Acad Sci* (2007) 1114:23–35. doi:10.1196/annals.1396.043
27. Gallimore A, Glithero A, Godkin A, Tissot AC, Pluckthun A, Elliott T, et al. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J Exp Med* (1998) 187(9):1383–93. doi:10.1084/jem.187.9.1383
28. Moskophidis D, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* (1993) 362(6422):758–61. doi:10.1038/362758a0
29. Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, et al. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* (1998) 188(12):2205–13. doi:10.1084/jem.188.12.2205
30. Goepfert PA, Bansal A, Edwards BH, Ritter GD Jr, Tellez I, McPherson SA, et al. A significant number of human immunodeficiency virus epitope-specific cytotoxic T lymphocytes detected by tetramer binding do not

produce gamma interferon. *J Virol* (2000) 74(21):10249–55. doi:10.1128/JVI.74.21.10249-10255.2000
31. Shankar P, Russo M, Harnisch B, Patterson M, Skolnik P, Lieberman J. Impaired function of circulating HIV-specific CD8(+) T cells in chronic human immunodeficiency virus infection. *Blood* (2000) 96(9):3094–101.
32. Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. *J Virol* (2007) 81(8):4215–25. doi:10.1128/JVI.02844-06
33. Radziewicz H, Ibegbu CC, Fernandez ML, Workowski KA, Obideen K, Wehbi M, et al. Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. *J Virol* (2007) 81(6):2545–53. doi:10.1128/JVI.02021-06
34. Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, et al. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nat Med* (1999) 5(6):677–85. doi:10.1038/9525
35. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* (2010) 28(19):3167–75. doi:10.1200/JCO.2009.26.7609
36. Kim PS, Ahmed R. Features of responding T cells in cancer and chronic infection. *Curr Opin Immunol* (2010) 22(2):223–30. doi:10.1016/j.coi.2010.02.005
37. Fuller MJ, Zajac AJ. Ablation of CD8 and CD4 T cell responses by high viral loads. *J Immunol* (2003) 170(1):477–86. doi:10.4049/jimmunol.170.1.477
38. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol* (2003) 77(8):4911–27. doi:10.1128/JVI.77.8.4911-4927.2003
39. Mueller YM, De Rosa SC, Hutton JA, Witek J, Roederer M, Altman JD, et al. Increased CD95/Fas-induced apoptosis of HIV-specific CD8(+) T cells. *Immunity* (2001) 15(6):871–82. doi:10.1016/S1074-7613(01)00246-1
40. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* (2009) 10(1):29–37. doi:10.1038/ni.1679
41. Yamamoto T, Price DA, Casazza JP, Ferrari G, Nason M, Chattopadhyay PK, et al. Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. *Blood* (2011) 117(18):4805–15. doi:10.1182/blood-2010-11-317297
42. Peretz Y, He Z, Shi Y, Yassine-Diab B, Goulet JP, Bordi R, et al. CD160 and PD-1 co-expression on HIV-specific CD8 T cells defines a subset with advanced dysfunction. *PLoS Pathog* (2012) 8(8):e1002840. doi:10.1371/journal.ppat.1002840
43. Raziorrouh B, Schraut W, Gerlach T, Nowack D, Gruner NH, Ulsenheimer A, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology* (2010) 52(6):1934–47. doi:10.1002/hep.23936
44. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med* (2006) 203(10):2281–92. doi:10.1084/jem.20061496
45. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* (2006) 443(7109):350–4. doi:10.1038/nature05115
46. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med* (2006) 12(10):1198–202. doi:10.1038/nm1106-1329b
47. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* (2006) 439(7077):682–7. doi:10.1038/nature04444
48. Gardiner D, Lalezari J, Lawitz E, DiMicco M, Ghalib R, Reddy KR, et al. A randomized, double-blind, placebo-controlled assessment of BMS-936558, a fully human monoclonal antibody to programmed death-1 (PD-1), in patients with chronic hepatitis C virus infection. *PLoS One* (2013) 8(5):e63818. doi:10.1371/journal.pone.0063818
49. Fuller MJ, Callendret B, Zhu B, Freeman GJ, Hasselschwert DL, Satterfield W, et al. Immunotherapy of chronic hepatitis C virus infection with antibodies against programmed cell death-1 (PD-1). *Proc Natl Acad Sci U S A* (2013) 110(37):15001–6. doi:10.1073/pnas.1312772110
50. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature* (2009) 458(7235):206–10. doi:10.1038/nature07662
51. Gay CL, Bosch RJ, Ritz J, Hataye JM, Aga E, Tressler RL, et al. Clinical trial of the anti-PD-L1 antibody BMS-936559 in HIV-1 infected participants on suppressive antiretroviral therapy. *J Infect Dis* (2017) 215(11):1725–33. doi:10.1093/infdis/jix191
52. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* (2012) 12(4):252–64. doi:10.1038/nrc3239
53. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* (2015) 348(6230):56–61. doi:10.1126/science.aaa8172
54. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kdelta and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16(11):702–14. doi:10.1038/nri.2016.93
55. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
56. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110delta cause activated PI3K-delta syndrome 1 (APDS1). *Haematologica* (2017) 102(7):e278–81. doi:10.3324/haematol.2017.167601
57. Petrovas C, Chaon B, Ambrozak DR, Price DA, Melenhorst JJ, Hill BJ, et al. Differential association of programmed death-1 and CD57 with ex vivo survival of CD8+ T cells in HIV infection. *J Immunol* (2009) 183(2):1120–32. doi:10.4049/jimmunol.0900182
58. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* (2002) 297(5583):1031–4. doi:10.1126/science.1073560
59. Bucks CM, Norton JA, Boesteanu AC, Mueller YM, Katsikis PD. Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion. *J Immunol* (2009) 182(11):6697–708. doi:10.4049/jimmunol.0800997
60. Aken BL, Achuthan P, Akanni W, Amode MR, Bernsdorff F, Bhai J, et al. Ensembl 2017. *Nucleic Acids Res* (2017) 45(D1):D635–42. doi:10.1093/nar/gkw1104
61. Giorgi JV, Detels R. T-cell subset alterations in HIV-infected homosexual men: NIAID multicenter AIDS cohort study. *Clin Immunol Immunopathol* (1989) 52(1):10–8. doi:10.1016/0090-1229(89)90188-8
62. Pereira BI, Akbar AN. Convergence of innate and adaptive immunity during human aging. *Front Immunol* (2016) 7:445. doi:10.3389/fimmu.2016.00445
63. Wherry EJ. T cell exhaustion. *Nat Immunol* (2011) 12(6):492–9. doi:10.1038/ni.2035
64. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* (2015) 15(8):486–99. doi:10.1038/nri3862
65. Youngblood B, Oestreich KJ, Ha SJ, Duraiswamy J, Akondy RS, West EE, et al. Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T cells. *Immunity* (2011) 35(3):400–12. doi:10.1016/j.immuni.2011.06.015
66. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* (2016) 354(6316):1160–5. doi:10.1126/science.aaf2807
67. Angelosanto JM, Blackburn SD, Crawford A, Wherry EJ. Progressive loss of memory T cell potential and commitment to exhaustion during chronic viral infection. *J Virol* (2012) 86(15):8161–70. doi:10.1128/JVI.00889-12
68. Utzschneider DT, Legat A, Fuertes Marraco SA, Carrie L, Luescher I, Speiser DE, et al. T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. *Nat Immunol* (2013) 14(6):603–10. doi:10.1038/ni.2606
69. Popkie AP, Zeidner LC, Albrecht AM, D'Ippolito A, Eckardt S, Newsom DE, et al. Phosphatidylinositol 3-kinase (PI3K) signaling via glycogen synthase kinase-3 (Gsk-3) regulates DNA methylation of imprinted loci. *J Biol Chem* (2010) 285(53):41337–47. doi:10.1074/jbc.M110.170704
70. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* (2013) 13(4):227–42. doi:10.1038/nri3405

71. Odorizzi PM, Wherry EJ. Inhibitory receptors on lymphocytes: insights from infections. *J Immunol* (2012) 188(7):2957–65. doi:10.4049/jimmunol.1100038
72. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med* (2006) 203(4):883–95. doi:10.1084/jem.20051776
73. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. *Trends Immunol* (2006) 27(4):195–201. doi:10.1016/j.it.2006.02.001
74. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* (2010) 236:219–42. doi:10.1111/j.1600-065X.2010.00923.x
75. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol* (2005) 25(21):9543–53. doi:10.1128/MCB.25.21.9543-9553.2005
76. Hodi FS, Chesney J, Pavlick AC, Robert C, Grossmann KF, McDermott DF, et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol* (2016) 17(11):1558–68. doi:10.1016/S1470-2045(16)30366-7
77. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* (2015) 373(1):23–34. doi:10.1056/NEJMoa1504030
78. Rao VK, Webster S, Dalm V, Sediva A, van Hagen PM, Holland S, et al. Effective 'activated PI3Kdelta syndrome'-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 07 March 2018
doi: 10.3389/fimmu.2018.00445

Phosphoinositide-3-Kinase Signaling in Human Natural Killer Cells: New Insights from Primary Immunodeficiency

*Emily M. Mace**

Department of Pediatrics, Baylor College of Medicine, Center for Human Immunobiology, Texas Children's Hospital, Houston, TX, United States

Human natural killer (NK) cells play a critical role in the control of viral infections and malignancy. Their importance in human health and disease is illustrated by severe viral infections in patients with primary immunodeficiencies that affect NK cell function and/or development. The recent identification of patients with phosphoinositide-3-kinase (PI3K)-signaling pathway mutations that can cause primary immunodeficiency provides valuable insight into the role that PI3K signaling plays in human NK cell maturation and lytic function. There is a rich literature that demonstrates a requirement for PI3K in multiple key aspects of NK cell biology, including development/maturation, homing, priming, and function. Here, I briefly review these previous studies and place them in context with recent findings from the study of primary immunodeficiency patients, particularly those with hyperactivating mutations in PI3Kδ signaling.

Keywords: phosphoinositide-3-kinase signaling, primary immunodeficiency, natural killer cell biology, human natural killer cells, natural killer cell development, natural killer cell cytotoxicity

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Jane Oliaro, Peter MacCallum Cancer Centre, Australia
Tri Giang Phan, Garvan Institute of Medical Research, Australia

***Correspondence:**
Emily M. Mace
mace@bcm.edu

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *15 January 2018*
Accepted: *19 February 2018*
Published: *07 March 2018*

Citation:
Mace EM (2018) Phosphoinositide-3-Kinase Signaling in Human Natural Killer Cells: New Insights from Primary Immunodeficiency. Front. Immunol. 9:445. doi: 10.3389/fimmu.2018.00445

INTRODUCTION

Human Natural killer (NK) Cell Development, Lytic Function, and Migration

Human NK cells are derived from bone marrow precursors and mature in the peripheral tissues, particularly the secondary lymphoid tissue (1–3). Their development can be defined by select cell surface receptor and transcription factor expression in combination with increasingly restricted lineage potential of developmental intermediates (4). Despite an increasing understanding of the relationship between NK cells and other innate lymphoid cell subsets, however, the exact nature of the steps of NK cell development is incompletely understood. This likely reflects the importance of the local microenvironment in tuning NK cell development and plasticity of NK cell developmental intermediates, as discrete tissue sites have unique resident NK cell populations (1).

Within peripheral blood, human NK cells comprise approximately 10% of lymphocytes and are broadly classified as $CD56^{bright}$ or $CD56^{dim}$, two subsets with distinct phenotypic and functional properties (5). $CD56^{bright}$ are considered less mature than the $CD56^{dim}$ subset, and their lesser frequency within peripheral blood is converse to their predominance in the secondary lymphoid tissue, where they are thought to develop (6). $CD56^{dim}$ NK cells are cited as having the greatest capacity for lytic function; however, similar capacity for lytic function can be elicited from $CD56^{bright}$ NK cells with cytokine priming or activation (5, 7–10). $CD56^{bright}$ cells are strong producers of

cytokines, particularly IFNγ and TNFα and are frequently considered more immunoregulatory than CD56^{dim} NK cells. In addition, CD56^{dim} NK cell subsets can be further dissected to terminally mature subsets that can include adaptive NK cells with memory-like function for rapid response to previously encountered antigen (11–17).

Natural killer cell lytic function is mediated through the formation of an immunological synapse, a specialized signaling platform that directs the secretion of specialized secretory lysosomes containing perforin and granzymes (18, 19). Many of the steps leading to the formation of an NK cell lytic immunological synapse are common to other immunological synapses, including T cell synapses (20). There are also distinct features of the NK cell immunological synapse that are likely a function of unique mechanisms of NK cell activation and sensing, as NK cells use germline-encoded activating and inhibitory receptors to integrate signals that can lead to lysis of non-self, stressed, transformed, or virally infected cells (21). While an in-depth description of this process is beyond the scope of this review, key events in this process include firm adhesion to a target cell, actin polymerization and reorganization at the immunological synapse, lytic granule convergence, microtubule-organizing center (MTOC) (and lytic granule), polarization toward the synapse, granule exocytosis, and termination of the immunological synapse following target cell death (22). Relative to lytic synapses formed by cytolytic effector CD8$^+$ T cells, NK cell lytic synapses are less rapidly formed and seemingly have a greater number of regulated steps to cytotoxicity, likely due to their non-antigen-restricted mechanism of sensing target cells and "missing-self" recognition (21). Their capacity for autologous killing is restrained by a process termed licensing, in which NK cells are licensed for lytic potential through the engagement of inhibitory receptors with self-MHC class I (23–25). While the molecular mechanisms of licensing are not fully understood, this process is reversible, and even unlicensed cells ultimately have the potential to be fully lytic (26–29). Through an understanding of licensing, priming, and cytokine-induced memory, it seems that NK cell function can be tuned for responsiveness through multiple mechanisms.

While less understood at a molecular level than immunological synapse formation, NK cell migration and homing are intrinsic components of both development and function. The accepted paradigm of NK cell development suggests that NK cells enter circulation to traffic to tissue at both an early developmental stage and ultimately as terminally mature cells. Intravital imaging of mouse lymph nodes demonstrates that NK cells patrol the T cell zone and make transient, yet direct, contacts with dendritic cells and T cells (30–32). This significant migratory behavior has obviously not been directly visualized in human tissue; however, human NK cells undergo spontaneous migration on developmentally supportive stromal cells (33). This intrinsic migratory capacity can be recapitulated by the differentiation of NK cells *in vitro*, and developmental intermediates have intermediate migratory phenotypes (33, 34). In the context of lytic function, NK cell migration plays a key role in the serial killing capacity of activated cells, which often kill up to 10 sequential targets (35–40). Activated NK cells have a more dynamic migration, with modes of migration that are distinct from resting cells (40). This includes a more motile scanning of targets, a specifically greater directional persistence, less time spent in arrest, and a greater migration speed. These changes in migration mode have been described between resting and cytokine-activated (40), or unlicensed and licensed human NK cells (39), suggesting a link between multiple forms of activation and changes in migratory phenotype; however, the mechanism by which these changes are induced is unknown.

Human primary immunodeficiency is a powerful model to determine the requirements for human NK cell function and development. In particular, studies of patients with Wiskott–Aldrich syndrome (41, 42), MyH9-related disorders (43, 44), DOCK8 deficiency (45), and Coronin 1A deficiency (46) have led to the definition of these as critical mediators of NK cell immune synapse formation and function and defined the molecular basis of their function in a uniquely human setting. A similar approach can be taken to determine the requirements for human NK cell development through the study of patients with NK cell deficiency as a result of loss of NK cells or NK cell subsets in peripheral blood [reviewed in Ref. (47, 48)]. In particular, a decreased frequency of the CD56^{dim} subset has been used as a readout for the impaired terminal maturation of NK cells. Using this approach, unexpected requirements for the eukaryotic DNA helicase complex components MCM4 and GINS1 specifically in NK cell development have been identified (49–51). Similarly, biallelic mutations in *IRF8* lead to specific loss of the CD56^{dim} subset, with accompanying severe viral susceptibility in affected patients (52), and *RTEL1* mutations can lead to the absence of NK cells in peripheral blood (53, 54). More puzzling are mutations in *GATA2*, which lead to specific loss of the CD56^{bright} subset with variable effects on absolute NK cell frequencies, although this effect may be due to depletion of all but adaptive NK cells in affected patients (55–58).

Cases of isolated NK cell deficiency, in which NK cells are the primary or only affected immunological subset, are relatively rare. However, the extreme susceptibility of these patients to viral infections, particularly of the herpesvirus family, underscores the importance of NK cells in human health and disease (47, 48). Much more common are primary immmunodeficiencies that may include deregulated NK cell function or phenotype as part of their spectrum of disease. These can be illustrative of requirements for NK cell development or function, despite involvement of other immune compartments. These primary immunodeficiencies that affect NK cells range from severe combined immune deficiency as a result of *IL2RG* (59) or *JAK3* (60) mutation, which defines the requirement for common gamma chain cytokine signaling in human NK cell development, to diseases including STAT1 gain-of-function (GOF) mutations (61) and STAT3 deficiency (62). In each of these cases, it is important to consider that other affected immune compartments can also impact NK cell phenotype and function. It can also be difficult to delineate between primary immmunodeficiencies that seemingly lead to a "hard stop" in NK cell maturation, such as MCM4 and GINS1 deficiencies and those that deregulate specific receptor expression or aspects of homeostasis, such as STAT1 GOF mutations. Regardless, in each case, the phenotype of deregulated NK cell

development is accompanied by an effect on NK cell function that translates to susceptibility to infection and, in some cases, malignancy. Moving forward, however, it will be important to recognize these distinctions through the careful definition of what truly phenotypically and functionally defines NK cells and their subsets. In addition, determining the NK cell-intrinsic component to these mutations, such as by cell line modeling, is important for the proof of concept to define a particular gene as being required for human NK cell function.

The phosphoinositide-3-kinase (PI3K)-signaling axis plays a key role in a multitude of cellular functions. It is increasingly being recognized for its importance in the control of inflammation and cancer and is a particularly exciting target for new small molecule inhibitors designed to modulate its key players. Given its ubiquitous expression, perturbations in this pathway are predicted to impact a number of cellular functions. However, there are specific requirements for PI3K signaling in NK cell function, the importance of which are underscored by model organisms and recently described human mutations in *PIK3CD* that lead to significant defects in NK cell maturation and function (**Table 1**).

PI3K Signaling in Human NK Cells

Class IA PI3K are heterodimeric enzymes that consist of a regulatory p85 subunit and a catalytic p110α, -β, or -δ subunit; class 1B PI3K is composed of the p110γ subunit and p101 or p84 regulatory subunit. While all PI3K isoenzymes play a key role in catalyzing the production of PtdIns(3,4,5)P3 from PtdIns(4,5)P2, evidence suggests that they have unique functions (81). In addition, while p110α and p110β are widely expressed, p110γ and p110δ expression is primarily restricted to lymphocytes. Activating signaling leads to PI3K-mediated generation of PtdIns(3,4,5)P3, the accumulation of which in the cell membrane provides a platform for pleckstrin homology (PH)-domain-containing proteins, including AKT, phosphoinositide-dependent kinase-1 (PDK1), and Tec family kinases such as BTK. This pathway is additionally regulated by phosphatases including phosphatase and tensin homolog (PTEN) and SH2-containing inositol phosphatase 1 (SHIP-1).

In human NK cells, the PI3K-signaling pathway plays a direct role in signaling downstream from activating receptors, including 2B4 and KIR receptors (82–84). The recruitment of p85, in combination with Grb2, is also necessary and sufficient for the propagation of signaling, leading to cytotoxicity downstream of NKG2D ligation association with the non-ITAM containing DAP10 adaptor (85–88). PI3K activity following recruitment to membrane proximal receptors leads to the production of PtdIns(3,4,5)P3 and the subsequent recruitment of PH domain-containing proteins such as PLCγ1, PLCγ2, Vav1, and Tec kinases. Antibody-dependent cellular cytotoxicity (ADCC) is mediated by PI3K- and ITAM-dependent signaling by CD16 through FcγR and/or TCRζ (89); PI3K signaling plays an additional role following FcγR ligation by activating ADP-ribosylation factor, which leads to PtdIns(4,5)P2 production by PI5K and phospholipase D activation (90).

Phosphoinositide-3-kinase activation and subsequent recruitment of PLCγ1 and PLCγ2 leads to mobilization of intracellular Ca^{++} stores. In addition, PI3K activates a Rac1–MEK–ERK pathway that is a key signaling pathway for actin reorganization and cellular polarization (91). The central role of PI3K in mediating cell polarization can be defined by its control of Cdc42 activation at the NK cell immune synapse; in particular, p85α acts as a scaffold to target and position PI3K and subsequently recruit guanine nucleotide exchange factors to the membrane (92). As such, the role of PI3K signaling in cytotoxicity and NK cell migration can be through the control of actin remodeling, polarization, and even granule exocytosis, which requires intracellular calcium store mobilization.

In addition to activating for cytotoxicity, PI3K plays a pivotal role in both priming and signaling downstream of cytokine activation. It is particularly important for the attenuation of signaling through IL-15, the critical NK cell development and survival cytokine (93). The activation of PI3K following IL-15 receptor ligation leads to the production of PtdIns(3,4,5)P3 and the recruitment of AKT to the cell membrane. AKT activation leads to survival and proliferation through the inhibition of pro-apoptotic Bcl2- and PDK1-dependent activation of mTOR, which promotes translation directly through the phosphorylation of S6 kinase and the initiation factor eIF4E-binding protein (94). In mice, PI3K-dependent PDK1 activation *via* IL-15 signaling may also directly help to direct NK cell lineage commitment through the induction of E4BP4 and Eomes, and PDK1-deficient mice have loss of NK cell cellularity and function (95).

The critical role of PI3K in JAK–STAT signaling makes it key in potentiating the effects of cytokine priming, in which the threshold for NK cell activation is lowered by stimulation with common gamma chain cytokines (IL-2, -15, -21) or IL-12 and IL-18 (96–98). The therapeutic potential of cytokine priming is highlighted by recent studies of human memory-like NK cells with enhanced lytic function that can be generated by cytokine priming and can be reactivated after even extended periods of rest (99–101). These cells are of extreme interest for immune therapy and also highlight the importance of cytokine priming in generating NK cells that can rise to further challenge (102).

TABLE 1 | Effect of phosphoinositide-3-kinase (PI3K) mutations relevant to activated PI3K delta syndrome on natural killer (NK) cell development and function.

Gene (protein)	Mutation type	NK function	NK number/ phenotype	Reference
Human				
PIK3CD (p110δ)	GOF	Impaired	Decreased/affected	(63–67)
PIK3CD (p110δ)	LOF	ND	ND	(68)
PIK3R1 (p85α)	GOF	ND	Decreased/ND	(69)
PIK3R1 (p85α)	LOF	ND	Decreased	(70)
PTEN (PTEN)	LOF	ND	Decreased/ND	(71–73)
PTEN (PTEN)	OE	Decreased	ND	(74)
Mouse				
Pik3cd (p110δ)	Deletion	Impaired	Decreased/affected	(75, 76)
Pik3cd (p110δ)	Inactive	Impaired	Decreased/affected	(77)
Pik3r1 (p85α)	Deletion	Impaired	Decreased/affected	(78)
PTEN (PTEN)	Deletion	Impaired	Increased/affected	(79)
Inpp5d (SHIP-1)	Deletion	Decreased (cytokine)	Decreased/affected	(80)
PTEN (PTEN)	OE	Decreased	Unaffected	(74)

ND, not determined; GOF, gain-of-function; LOF, loss of function; OE, overexpression.

Physiologically, priming leads to an increased antitumor effect of NK cells, including an increased production of cytotoxic effector molecules, an increased conjugate formation with target cells, and an increased baseline activation of integrins (10). Interestingly, this effect in humans is primarily mediated by the $CD56^{bright}$ NK cell subset, as opposed to $CD56^{dim}$ NK cells, which are traditionally considered the more cytolytic subset. Small molecule inhibition of the PI3K-signaling pathway blocks this priming effect and attenuates the antitumor response, underscoring its importance in modulating NK cell function (10, 103). The importance of the PI3K-signaling pathway in NK cell priming and its implication in NK cell licensing (104) underscore its importance as a master regulator of NK cell-functional capacity.

Finally, PI3K signaling is required for NK cell chemotaxis to chemokines including CC chemokine ligand (CC)L2, CCL5, CXCL10, and SDF1α (105). While lymphocyte migration is thought to be mainly controlled by p110γ, p110δ is required specifically for chemotaxis mediated by the G-protein-coupled receptor sphingosine 1-phosphate receptor 5, which plays a key role in NK cell tissue localization (106, 107). Both p110δ and p110γ are activated for chemotaxis to CXCL12 and CCL3 and mediate NK cell migration to tissue and to the uterus during pregnancy (106). Conversely, NK cells from PTEN-deficient mice have hyperresponsive signaling in response to sphingosine 1-phosphate, leading to an increase in NK cells in peripheral blood as a result of aberrant trafficking to the tissue (79).

Taken together, these studies underscore the importance of PI3K signaling in NK cell development, function, and homeostasis. This importance can be further tested by studying patients with rare mutations in this pathway, particularly when these patients are considered in the context of informative mouse models.

The Requirement for PI3K in NK Cell Lytic Function

The role of PI3K signaling in immunological synapse formation and function was first tested broadly by using relatively promiscuous inhibitors such as Ly294002 and wortmannin. These studies showed that the broad inhibition of PI3K signaling prevented NK cell cytotoxicity in NK cell lines killing *via* natural cytotoxicity and in primary cells mediating ADCC (86, 89, 108, 109). While initial studies suggested that PI3K signaling wasn't required for primary NK cell-mediated lysis of K562 targets (109), the pretreatment of IL-2-activated primary NK cells with wortmannin significantly decreases cytotoxicity against 721.221 targets, at least in part by modulating LFA-1 function (110, 111). Further studies identified PI3K–Rac1–PAK1–MEK–ERK signaling that is required for polarization and cytotoxicity of human NK cell lines and freshly isolated peripheral blood NK cells (91). Knockdown (KD) of p85α or AKT prevents lytic granule polarization to the immune synapse and inhibits the activity of Cdc42 (92). In addition, p110δ interacts directly with the SH3 domain of CrkL during NKG2D-mediated NK cell cytotoxicity and controls LFA-1-mediated conjugate formation downstream of NKG2D ligation on human NK cells (86).

Mice deficient for PI3Kδ have impaired NK cell function against tumor targets, including defects in exocytosis (75, 76). Similarly, p85α-deficient mice have impaired NK cell cytotoxicity and cytokine production (78). Specific inhibitors of PI3K class I isoforms show that while pan-PI3K inhibition impairs mature human NK cell function, the selective inhibition of PI3Kα, -β, -γ, or -δ does not have a significant effect (112). These results suggest that the genetic loss of PI3Kδ may lead to NK cell developmental defects that are not present when PI3Kδ function is inhibited in mature cells. This is supported further by studies of a mouse expressing catalytically inactive PI3Kδ, in which signaling is impaired downstream of activating receptors and NK cells fail to mature and are unable to mediate lytic function (77). Overall, however, this is a field that has been complicated by differential findings regarding the requirement for PI3K isoforms in NK cell development, in part due to differences between mouse strains (113).

While human loss-of-function mutations in PI3K110δ (68) and p85α (70) have been reported, they are overwhelmingly rare. Both defects lead to primary immunodeficiency, and NK cell number in the patient reported with p85α deficiency was significantly decreased when compared to healthy ranges (70).What have emerged as a much more common variation are GOF mutations in *PIK3CD* or *PIK3R1*. *PIK3CD* GOF mutations were independently reported in 2014 by Lucas et al. (65) and Angulo et al. (114) and lead to hyperactivation of PI3K110δ signaling by interrupting the interaction between PI3K110δ and the p85α-regulatory subunit, or by constitutive membrane association and activation (65, 114, 115). This hyperactivation can be detected on a cellular basis as hyperphosphorylation of S6, mTOR, and AKT. Clinically, these mutations can lead to varied phenotypes, and original *PIK3CD* cohorts were identified by screening patients with recurrent chest infections (114) and herpesviral infections (65), respectively. Patients with activating mutations in *PIK3R1* leading to an increased PI3Kδ activity also have primary immunodeficiency (67, 69, 116–123), and many studies, including large cohort studies, have defined new mutations in *PIK3CD* leading to activated PI3K delta syndrome (APDS) and expanded the phenotype of disease (64, 66, 67, 124–131). While the clinical features of patients with mutations in *PIK3CD* and *PIK3R1* can be similar, there is evidence that *PIK3R1* mutations can also have extra-immune phenotypes (118). To delineate the two where necessary, *PIK3CD* mutations are referred to as leading to APDS1, whereas mutations in *PIK3R1* cause APDS2. Finally, patients have also been identified with PTEN loss-of-function (LOF) mutations that can also lead to features of APDS (71–73).

Immunologically, APDS patients have combined immune deficiency due to impairment in multiple compartments, including B cells, T cells, and more recently described NK cells (63, 65, 114, 132). B cell function is generally impaired, and an increase in transitional B cell number is a highly conserved phenotype of APDS patients. There are also significant T cell defects in APDS patients, including increased T cell senescence likely driven by hyperproliferation, at least in some part as a result of an increased mTOR metabolism (65).

A decreased NK cell frequency has been reported in these APDS cohorts (64–67, 69); however, a detailed analysis was recently performed that identified multiple facets of deregulated

NK cell phenotype and function in patients with PI3K GOF mutations (63). This includes a decreased expression of CD16 and an increased expression of CD62L in peripheral blood NK cells, suggesting incomplete terminal maturation. NK cell function is impaired, and the source of this impairment is due to multiple defects in immune synapse formation and function. These include a decreased conjugation with target cells and a decreased phosphorylation of ERK in response to activating signaling. When forming conjugates with susceptible target cells, NK cells from APDS patients have impaired MTOC polarization and actin accumulation at the immunological synapse (63). Collectively, these effects lead to impaired NK cell lytic function mediated against susceptible class I negative, as well as antibody-coated targets. These effects were demonstrated in patients with previously described E525K, E1021K, and N334K mutations, with patients with E525K mutations interestingly having a more severe impairment of NK cell function.

The treatment of patients with the mTOR inhibitor rapamycin led to improved NK cell function and partial restoration of immune synapse formation, suggesting that tonic hyperactivation of the mTOR–AKT pathway is contributing to the functional impairment of NK cells (63). The mechanism of this is currently unclear, however, and the similarity between the effect of PI3K GOF mutations and the previously reported loss-of-function models, namely impaired NK cell cytotoxicity, can be confusing. However, of note, ERK1/2 phosphorylation in response to activation was decreased in NK cells from APDS patients, counter to the expected hyperphosphorylation predicted by an increased AKT phosphorylation (and its responsiveness to rapamycin). The decreased effector function of APDS patient NK cells, combined with a decreased ERK phosphorylation, suggests that long-term hyperactivation of these pathways leads to NK cellular hyporesponsiveness. The partial reversibility of the patients' NK cell function after the initiation of rapamycin treatment suggests that there is tunable signaling in these patient cells that can be responsive to modulation; however, this is also in addition to seemingly hardwired NK cell-developmental defects that were not affected by rapamycin treatment. There also may be differential effects of hyperactive PI3K signaling on the MAPK and AKT pathways. It would be of interest to probe downstream signalosomes with greater detail in APDS patients to determine the localization and activity of key activating and inhibitory mediators such as Vav1, SAP, and SHIP-1. In addition, while not tested in the current study, it would be of interest to test the short-term incubation of patient cells with rapamycin to determine whether NK cell function can similarly be restored by the temporary reversal of mTOR and AKT hyperphosphorylation.

Enhanced signaling through mTOR also leads to enhanced cellular metabolism, and a better understanding of how glycolysis and oxidative phosphorylation shape NK cell function is emerging (133). Consistent with the responsiveness of $CD56^{bright}$ NK cells in response to priming, $CD56^{bright}$ NK cells become highly metabolically active following cytokine stimulation, which enables their robust production of IFNγ. The treatment of healthy donor NK cells with rapamycin leads to a decreased production of IFNγ and a reduced expression of nutrient receptors such as the transferrin receptor (103, 134). mTor-deficient mice have impaired development due to impaired IL-15 responses, and it is likely that metabolic regulation is an important component of PI3K–mTOR-mediated IL-15 signaling and an unexplored component of the NK cell phenotype in APDS patients.

While the standard of care for many APDS patients has been rapamycin, the recent development of selective PI3Kδ inhibitors for the purpose of treating APDS patients has led to the availability of these and preliminary data about their efficacy and modes of action. The treatment of six patients with E525K or E1021K mutations with the selective PI3Kδ inhibitor leniolisib led to reduced phospho-AKT and -S6 in T cells (135, 136). While NK cells were not explicitly examined in this study, it will be of interest to determine whether, like rapamycin, specific PI3Kδ inhibition leads to restored function and whether, unlike rapamycin, this treatment also has an effect on NK cell phenotype and maturation.

PTEN, SHIP-1, and SAP Mutations and NK Cell Function

As APDS occurs as a result of hyperactivation of the PI3K-signaling pathway, it can be informative to also consider the consequence of LOF mutations in negative regulators of PI3K signaling. Autosomal-dominant mutations in *PTEN* are a previously described cause of hamartoma tumor syndromes, with a range of clinical effects that include susceptibility to malignancy, mucocutaneous lesions, and macrocephaly (137). Whole-exome sequencing identification of patients with LOF heterozygous mutations in *PTEN* has identified patients with APDS-like characteristics (71–73). These may include many of the clinical and immunological hallmarks of APDS, including recurrent infections, lymphadenopathy, hepatosplenomegaly, and cytopenias. In addition, APDS PTEN patients had previously described features of PTEN hamartoma tumor syndrome, including macrocephaly and mental retardation. A decreased PTEN protein expression in these patients was accompanied by an increased phospho-S6 and phospho-AKT as predicted (71, 72); however, basal PtdIns (3,4,5)P3 levels were surprisingly unaffected in one patient tested (72). NK cell function in these patients has not been specifically interrogated; however, decreased NK cell numbers were reported in some PTEN patients with immune deficiency (71–73). The variable penetrance of PTEN mutations is not completely understood; however, the capacity for these mutations to phenocopy activating mutations in *PIK3CD* or *PIK3R1* speaks of the importance of modulation of this signaling pathway.

In addition, the expression and functional role of PTEN in healthy donor human NK cells has been specifically interrogated. Overexpression (OE) of PTEN in an NK cell line or primary human or transgenic mouse NK cells leads to loss of NK cell function (74). Mechanistically, this is accompanied by a decreased accumulation of F-actin at the immunological synapse and impaired granule convergence and polarization. PTEN KD in primary mouse or human cells leads to a modest increase in NK cell lytic function, underscoring the role of PTEN as a negative regulator of NK cell cytotoxicity and contrasting the effect of PI3K GOF mutations. Interestingly, unlike mouse models of SHIP-1 and human patients with APDS, in which deregulation

of this pathway impairs NK cell maturation, there was no reported effect of PTEN OE on NK cell development in the transgenic super-PTEN mouse model. By contrast, SHIP-1-deficient mice have reduced NK cell numbers in the periphery, specifically due to impaired terminal maturation from immature precursors (80). In human NK cells, both SHIP-1 and PTEN have differential expression in mature subsets, with PTEN being highly expressed in $CD56^{bright}$ NK cells (74) and SHIP-1 more highly expressed in the $CD56^{dim}$ subset (138). SHIP-1 additionally plays a role in modulating signaling downstream of CD16 as it is recruited to the TCRζ chain during ADCC and can negatively regulate cytotoxicity (139).

Finally, the activating receptor 2B4 associates with SAP/SH2D1A, an SH2-domain-containing adaptor molecule. In addition to other immune defects, patients with X-linked lymphoproliferative disease (XLP) as a result of mutations in SAP have impaired NK cell function (140). The inhibition of PI3K function disrupts the 2B4–SAP interaction, and conversely PI3K function is impaired in patients with SAP mutations (82). As a result, the treatment of NK cells from patients with XLP with PI3K inhibitors does not further affect NK cell-cytotoxic function, whereas in healthy donors, PI3K inhibition impairs NK cell lytic function.

NK Cell Maturation and Homing

The role of PI3K in cytokine signaling, as well as its role in Rac signaling and actin remodeling, points strongly to a critical role in governing NK cell migration and maturation. In addition, PI3Kγ mediates migration through its association with G-protein-coupled receptors, and p110α and p110δ play a role in lymphocyte chemotaxis and migration. Pan-PI3K inhibition reduces NK cell migration in response to chemokines, and the selective inhibition of p110γ or p110δ shows that both play a role in CXCL12-mediated NK cell migration (106). The deletion of the p110δ isoform in mice leads to a significant decrease in NK cell number in peripheral organs, with distinct phenotypic abnormalities including a decreased expression of Ly49G2, Ly49C/I, and CD11b/CD43 (75). In remaining cells, cytokine secretion, but not production, is impaired, demonstrating a role for p110δ in both maturation and function. Transgenic mice expressing catalytically inactive p110δ also have reduced numbers of mature NK cells in the periphery, although NK cell progenitors in bone marrow are present at normal frequency (77). As with p110δ-deficient mice, catalytically inactive p110δ mice have a decreased frequency of inhibitory Ly49C/I-positive NK cells, indicating impaired terminal maturation or receptor regulation. Discrepancies between isoform-specific knockout mice and catalytically inactive mutants may be due to altered expression of other PI3K subunits following single isoform deletion, including altered p85α, p110β, and p110γ expression in p110δ-knockout mice (141, 142). PTEN also plays a critical role in NK cell homing, and NK cell-specific PTEN deletion in mice leads to increased NK cell numbers in peripheral blood due to premature egress from the bone marrow and altered response to S1P signals (79).

Whether NK cells from patients with activating PI3K mutations have alterations in NK cell maturation, tissue distribution, homeostasis, or migration is not fully understood. A direct correlation to the effect seen with PTEN deletion in mice is not seen, as abnormal NK cell numbers in peripheral blood of APDS patients have been reported as decreased, not increased (63–67, 69). Despite a decreased NK cell number, the ratio of $CD56^{bright}$ to $CD56^{dim}$ NK cells is not significantly affected in these patients, suggesting that terminal maturation occurs (63). However, there are receptors that are associated with NK cell development that have significantly altered expression in APDS patient NK cells. These include a decreased expression of CD16 on $CD56^{dim}$ NK cells and a decreased expression of CD62L on $CD56^{bright}$ NK cells. Altered expression of CD62L could lead to impaired localization in secondary lymphoid tissue, and PTEN knockout mice also have a significantly decreased CD62L expression (79). Notably, NK cells from APDS patients had a significantly decreased expression of CD122, the common gamma chain, and CD127, the IL-7 receptor. Finally, an increased expression of NKG2A on $CD56^{dim}$ NK cells from APDS patients is also suggestive of dysregulated maturation. Taken together, however, it is difficult to interpret these selective receptor anomalies with a cohesive defect in a specific aspect of NK cell maturation. Additional studies, including gene expression and, if possible, the study of tissues beyond peripheral blood, are required to definitively isolate defects in NK cell maturation and homing in patients with activating *PIK3CD* mutations.

What Can Patients with PI3K Mutations Teach Us about NK Cell Function?

What can we learn about the requirement for, and role of, PI3K signaling in human NK cells from patients with primary immunodeficiency? The discovery of APDS provides us with a robust number of patients to study and underscore the biological complexity of this signaling pathway. While these mutations are termed GOF, and rightly so based upon the hyperphosphorylation of key signaling molecules, they are not represented by an increased NK cell function. This can be partially explained by signs of impaired activation of signaling intermediates such as phospho-ERK and phospho-JNK (63), suggesting that NK cell hyporesponsiveness may result from constitutive overactivation of the pathway. This apparent hyporesponsiveness is also in contrast to studies of human cells in which PTEN levels have been transiently manipulated, with KD of PTEN leading to an increased NK cell function in this system, as would be predicted by the loss of inhibition of the PI3K-signaling pathway (**Figure 1**) (74). The mechanism of hyporesponsiveness in APDS patient NK cells is unclear, but the restoration of function following rapamycin treatment suggests that this may be a reversible condition. Currently, however, we do not know whether the effect of rapamycin is on long-term NK cell development or on survival, or whether short-term rapamycin treatment would lead to similar regained function. Treatment of patients with rapamycin may lead to the replenishment over time of NK cell subsets that have developed with modulated IL-15–mTOR signaling, leading to the restoration of functional capacity. Given the particular susceptibility of these patients to herpesviral infection (including Epstein–Barr virus, cytomegalovirus, and varicella zoster), it is likely that their NK cell dysfunction contributes to this clinical phenotype.

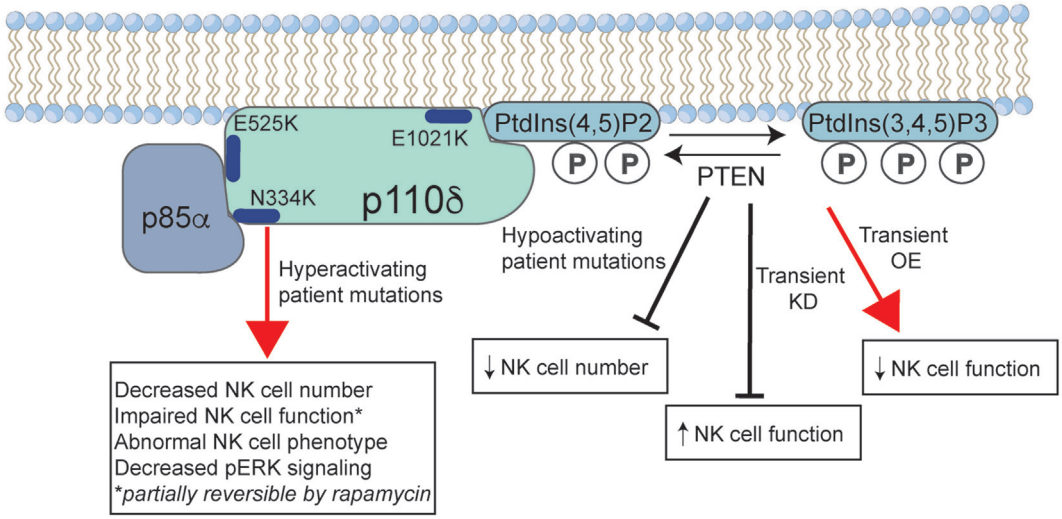


FIGURE 1 | The effect of activated PI3K delta syndrome (APDS)-causing mutations on human natural killer (NK) cell function. Common mutations in p110δ that lead to disease are shown, including those that lead to loss of negative regulation by the p85α-regulatory subunit (E525K, N334K), and the E1021K mutation that leads to constitutive membrane association. Phosphoinositide-3-kinase (PI3K) p110δ catalyzes the conversion of PtdIns(3,4)P2 to PtdIns(3,4,5)P3 at the cell membrane, a reaction that is negatively regulated by phosphatase and tensin homolog (PTEN). Gain-of-function mutations in PI3Kδ lead to a decreased NK cell number and aberrant phenotype. While less well described, patients with loss-of-function mutations in PTEN may have an APDS phenotype that is accompanied by a decreased NK cell number. Studies of short-term KD or OE of PTEN in human NK cells lead to increased and decreased NK cell functions, respectively. P, phosphate; KD, knockdown; OE, overexpression.

It is also important to consider the overall immune environment in these patients. Deregulation of the B and T cell subsets may additionally affect the generation or homeostasis of NK cell subsets, through direct or indirect mechanisms. It will be of value to study the NK cells of these patients more closely to better determine the molecular basis of dysfunction. In addition, a mouse model of APDS would enable the further dissection of the effect of activated PI3K on NK cell development, migration, and cytotoxicity. Better understanding of the effect of these mutations specifically on NK cells will be important for better understanding and implementing the next generation of therapies, including targeted small molecule inhibitors. As always in the case of primary immunodeficiency, these patients also provide us the rare opportunity to better understand the requirements for human immunity through the study of a uniquely human model.

AUTHOR CONTRIBUTIONS

All the work was performed by EM.

ACKNOWLEDGMENTS

I would like to thank all the patients and their families who participated in scientific research and colleagues within the primary immunodeficiency community for their engaging and meaningful collaboration.

FUNDING

This work was supported by an American Society of Hematology Junior Scholar Award to EM.

REFERENCES

1. Bjorkstrom NK, Ljunggren HG, Michaelsson J. Emerging insights into natural killer cells in human peripheral tissues. *Nat Rev Immunol* (2016) 16(5):310–20. doi:10.1038/nri.2016.34
2. Freud AG, Becknell B, Roychowdhury S, Mao HC, Ferketich AK, Nuovo GJ, et al. A human CD34(+) subset resides in lymph nodes and differentiates into CD56^{bright} natural killer cells. *Immunity* (2005) 22(3): 295–304. doi:10.1016/j.immuni.2005.01.013
3. Freud AG, Caligiuri MA. Human natural killer cell development. *Immunol Rev* (2006) 214:56–72. doi:10.1111/j.1600-065X.2006.00451.x
4. Yu J, Freud AG, Caligiuri MA. Location and cellular stages of natural killer cell development. *Trends Immunol* (2013) 34(12):573–82. doi:10.1016/j.it.2013.07.005
5. Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, et al. Human CD56^{bright} NK cells: an update. *J Immunol* (2016) 196(7):2923–31. doi:10.4049/jimmunol.1502570
6. Ferlazzo G, Thomas D, Lin SL, Goodman K, Morandi B, Muller WA, et al. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *J Immunol* (2004) 172(3):1455–62. doi:10.4049/jimmunol.172.3.1455
7. Nielsen N, Odum N, Urso B, Lanier LL, Spee P. Cytotoxicity of CD56 (bright) NK cells towards autologous activated CD4^{+} T cells is mediated through NKG2D, LFA-1 and TRAIL and dampened *via* CD94/NKG2A. *PLoS One* (2012) 7(2):e31959. doi:10.1371/journal.pone.0031959
8. Ellis TM, Fisher RI. Functional heterogeneity of Leu 19$^{"bright"}$+ and Leu 19$^{"dim"}$+ lymphokine-activated killer cells. *J Immunol* (1989) 142(8): 2949–54.

9. Nagler A, Lanier LL, Cwirla S, Phillips JH. Comparative studies of human FcRIII-positive and negative natural killer cells. *J Immunol* (1989) 143(10): 3183–91.
10. Wagner JA, Rosario M, Romee R, Berrien-Elliott MM, Schneider SE, Leong JW, et al. CD56^{bright} NK cells exhibit potent antitumor responses following IL-15 priming. *J Clin Invest* (2017) 127(11):4042–58. doi:10.1172/JCI90387
11. Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, et al. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56^{dim} NK-cell differentiation uncoupled from NK-cell education. *Blood* (2010) 116(19):3853–64. doi:10.1182/blood-2010-04-281675
12. Guma M, Budt M, Saez A, Brckalo T, Hengel H, Angulo A, et al. Expansion of CD94/NKG2C^{+} NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* (2006) 107(9):3624–31. doi:10.1182/blood-2005-09-3682
13. Lopez-Verges S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H, et al. CD57 defines a functionally distinct population of mature NK cells in the human CD56^{dim}CD16+ NK-cell subset. *Blood* (2010) 116(19):3865–74. doi:10.1182/blood-2010-04-282301
14. Lopez-Verges S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA, et al. Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. *Proc Natl Acad Sci U S A* (2011) 108(36):14725–32. doi:10.1073/pnas.1110900108
15. Beziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Bjorklund AT, et al. NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. *Blood* (2013) 121(14):2678–88. doi:10.1182/blood-2012-10-459545
16. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. *Immunity* (2015) 42(3): 443–56. doi:10.1016/j.immuni.2015.02.008
17. Lee J, Zhang T, Hwang I, Kim A, Nitschke L, Kim M, et al. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. *Immunity* (2015) 42(3): 431–42. doi:10.1016/j.immuni.2015.02.013
18. Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* (1998) 395(6697):82–6. doi:10.1038/25764
19. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, et al. The immunological synapse: a molecular machine controlling T cell activation. *Science* (1999) 285(5425):221–7. doi:10.1126/science.285.5425.221
20. Davis DM, Chiu I, Fassett M, Cohen GB, Mandelboim O, Strominger JL. The human natural killer cell immune synapse. *Proc Natl Acad Sci U S A* (1999) 96(26):15062–7. doi:10.1073/pnas.96.26.15062
21. Wulfing C, Purtic B, Klem J, Schatzle JD. Stepwise cytoskeletal polarization as a series of checkpoints in innate but not adaptive cytolytic killing. *Proc Natl Acad Sci U S A* (2003) 100(13):7767–72. doi:10.1073/pnas.1336920100
22. Mace EM, Dongre P, Hsu HT, Sinha P, James AM, Mann SS, et al. Cell biological steps and checkpoints in accessing NK cell cytotoxicity. *Immunol Cell Biol* (2014) 92(3):245–55. doi:10.1038/icb.2013.96
23. Yokoyama WM, Kim S. Licensing of natural killer cells by self-major histocompatibility complex class I. *Immunol Rev* (2006) 214:143–54. doi:10.1111/j.1600-065X.2006.00458.x
24. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* (2005) 436(7051):709–13. doi:10.1038/nature03847
25. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* (2005) 105(11): 4416–23. doi:10.1182/blood-2004-08-3156
26. Elliott JM, Wahle JA, Yokoyama WM. MHC class I-deficient natural killer cells acquire a licensed phenotype after transfer into an MHC class I-sufficient environment. *J Exp Med* (2010) 207(10):2073–9. doi:10.1084/jem.20100986
27. Joncker NT, Shifrin N, Delebecque F, Raulet DH. Mature natural killer cells reset their responsiveness when exposed to an altered MHC environment. *J Exp Med* (2010) 207(10):2065–72. doi:10.1084/jem.20100570
28. Chalifour A, Scarpellino L, Back J, Brodin P, Devevre E, Gros F, et al. A Role for cis interaction between the inhibitory Ly49A receptor and MHC class I for natural killer cell education. *Immunity* (2009) 30(3):337–47. doi:10.1016/j.immuni.2008.12.019
29. Brodin P, Lakshmikanth T, Johansson S, Karre K, Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. *Blood* (2009) 113(11): 2434–41. doi:10.1182/blood-2008-05-156836
30. Beuneu H, Deguine J, Breart B, Mandelboim O, Di Santo JP, Bousso P. Dynamic behavior of NK cells during activation in lymph nodes. *Blood* (2009) 114(15):3227–34. doi:10.1182/blood-2009-06-228759
31. Bajenoff M, Breart B, Huang AY, Qi H, Cazareth J, Braud VM, et al. Natural killer cell behavior in lymph nodes revealed by static and real-time imaging. *J Exp Med* (2006) 203(3):619–31. doi:10.1084/jem.20051474
32. Garrod KR, Wei SH, Parker I, Cahalan MD. Natural killer cells actively patrol peripheral lymph nodes forming stable conjugates to eliminate MHC-mismatched targets. *Proc Natl Acad Sci U S A* (2007) 104(29):12081–6. doi:10.1073/pnas.0702867104
33. Mace EM, Gunesch JT, Dixon A, Orange JS. Human NK cell development requires CD56-mediated motility and formation of the developmental synapse. *Nat Commun* (2016) 7:12171. doi:10.1038/ncomms12171
34. Lee BJ, Mace EM. Acquisition of cell migration defines NK cell differentiation from hematopoietic stem cell precursors. *Mol Biol Cell* (2017) 28(25): 3573–81. doi:10.1091/mbc.E17-08-0508
35. Khorshidi MA, Vanherberghen B, Kowalewski JM, Garrod KR, Lindstrom S, Andersson-Svahn H, et al. Analysis of transient migration behavior of natural killer cells imaged *in situ* and *in vitro*. *Integr Biol (Camb)* (2011) 3(7): 770–8. doi:10.1039/c1ib00007a
36. Vanherberghen B, Olofsson PE, Forslund E, Sternberg-Simon M, Khorshidi MA, Pacouret S, et al. Classification of human natural killer cells based on migration behavior and cytotoxic response. *Blood* (2013) 121(8):1326–34. doi:10.1182/blood-2012-06-439851
37. Bhat R, Watzl C. Serial killing of tumor cells by human natural killer cells—enhancement by therapeutic antibodies. *PLoS One* (2007) 2(3):e326. doi:10.1371/journal.pone.0000326
38. Choi PJ, Mitchison TJ. Imaging burst kinetics and spatial coordination during serial killing by single natural killer cells. *Proc Natl Acad Sci U S A* (2013) 110(16):6488–93. doi:10.1073/pnas.1221312110
39. Forslund E, Sohlberg E, Enqvist M, Olofsson PE, Malmberg KJ, Onfelt B. Microchip-based single-cell imaging reveals that CD56^{dim}CD57–KIR–NKG2A^{+} NK cells have more dynamic migration associated with increased target cell conjugation and probability of killing compared to CD56^{dim}CD57–KIR–NKG2A^{-} NK cells. *J Immunol* (2015) 195(7):3374–81. doi:10.4049/jimmunol.1500171
40. Olofsson PE, Forslund E, Vanherberghen B, Chechet K, Mickelin O, Ahlin AR, et al. Distinct migration and contact dynamics of resting and IL-2-activated human natural killer cells. *Front Immunol* (2014) 5:80. doi:10.3389/fimmu.2014.00080
41. Orange JS, Ramesh N, Remold-O'Donnell E, Sasahara Y, Koopman L, Byrne M, et al. Wiskott–Aldrich syndrome protein is required for NK cell cytotoxicity and colocalizes with actin to NK cell-activating immunologic synapses. *Proc Natl Acad Sci U S A* (2002) 99(17):11351–6. doi:10.1073/pnas.162376099
42. Orange JS, Roy-Ghanta S, Mace EM, Maru S, Rak GD, Sanborn KB, et al. IL-2 induces a WAVE2-dependent pathway for actin reorganization that enables WASp-independent human NK cell function. *J Clin Invest* (2011) 121(4):1535–48. doi:10.1172/JCI44862
43. Sanborn KB, Rak GD, Maru SY, Demers K, Difeo A, Martignetti JA, et al. Myosin IIA associates with NK cell lytic granules to enable their interaction with F-actin and function at the immunological synapse. *J Immunol* (2009) 182(11):6969–84. doi:10.4049/jimmunol.0804337
44. Sanborn KB, Mace EM, Rak GD, Difeo A, Martignetti JA, Pecci A, et al. Phosphorylation of the myosin IIA tailpiece regulates single myosin IIA molecule association with lytic granules to promote NK-cell cytotoxicity. *Blood* (2011) 118(22):5862–71. doi:10.1182/blood-2011-03-344846
45. Mizesko MC, Banerjee PP, Monaco-Shawver L, Mace EM, Bernal WE, Sawalle-Belohradsky J, et al. Defective actin accumulation impairs human natural killer cell function in patients with dedicator of cytokinesis 8

deficiency. *J Allergy Clin Immunol* (2013) 131(3):840–8. doi:10.1016/j.jaci.2012.12.1568
46. Mace EM, Orange JS. Lytic immune synapse function requires filamentous actin deconstruction by Coronin 1A. *Proc Natl Acad Sci U S A* (2014) 111(18):6708–13. doi:10.1073/pnas.1314975111
47. Mace EM, Orange JS. Genetic causes of human NK cell deficiency and their effect on NK cell subsets. *Front Immunol* (2016) 7:545. doi:10.3389/fimmu.2016.00545
48. Voss M, Bryceson YT. Natural killer cell biology illuminated by primary immunodeficiency syndromes in humans. *Clin Immunol* (2017) 177:29–42. doi:10.1016/j.clim.2015.11.004
49. Hughes CR, Guasti L, Meimaridou E, Chuang CH, Schimenti JC, King PJ, et al. MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. *J Clin Invest* (2012) 122(3):814–20. doi:10.1172/JCI60224
50. Gineau L, Cognet C, Kara N, Lach FP, Dunne J, Veturi U, et al. Partial MCM4 deficiency in patients with growth retardation, adrenal insufficiency, and natural killer cell deficiency. *J Clin Invest* (2012) 122(3):821–32. doi:10.1172/JCI61014
51. Cottineau J, Kottemann MC, Lach FP, Kang YH, Vely F, Deenick EK, et al. Inherited GINS1 deficiency underlies growth retardation along with neutropenia and NK cell deficiency. *J Clin Invest* (2017) 127(5):1991–2006. doi:10.1172/JCI90727
52. Mace EM, Bigley V, Gunesch JT, Chinn IK, Angelo LS, Care MA, et al. Biallelic mutations in IRF8 impair human NK cell maturation and function. *J Clin Invest* (2017) 127(1):306–20. doi:10.1172/JCI86276
53. Hanna S, Beziat V, Jouanguy E, Casanova JL, Etzioni A. A homozygous mutation of RTEL1 in a child presenting with an apparently isolated natural killer cell deficiency. *J Allergy Clin Immunol* (2015) 136(4):1113–4. doi:10.1016/j.jaci.2015.04.021
54. Etzioni A, Eidenschenk C, Katz R, Beck R, Casanova JL, Pollack S. Fatal varicella associated with selective natural killer cell deficiency. *J Pediatr* (2005) 146(3):423–5. doi:10.1016/j.jpeds.2004.11.022
55. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123(6):809–21. doi:10.1182/blood-2013-07-515528
56. Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. *Blood* (2013) 121(14):2669–77. doi:10.1182/blood-2012-09-453969
57. Schlums H, Jung M, Han H, Theorell J, Bigley V, Chiang SC, et al. Adaptive NK cells can persist in patients with GATA2 mutation depleted of stem and progenitor cells. *Blood* (2017) 129(14):1927–39. doi:10.1182/blood-2016-08-734236
58. Maciejewski-Duval A, Meuris F, Bignon A, Aknin ML, Balabanian K, Faivre L, et al. Altered chemotactic response to CXCL12 in patients carrying GATA2 mutations. *J Leukoc Biol* (2016) 99(6):1065–76. doi:10.1189/jlb.5MA0815-388R
59. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. *Cell* (1993) 73(1):147–57. doi:10.1016/0092-8674(93)90167-O
60. Roberts JL, Lengi A, Brown SM, Chen M, Zhou YJ, O'Shea JJ, et al. Janus kinase 3 (JAK3) deficiency: clinical, immunologic, and molecular analyses of 10 patients and outcomes of stem cell transplantation. *Blood* (2004) 103(6):2009–18. doi:10.1182/blood-2003-06-2104
61. Vargas-Hernández A, Mace EM, Zimmerman O, Zerbe CS, Freeman AF, Rosenzweig S, et al. Ruxolitinib partially reverses functional natural killer cell deficiency in patients with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations. *J Allergy Clin Immunol* (2017):31647. doi:10.1016/j.jaci.2017.08.040
62. Zhu S, Phatarpekar PV, Denman CJ, Senyukov VV, Somanchi SS, Nguyen-Jackson HT, et al. Transcription of the activating receptor NKG2D in natural killer cells is regulated by STAT3 tyrosine phosphorylation. *Blood* (2014) 124(3):403–11. doi:10.1182/blood-2013-05-499707
63. Ruiz-García R, Vargas-Hernández A, Chinn IK, Angelo LS, Cao TN, Coban-Akdemir Z, et al. Mutations in PI3K110δ cause impaired natural killer cell function partially rescued by rapamycin treatment. *J Allergy Clin Immunol* (2018). doi:10.1016/j.jaci.2017.11.042
64. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
65. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
66. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol* (2014) 134(1):233–6. doi:10.1016/j.jaci.2014.02.020
67. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Scholvinck L, Kalina T, et al. Genetic defects in PI3Kdelta affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
68. Zhang KJ, Husami A, Marsh R, Jordan MB. Identification of a phosphoinositide 3-kinase (PI-3K) p110δ (PIK3CD) deficient individual. *J Clin Immunol* (2013) 33:673–4.
69. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923–8. doi:10.1172/JCI75746
70. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang YD, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85alpha subunit of PI3K. *J Exp Med* (2012) 209(3):463–70. doi:10.1084/jem.20112533
71. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138(6):1672–80.e10. doi:10.1016/j.jaci.2016.03.055
72. Browning MJ, Chandra A, Carbonaro V, Okkenhaug K, Barwell J. Cowden's syndrome with immunodeficiency. *J Med Genet* (2015) 52(12):856–9. doi:10.1136/jmedgenet-2015-103266
73. Driessen GJ, IJspeert H, Wentink M, Yntema HG, van Hagen PM, van Strien A, et al. Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency. *J Allergy Clin Immunol* (2016) 138(6):1744–7.e5. doi:10.1016/j.jaci.2016.07.010
74. Briercheck EL, Trotta R, Chen L, Hartlage AS, Cole JP, Cole TD, et al. PTEN is a negative regulator of NK cell cytolytic function. *J Immunol* (2015) 194(4):1832–40. doi:10.4049/jimmunol.1401224
75. Kim N, Saudemont A, Webb L, Camps M, Ruckle T, Hirsch E, et al. The p110delta catalytic isoform of PI3K is a key player in NK-cell development and cytokine secretion. *Blood* (2007) 110(9):3202–8. doi:10.1182/blood-2007-02-075366
76. Zebedin E, Simma O, Schuster C, Putz EM, Fajmann S, Warsch W, et al. Leukemic challenge unmasks a requirement for PI3Kdelta in NK cell-mediated tumor surveillance. *Blood* (2008) 112(12):4655–64. doi:10.1182/blood-2008-02-139105
77. Guo H, Samarakoon A, Vanhaesebroeck B, Malarkannan S. The p110 delta of PI3K plays a critical role in NK cell terminal maturation and cytokine/chemokine generation. *J Exp Med* (2008) 205(10):2419–35. doi:10.1084/jem.20072327
78. Awasthi A, Samarakoon A, Dai X, Wen R, Wang D, Malarkannan S. Deletion of PI3K-p85alpha gene impairs lineage commitment, terminal maturation, cytokine generation and cytotoxicity of NK cells. *Genes Immun* (2008) 9(6):522–35. doi:10.1038/gene.2008.45
79. Leong JW, Schneider SE, Sullivan RP, Parikh BA, Anthony BA, Singh A, et al. PTEN regulates natural killer cell trafficking *in vivo*. *Proc Natl Acad Sci U S A* (2015) 112(7):E700–9. doi:10.1073/pnas.1413886112
80. Banh C, Miah SM, Kerr WG, Brossay L. Mouse natural killer cell development and maturation are differentially regulated by SHIP-1. *Blood* (2012) 120(23):4583–90. doi:10.1182/blood-2012-04-425009
81. Vanhaesebroeck B, Whitehead MA, Pineiro R. Molecules in medicine mini-review: isoforms of PI3K in biology and disease. *J Mol Med (Berl)* (2016) 94(1):5–11. doi:10.1007/s00109-015-1352-5

82. Aoukaty A, Tan R. Association of the X-linked lymphoproliferative disease gene product SAP/SH2D1A with 2B4, a natural killer cell-activating molecule, is dependent on phosphoinositide 3-kinase. *J Biol Chem* (2002) 277(15): 13331–7. doi:10.1074/jbc.M112029200
83. Marti F, Xu CW, Selvakumar A, Brent R, Dupont B, King PD. LCK-phosphorylated human killer cell-inhibitory receptors recruit and activate phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* (1998) 95(20): 11810–5. doi:10.1073/pnas.95.20.11810
84. Eissmann P, Beauchamp L, Wooters J, Tilton JC, Long EO, Watzl C. Molecular basis for positive and negative signaling by the natural killer cell receptor 2B4 (CD244). *Blood* (2005) 105(12):4722–9. doi:10.1182/blood-2004-09-3796
85. Upshaw JL, Arneson LN, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ. NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. *Nat Immunol* (2006) 7(5):524–32. doi:10.1038/ni1325
86. Segovis CM, Schoon RA, Dick CJ, Nacusi LP, Leibson PJ, Billadeau DD. PI3K links NKG2D signaling to a CrkL pathway involved in natural killer cell adhesion, polarity, and granule secretion. *J Immunol* (2009) 182(11): 6933–42. doi:10.4049/jimmunol.0803840
87. Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, et al. An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* (1999) 285(5428):730–2. doi:10.1126/science.285.5428.730
88. Billadeau DD, Upshaw JL, Schoon RA, Dick CJ, Leibson PJ. NKG2D–DAP10 triggers human NK cell-mediated killing *via* a Syk-independent regulatory pathway. *Nat Immunol* (2003) 4(6):557–64. doi:10.1038/ni929
89. Kanakaraj P, Duckworth B, Azzoni L, Kamoun M, Cantley LC, Perussia B. Phosphatidylinositol-3 kinase activation induced upon Fc gamma RIIIA–ligand interaction. *J Exp Med* (1994) 179(2):551–8. doi:10.1084/jem.179.2.551
90. Galandrini R, Micucci F, Tassi I, Cifone MG, Cinque B, Piccoli M, et al. Arf6: a new player in FcgammaRIIIA lymphocyte-mediated cytotoxicity. *Blood* (2005) 106(2):577–83. doi:10.1182/blood-2004-10-4100
91. Jiang K, Zhong B, Gilvary DL, Corliss BC, Hong-Geller E, Wei S, et al. Pivotal role of phosphoinositide-3 kinase in regulation of cytotoxicity in natural killer cells. *Nat Immunol* (2000) 1(5):419–25. doi:10.1038/80859
92. Carlin LM, Evans R, Milewicz H, Fernandes L, Matthews DR, Perani M, et al. A targeted siRNA screen identifies regulators of Cdc42 activity at the natural killer cell immunological synapse. *Sci Signal* (2011) 4(201):ra81. doi:10.1126/scisignal.2001729
93. Ma A, Koka R, Burkett P. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. *Annu Rev Immunol* (2006) 24:657–79. doi:10.1146/annurev.immunol.24.021605.090727
94. Ali AK, Nandagopal N, Lee SH. IL-15–PI3K–AKT–mTOR: a critical pathway in the life journey of natural killer cells. *Front Immunol* (2015) 6:355. doi:10.3389/fimmu.2015.00355
95. Yang M, Li D, Chang Z, Yang Z, Tian Z, Dong Z. PDK1 orchestrates early NK cell development through induction of E4BP4 expression and maintenance of IL-15 responsiveness. *J Exp Med* (2015) 212(2):253–65. doi:10.1084/jem.20141703
96. Fehniger TA, Cai SF, Cao X, Bredemeyer AJ, Presti RM, French AR, et al. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. *Immunity* (2007) 26(6):798–811. doi:10.1016/j.immuni.2007.04.010
97. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* (2007) 26(4):503–17. doi:10.1016/j.immuni.2007.03.006
98. Chaix J, Tessmer MS, Hoebe K, Fuseri N, Ryffel B, Dalod M, et al. Cutting edge: priming of NK cells by IL-18. *J Immunol* (2008) 181(3):1627–31. doi:10.4049/jimmunol.181.3.1627
99. Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, et al. Cytokine activation induces human memory-like NK cells. *Blood* (2012) 120(24):4751–60. doi:10.1182/blood-2012-04-419283
100. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A* (2009) 106(6):1915–9. doi:10.1073/pnas.0813192106
101. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Transl Med* (2016) 8(357):357ra123. doi:10.1126/scitranslmed.aaf2341
102. Romee R, Leong JW, Fehniger TA. Utilizing cytokines to function-enable human NK cells for the immunotherapy of cancer. *Scientifica (Cairo)* (2014) 2014:205796. doi:10.1155/2014/205796
103. Nandagopal N, Ali AK, Komal AK, Lee SH. The critical role of IL-15–PI3K–mTOR pathway in natural killer cell effector functions. *Front Immunol* (2014) 5:187. doi:10.3389/fimmu.2014.00187
104. Fortenbery NR, Paraiso KH, Taniguchi M, Brooks C, Ibrahim L, Kerr WG. SHIP influences signals from CD48 and MHC class I ligands that regulate NK cell homeostasis, effector function, and repertoire formation. *J Immunol* (2010) 184(9):5065–74. doi:10.4049/jimmunol.0901862
105. al-Aoukaty A, Rolstad B, Maghazachi AA. Recruitment of pleckstrin and phosphoinositide 3-kinase gamma into the cell membranes, and their association with G beta gamma after activation of NK cells with chemokines. *J Immunol* (1999) 162(6):3249–55.
106. Saudemont A, Garcon F, Yadi H, Roche-Molina M, Kim N, Segonds-Pichon A, et al. p110gamma and p110delta isoforms of phosphoinositide 3-kinase differentially regulate natural killer cell migration in health and disease. *Proc Natl Acad Sci U S A* (2009) 106(14):5795–800. doi:10.1073/pnas.0808594106
107. Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, et al. T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J Exp Med* (2009) 206(11):2469–81. doi:10.1084/jem.20090525
108. Zhong B, Liu JH, Gilvary DL, Jiang K, Kasuga M, Ritchey CA, et al. Functional role of phosphatidylinositol 3-kinase in direct tumor lysis by human natural killer cells. *Immunobiology* (2002) 205(1):74–94. doi:10.1078/0171-2985-00112
109. Bonnema JD, Rivlin KA, Ting AT, Schoon RA, Abraham RT, Leibson PJ. Cytokine-enhanced NK cell-mediated cytotoxicity. Positive modulatory effects of IL-2 and IL-12 on stimulus-dependent granule exocytosis. *J Immunol* (1994) 152(5):2098–104.
110. Barber DF, Long EO. Coexpression of CD58 or CD48 with intercellular adhesion molecule 1 on target cells enhances adhesion of resting NK cells. *J Immunol* (2003) 170(1):294–9. doi:10.4049/jimmunol.170.1.294
111. Barber DF, Faure M, Long EO. LFA-1 contributes an early signal for NK cell cytotoxicity. *J Immunol* (2004) 173(6):3653–9. doi:10.4049/jimmunol.173.6.3653
112. Yea SS, So L, Mallya S, Lee J, Rajasekaran K, Malarkannan S, et al. Effects of novel isoform-selective phosphoinositide 3-kinase inhibitors on natural killer cell function. *PLoS One* (2014) 9(6):e99486. doi:10.1371/journal.pone.0099486
113. Kerr WG, Colucci F. Inositol phospholipid signaling and the biology of natural killer cells. *J Innate Immun* (2011) 3(3):249–57. doi:10.1159/000323920
114. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
115. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kdelta regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114(8): 1982–7. doi:10.1073/pnas.1617244114
116. Martinez-Saavedra MT, Garcia-Gomez S, Dominguez Acosta A, Mendoza Quintana JJ, Paez JP, Garcia-Reino EJ, et al. Gain-of-function mutation in PIK3R1 in a patient with a narrow clinical phenotype of respiratory infections. *Clin Immunol* (2016) 173:117–20. doi:10.1016/j.clim.2016.09.011
117. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
118. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kdelta syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27(6):640–4. doi:10.1111/pai.12585
119. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210–8.e9. doi:10.1016/j.jaci.2016.03.022

120. Kuhlen M, Honscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. *De novo* PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
121. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159(1):33–6. doi:10.1016/j.clim.2015.04.014
122. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36(5):462–71. doi:10.1007/s10875-016-0281-6
123. Bravo Garcia-Morato M, Garcia-Minaur S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, Lopez-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
124. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kdelta syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
125. Buchbinder D, Seppanen M, Rao VK, Uzel G, Nugent D. Clinical challenges: identification of patients with novel primary immunodeficiency syndromes. *J Pediatr Hematol Oncol* (2017). doi:10.1097/MPH.0000000000001003
126. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183(2):221–9. doi:10.1111/cei.12706
127. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased *in vitro* anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2017) 178:20–8. doi:10.1016/j.clim.2015.12.008
128. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34(3):272–6. doi:10.1007/s10875-014-0012-9
129. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35(1):11–4. doi:10.1007/s10875-014-0109-1
130. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110delta cause activated PI3Kdelta syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140(4):1152–6.e10. doi:10.1016/j.jaci.2017.03.026
131. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase delta syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
132. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3K delta and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16(11):702–14. doi:10.1038/nri.2016.93
133. Gardiner CM, Finlay DK. What fuels natural killers? Metabolism and NK cell responses. *Front Immunol* (2017) 8:367. doi:10.3389/fimmu.2017.00367
134. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, et al. mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *J Immunol* (2014) 193(9):4477–84. doi:10.4049/jimmunol.1401558
135. Hoegenauer K, Soldermann N, Zecri F, Strang RS, Graveleau N, Wolf RM, et al. Discovery of CDZ173 (Leniolisib), representing a structurally novel class of PI3K delta-selective inhibitors. *ACS Med Chem Lett* (2017) 8(9):975–80. doi:10.1021/acsmedchemlett.7b00293
136. Rao VK, Webster S, Dalm V, Sediva A, van Hagen PM, Holland S, et al. Effective "activated PI3Kdelta syndrome"-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
137. Eng C. PTEN: one gene, many syndromes. *Hum Mutat* (2003) 22(3):183–98. doi:10.1002/humu.10257
138. Trotta R, Parihar R, Yu J, Becknell B, Allard J II, Wen J, et al. Differential expression of SHIP1 in CD56^{bright} and CD56^{dim} NK cells provides a molecular basis for distinct functional responses to monokine costimulation. *Blood* (2005) 105(8):3011–8. doi:10.1182/blood-2004-10-4072
139. Galandrini R, Tassi I, Mattia G, Lenti L, Piccoli M, Frati L, et al. SH2-containing inositol phosphatase (SHIP-1) transiently translocates to raft domains and modulates CD16-mediated cytotoxicity in human NK cells. *Blood* (2002) 100(13):4581–9. doi:10.1182/blood-2002-04-1058
140. Benoit L, Wang X, Pabst HF, Dutz J, Tan R. Defective NK cell activation in X-linked lymphoproliferative disease. *J Immunol* (2000) 165(7):3549–53. doi:10.4049/jimmunol.165.7.3549
141. Tassi I, Cella M, Gilfillan S, Turnbull I, Diacovo TG, Penninger JM, et al. p110gamma and p110delta phosphoinositide 3-kinase signaling pathways synergize to control development and functions of murine NK cells. *Immunity* (2007) 27(2):214–27. doi:10.1016/j.immuni.2007.07.014
142. Clayton E, Bardi G, Bell SE, Chantry D, Downes CP, Gray A, et al. A crucial role for the p110delta subunit of phosphatidylinositol 3-kinase in B cell development and activation. *J Exp Med* (2002) 196(6):753–63. doi:10.1084/jem.20020805

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer TP and the handling editor declared their shared affiliation.

frontiers in Immunology

PERSPECTIVE
published: 16 March 2018
doi: 10.3389/fimmu.2018.00543

Disease Evolution and Response to Rapamycin in Activated Phosphoinositide 3-Kinase δ Syndrome: The European Society for Immunodeficiencies-Activated Phosphoinositide 3-Kinase δ Syndrome Registry

Maria Elena Maccari[1,2], Hassan Abolhassani[3,4], Asghar Aghamohammadi[4], Alessandro Aiuti[5], Olga Aleinikova[6], Catherine Bangs[7], Safa Baris[8], Federica Barzaghi[5], Helen Baxendale[9], Matthew Buckland[10], Siobhan O. Burns[10], Caterina Cancrini[11,12], Andrew Cant[13], Pascal Cathébras[14], Marina Cavazzana[15,16,17], Anita Chandra[18,19], Francesca Conti[11,12], Tanya Coulter[20], Lisa A. Devlin[20], J. David M. Edgar[20], Saul Faust[21], Alain Fischer[17,22,23], Marina Garcia Prat[24], Lennart Hammarström[3], Maximilian Heeg[1,2], Stephen Jolles[25], Elif Karakoc-Aydiner[8], Gerhard Kindle[1], Ayca Kiykim[8], Dinakantha Kumararatne[17], Bodo Grimbacher[1], Hilary Longhurst[10], Nizar Mahlaoui[22,26], Tomas Milota[27], Fernando Moreira[10], Despina Moshous[17,22,23], Anna Mukhina[28], Olaf Neth[29], Benedicte Neven[17,22,30], Alexandra Nieters[1], Peter Olbrich[29], Ahmet Ozen[8], Jana Pachlopnik Schmid[31], Capucine Picard[32,33], Seraina Prader[31], William Rae[21], Janine Reichenbach[31], Stephan Rusch[1], Sinisa Savic[32], Alessia Scarselli[11,12], Raphael Scheible[1], Anna Sediva[27], Svetlana O. Sharapova[6], Anna Shcherbina[28], Mary Slatter[12], Pere Soler-Palacin[24], Aurelie Stanislas[15], Felipe Suarez[23], Francesca Tucci[5], Annette Uhlmann[1], Joris van Montfrans[34], Klaus Warnatz[1], Anthony Peter Williams[21], Phil Wood[35], Sven Kracker[16,17†], Alison Mary Condliffe[36†] and Stephan Ehl[1,2†]*

[1] Center for Chronic Immunodeficiency, Medical Center – University of Freiburg, Freiburg, Germany, [2] Department of Pediatrics and Adolescent Medicine, Medical Center – University of Freiburg, Freiburg, Germany, [3] Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden, [4] Research Center for Immunodeficiencies, Pediatric Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran, [5] San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), Pediatric Immunohematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy, [6] Research Department, Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk, Belarus, [7] Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom, [8] Division of Pediatric Allergy/Immunology, Marmara University, Istanbul, Turkey, [9] Cambridge Centre for Lung Defense, Papworth Hospital, Cambridge, United Kingdom, [10] Institute of Immunity and Transplantation, Royal Free Hospital, London, United Kingdom, [11] University Department of Pediatrics, Bambino Gesù Children's Hospital IRCCS, Rome, Italy, [12] Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, [13] Department of Paediatric Immunology, Newcastle upon Tyne Hospital NHS Foundation Trust, Newcastle upon Tyne, United Kingdom, [14] Internal Medicine, University Hospital of Saint-Etienne, Saint-Etienne, France, [15] Biotherapy Department, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Children's Hospital, Paris, France, [16] Laboratory of Human Lymphohematopoiesis, INSERM UMR 1163, Imagine Institute, Paris, France, [17] Paris Descartes-Sorbonne Paris Cité University, Paris, France, [18] Department of Clinical Immunology, Addenbrookes Hospital, Cambridge, United Kingdom, [19] Department of Medicine, University of Cambridge, Cambridge, United Kingdom, [20] Regional Immunology Service, The Royal Hospitals & Queen's University, Belfast, United Kingdom, [21] NIHR Clinical Research Facility, University Hospital Southampton NHSFT, Southampton, United Kingdom, [22] Department of Pediatric Immunology, Hematology and Rheumatology, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Children's Hospital, Paris, France, [23] INSERM UMR 1163, Imagine Institute, Paris, France, [24] Pediatric Infectious Diseases and

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Shigeaki Nonoyama, National Defense Medical College, Japan
Kahn Preece, The University of Queensland, Australia

***Correspondence:**
Maria Elena Maccari
maria.elena.maccari@uniklinik-freiburg.de

[†]These authors have contributed equally to the work.

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *30 December 2017*
Accepted: *02 March 2018*
Published: *16 March 2018*

Citation:
Maccari ME, Abolhassani H, Aghamohammadi A, Aiuti A, Aleinikova O, Bangs C, Baris S, Barzaghi F, Baxendale H, Buckland M, Burns SO, Cancrini C, Cant A, Cathébras P, Cavazzana M, Chandra A, Conti F, Coulter T, Devlin LA, Edgar JDM, Faust S,

Fischer A, Prat MG, Hammarström L, Heeg M, Jolles S, Karakoc-Aydiner E, Kindle G, Kiykim A, Kumararatne D, Grimbacher B, Longhurst H, Mahlaoui N, Milota T, Moreira F, Moshous D, Mukhina A, Neth O, Neven B, Nieters A, Olbrich P, Ozen A, Schmid JP, Picard C, Prader S, Rae W, Reichenbach J, Rusch S, Savic S, Scarselli A, Scheible R, Sediva A, Sharapova SO, Shcherbina A, Slatter M, Soler-Palacin P, Stanislas A, Suarez F, Tucci F, Uhlmann A, van Montfrans J, Warnatz K, Williams AP, Wood P, Kracker S, Condliffe AM and Ehl S (2018) Disease Evolution and Response to Rapamycin in Activated Phosphoinositide 3-Kinase δ Syndrome: The European Society for Immunodeficiencies-Activated Phosphoinositide 3-Kinase δ Syndrome Registry. Front. Immunol. 9:543. doi: 10.3389/fimmu.2018.00543

Immunodeficiencies Unit, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain, [25] Immunodeficiency Centre for Wales, University Hospital of Wales, Cardiff, United Kingdom, [26] French National Reference Center for Primary Immune Deficiencies (CEREDIH), Necker Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France, [27] Department of Immunology, 2nd Faculty of Medicine Charles University and Motol University Hospital, Prague, Czechia, [28] Department of Immunology, Research and Clinical Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia, [29] Sección de Infectología, Rheumatología and Inmunodeficiencias, Unidad de Pediatria, Hospital Virgen del Rocıo, Instituto de Biomedicina de Sevilla (IBiS), Sevilla, Spain, [30] Laboratory of Immunogenetics of Pediatric Autoimmunity, INSERM UMR 1163, Imagine Institute, Paris, France, [31] Division of Immunology, University Children's Hospital Zurich and Children's Research Centre, University Zurich, Zurich, Switzerland, [32] Study Center for Primary Immunodeficiencies, Necker-Enfants Malades Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Medical School, Paris, France, [33] Laboratory of Lymphocyte Activation and Susceptibility to EBV Infection, INSERM UMR 1163, Imagine Institute, Paris, France, [34] Wilhelmina Children's Hospital, Utrecht, Netherlands, [35] Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, United Kingdom, [36] Department of Infection, Immunity and Cardiovascular Science, University of Sheffield, Sheffield, United Kingdom

Activated phosphoinositide 3-kinase (PI3K) δ Syndrome (APDS), caused by autosomal dominant mutations in *PIK3CD* (APDS1) or *PIK3R1* (APDS2), is a heterogeneous primary immunodeficiency. While initial cohort-descriptions summarized the spectrum of clinical and immunological manifestations, questions about long-term disease evolution and response to therapy remain. The prospective European Society for Immunodeficiencies (ESID)-APDS registry aims to characterize the disease course, identify outcome predictors, and evaluate treatment responses. So far, 77 patients have been recruited (51 APDS1, 26 APDS2). Analysis of disease evolution in the first 68 patients pinpoints the early occurrence of recurrent respiratory infections followed by chronic lymphoproliferation, gastrointestinal manifestations, and cytopenias. Although most manifestations occur by age 15, adult-onset and asymptomatic courses were documented. Bronchiectasis was observed in 24/40 APDS1 patients who received a CT-scan compared with 4/15 APDS2 patients. By age 20, half of the patients had received at least one immunosuppressant, but 2–3 lines of immunosuppressive therapy were not unusual before age 10. Response to rapamycin was rated by physician visual analog scale as good in 10, moderate in 9, and poor in 7. Lymphoproliferation showed the best response (8 complete, 11 partial, 6 no remission), while bowel inflammation (3 complete, 3 partial, 9 no remission) and cytopenia (3 complete, 2 partial, 9 no remission) responded less well. Hence, non-lymphoproliferative manifestations should be a key target for novel therapies. This report from the ESID-APDS registry provides comprehensive baseline documentation for a growing cohort that will be followed prospectively to establish prognostic factors and identify patients for treatment studies.

Keywords: activated phosphoinositide 3-kinase δ syndrome, PIK3CD, PIK3R1, registry, natural history, rapamycin

INTRODUCTION

Heterozygous gain-of-phosphoinositide 3-kinase (PI3K) δ-function mutations in *PIK3CD* or *PIK3R1* cause an autosomal-dominant primary immunodeficiency (PID) called activated phosphoinositide 3-kinase δ syndrome (APDS) or PASLI (p110-delta-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency) 1 and 2, respectively (1–4). The main clinical and immunological characteristics of APDS 1 and 2 have been recently described in two major retrospective cohort studies (5, 6). Recurrent respiratory infections and benign lymphoproliferation emerged as key clinical aspects of the disease in both cohorts. Bronchiectasis was noted as a frequent complication with 60% in the APDS1 cohort and less frequently (18%) in the APDS2 cohort study. Additional immune dysregulation including cytopenias, glomerulonephritis, arthritis, and colitis was reported in these studies. An increased risk for lymphoma was also highlighted with 13% among the APDS1 patients and 28% in the APDS2 cohort. Non-immunological characteristics included neurodevelopmental delay (19% of APDS1 and 31% of APDS2) and growth impairment, especially among APDS2 patients (45%). Immunologically, hypogammaglobulinemia with increased IgM levels was frequent. B-cell lymphopenia, worsening with age, and expansion of transitional B cells were the main B-cell alterations. A reduction in the frequency of naïve $CD4^+$ and $CD8^+$ T cells with an increased frequency of effector/effector

memory CD8$^+$ T cells was reported. These first two important retrospective analyses of the disease illustrated clinical and immunological characteristics but did not address the dynamics of the disease evolution over time. Furthermore, although both reports showed that the majority of APDS patients receive supportive therapies in terms of immunoglobulin-replacement treatment (IGRT) or antimicrobial prophylaxes, data regarding immunosuppressive treatments were only reported for a limited number of patients. Here, we use an initial report from the European Society for Immunodeficiencies (ESID)-APDS prospective registry to address some of these questions.

METHODS

The ESID-APDS Registry: Goals and Design

The ESID is a not-for-profit association whose aim is to improve knowledge in the field of PIDs (www.esid.org). The ESID Registry is an international Internet-based database for basic epidemiological (level 1), and more extensive disease-specific (level 3) data on patients with PID. The APDS Registry is the first prospective level 3 project that was initiated to better define the natural history of patients with APDS. The study is carried out in accordance with the recommendations of Section 15 of the Code of Conduct of the General Medical Council of Baden-Württemberg, Germany. The protocol was approved by the Ethics committee of the University of Freiburg (IRB approval No. ESID registry: 493/14; IRB approval No. APDS registry: 458/15). All subjects gave written informed consent in accordance with the Declaration of Helsinki. The goals of the project are to characterize disease evolution over time, to establish prognostic factors and biomarkers, to assess the impact of various treatment strategies, and to identify patients who could be eligible for novel treatments and interventions. Entry into the database requires an initial retrospective documentation, followed by yearly prospective follow-ups. Because of required patient consent, deceased patients cannot be registered. Each patient is evaluated at entry for eligibility by one of the three chief investigators to ensure that only patients with functionally validated APDS-associated mutations are registered. The APDS registry is supported by the pharmaceutical companies Novartis, GlaxoSmithKline, and UCB UK, who financed development and maintenance of the online level 3-documentation-section for APDS as well as project management including ethics submission in all participating countries, data management, and quality controls.

RESULTS

Disease Manifestations and Their Evolution Over Time

By December 2017, 77 patients had been enrolled in the APDS Registry, 51 with APDS1, and 26 with APDS2. Detailed clinical and immunological information of 68 patients [39 of them not published in the cohort papers (5, 6)] from 59 unrelated families was available for this initial analysis. Forty-five of these 68 patients were diagnosed with APDS1 (43 with the E1021K and 2 with the C416R mutation) and 23 with APDS2 (all with mutations leading to skipping of exon 11). At the time of evaluation, living patients (65) had a mean age of 17.9 years (range 3–47 years). The main clinical features reported in APDS1 and APDS2 are summarized in **Figures 1A,B**. As in the previously reported cohorts, recurrent respiratory infections were by far the most frequent manifestation, occurring in 96% of the patients. Upper respiratory tract infections, otitis media, and sinusitis were the leading diagnoses, and, importantly, 59% of the patients had experienced at least one episode of pneumonia. Cumulative retrospective data highlight that the respiratory infections begin very early in life, with almost all patients being affected by the age of 15 (**Figure 1C**). The registry data confirmed the previously described (5, 6) high incidence of bronchiectasis (28 patients out of the 55 who underwent a CT-scan), which was documented early in life (age range: 2–39 years; mean: 11.2 years). As already suggested by a previous retrospective review of the literature (7), the majority of patients with bronchiectasis had APDS1 (24 patients out of the 40 who had a CT-scan). Abnormal lung function was noted in 17 out of 35 patients who performed these tests. Acute viral infections (with varicella and herpes simplex) as well as chronic viral infections/reactivations were frequently documented in APDS1 and APDS2 patients (**Figure 1A**). The most frequently reported chronic infection in both cohorts was Epstein–Barr virus infection (16/68). Among the non-respiratory bacterial infections, the most frequent was infectious lymphadenitis (14/68). Five patients suffered from chronic mucocutaneous candidiasis and three developed local infection following vaccination with bacillus Calmette–Guérin. Consistent with the two published cohorts, chronic non-neoplastic lymphoproliferation was reported in the majority of patients (87%). Persistent peripheral lymphoproliferation, splenomegaly, and lymphoid hyperplasia were frequent and they were often concomitantly reported in the same patients (**Figure 1D**). Across the cohort, lymphoproliferation occurred with later onset than respiratory infections (**Figure 1C**) but preceded gastrointestinal manifestations and the development of autoimmunity.

Benign lymphoproliferation may be difficult to distinguish from malignant disease, the risk of which is increased in APDS patients. Eight of the registry-documented patients (5 APDS1, 3 APDS2) developed lymphoma between the age of 11 and 25 years, including two patients with Hodgkin lymphoma, one of whom subsequently developed an intestinal diffuse large B-cell lymphoma. Six patients were diagnosed with non-Hodgkin lymphomas (two diffuse large B-cell lymphomas, one anaplastic lymphoma, one marginal zone lymphoma, two without detailed histologic information). Five patients achieved a complete remission on treatment, one patient achieved only a partial remission, one patient was still under treatment at the time of registration, while in the remaining case, the lymphoma was sadly fatal. One of these eight patients also had a benign ovarian serous cystadenoma. One patient developed a B-cell chronic lymphocytic leukemia at the age of 40 years. In addition to the established high incidence of hematological malignancy, 2 cases of solid organ malignancy or pre-malignancy were noted: one case of ductal breast carcinoma-*in situ* (diagnosed in an APDS2 patient at the

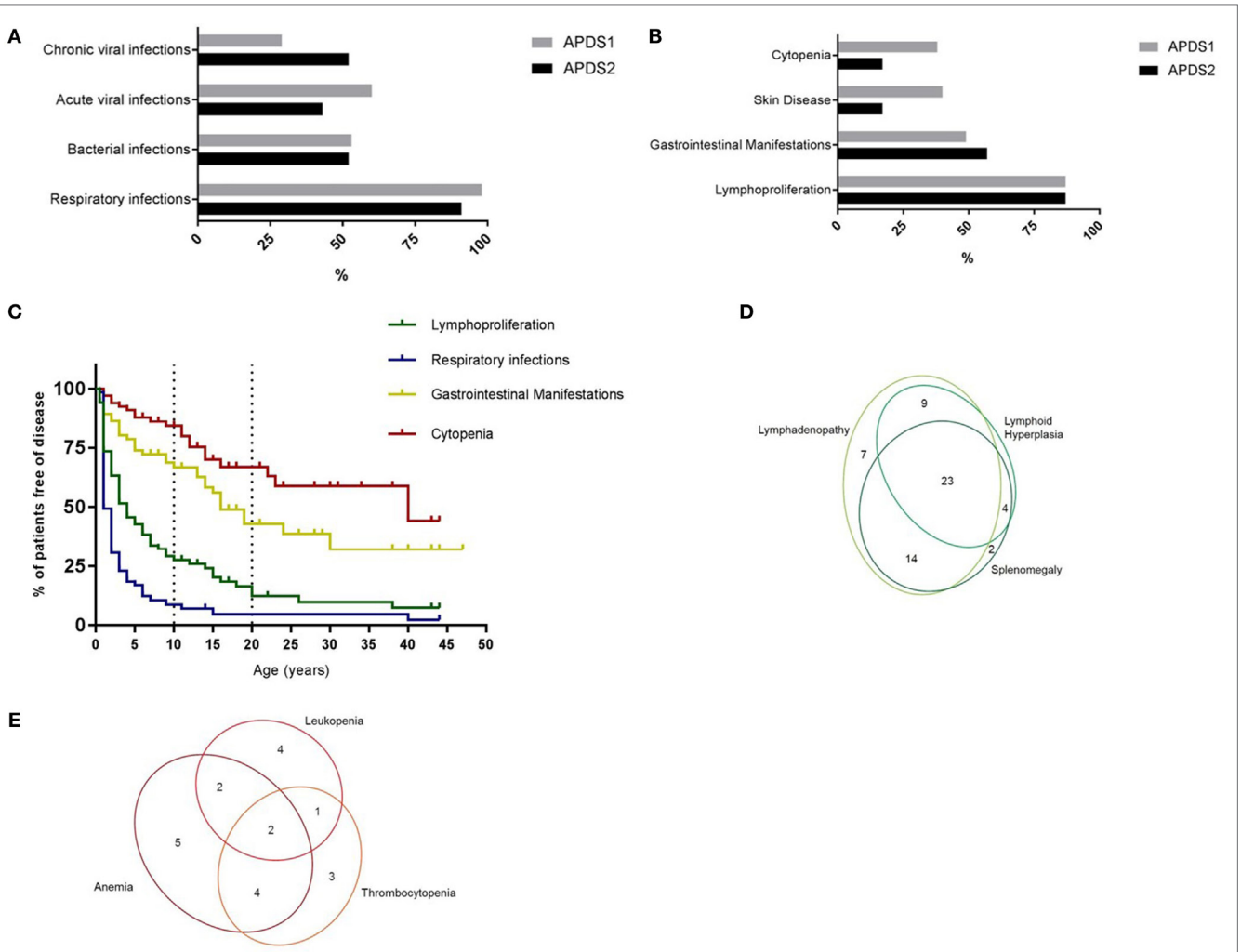


FIGURE 1 | **(A)** Incidence of infections in APDS1 and APDS2 patients. **(B)** Incidence of manifestations of immune dysregulation in APDS1 and APDS2 patients. **(C)** Evolution of disease manifestations over time. Information regarding age at onset available for: respiratory infections $n = 62/65$, lymphoproliferation $n = 59/59$, gastrointestinal manifestations $n = 33/35$, cytopenia $n = 20/21$ patients. **(D)** Diagram showing the different types of benign lymphoproliferative manifestations. **(E)** Diagram showing the different blood lineages affected in patients with cytopenias.

age of 33) and one case of rhabdomyosarcoma (diagnosed in an APDS1 patient at the age of 13).

Gastrointestinal manifestations were the third most frequent disease manifestation (51%) and across the cohort occurred before the other features of immune dysregulation, such as cytopenias or arthritis, but typically much later than the respiratory infections and the benign lymphoproliferation (**Figures 1B,C**). Small or large bowel inflammation was histologically confirmed in 17 patients, in 11 of them by the age of 10 years. Granulomas were reported in only one patient. Protracted diarrhea with no identified underlying cause was the second commonest reported gastrointestinal problem and was often severe enough to require hospitalization. Two patients were diagnosed with autoimmune hepatitis but no cases of sclerosing cholangitis were reported, in contrast with the two patients reported by Coulter et al. (5) and the two reported by Hartman et al. (8). Of note, 14/68 patients of the APDS-Registry cohort had eczema. Elkaim et al. (6) noted only three APDS2 patients with chronic eczema and no inflammatory skin disease was mentioned in the published APDS1 cohort (5). Cytopenias were the fourth major disease manifestation affecting around 30% of patients, usually later in life (**Figures 1B,C**) than the other main features and frequently affecting multiple blood lines (**Figure 1E**). The autoimmune origin of the cytopenias could be documented in the majority of the patients. Other autoimmune diseases were also reported, all occurring after the age of 10 years: two patients had autoimmune thyroiditis, three had arthritis, and three glomerulonephritis.

Concerning non-immunological manifestations, short stature (>2 SD) was reported in 11 patients, with a predominance of APDS2 individuals (8/13), consistent with previous reports (6, 7). Neurodevelopmental delay was diagnosed in three patients. Specific neuropsychiatric disorders were also reported: one patient had Asperger Syndrome, one had autism, one suffered from a mixed anxiety and depression disorder, and two other

patients had mild disorders of speech and language development. It is unclear if these findings reflect the impact of a severe physical illness or the impact of enhanced PI3Kδ signaling in the central nervous system.

Immunological Abnormalities

One of the objectives of the ESID-APDS registry is to collect immunological data prospectively. An initial analysis of the immunological profile in the registry cohort confirmed the already published T- and B-cell alterations. No clear difference between APDS1 and 2 was detected in the current cross-sectional data set. In the future, the longitudinal collection and analysis of these data will offer the possibility to explore associations between specific disease manifestations and immunological alterations, to evaluate the response of immunological alterations to the different types of treatment, and to establish the predictive value of immunological parameters for disease prognosis.

Current Therapies

Supportive therapy is a key component of the management of APDS patients. In the APDS-registry, 54 patients received antibiotic prophylaxis, whereas only eight received antifungal prophylaxis, which appears justified given the absence of reported invasive fungal infections. IGRT was administered in 44 patients (28/45 APDS1, 16/23 APDS2), was in general very well tolerated, and was started early in life (**Figure 2A**), mirroring the early presentation with respiratory infections. The majority of patients also received immunosuppressive treatments. Thirty-one patients received corticosteroids and 27 of them showed at least a partial clinical benefit. More than half

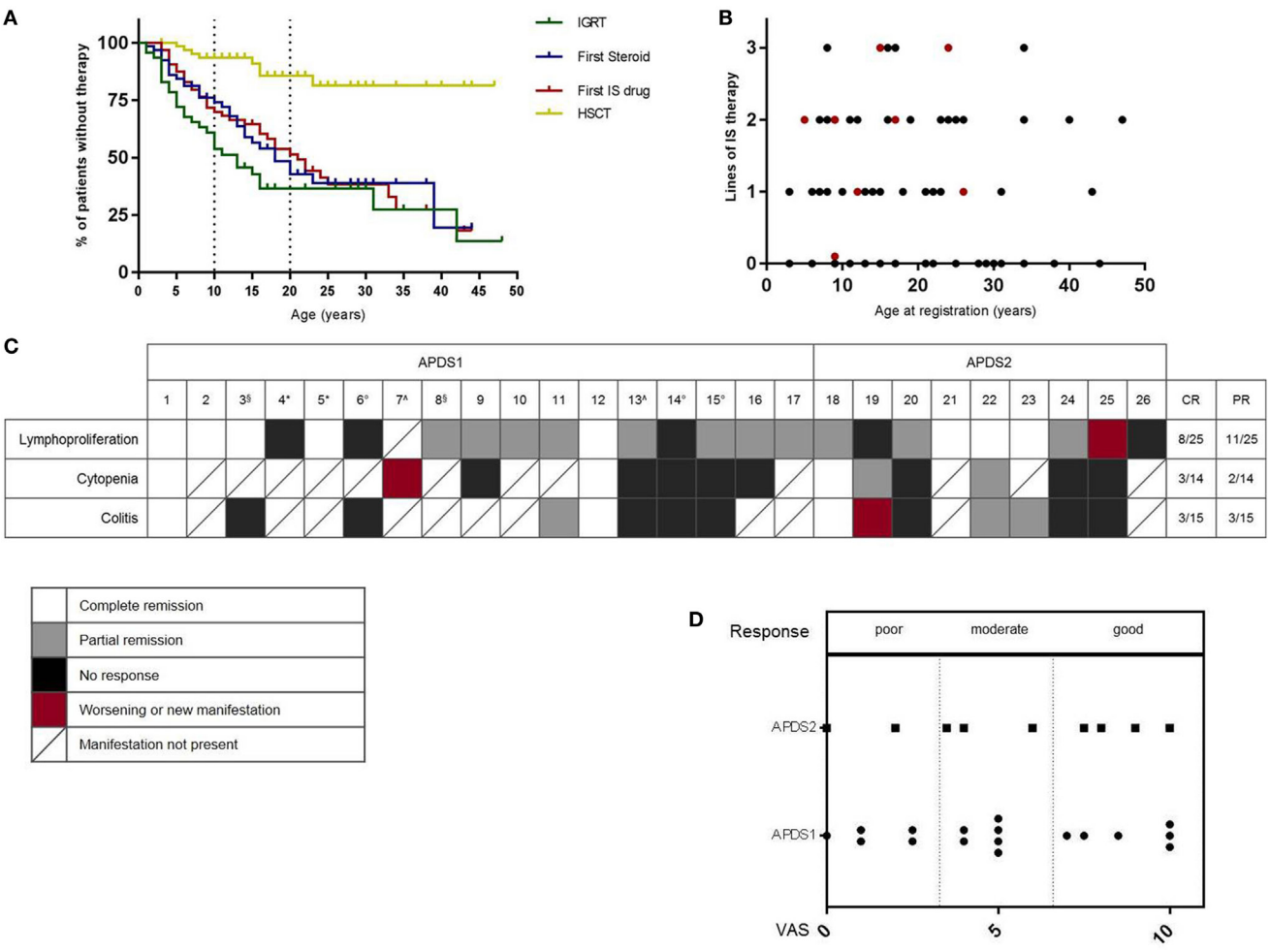


FIGURE 2 | **(A)** Use of treatment modalities over time. IGRT, immunoglobulin-replacement-treatment; IS, immunosuppressive drug; HSCT, hematopoietic stem cell transplantation. Information regarding age at first therapy available for: IGRT *n* = 28/44, steroid therapy *n* = 31/31, IS therapy *n* = 35/36, HSCT = 8/8. **(B)** Number of lines of immunosuppressive treatments (steroids, immunosuppressive drugs, rituximab) by the time of registration; red: patients who had undergone HSCT by the time of registration. **(C)** Response to rapamycin treatment. *White*: complete response; *gray*: partial response; *black*: no response; *red*: worsened or new manifestation; *boxes with a diagonal*: manifestation not present in this patient. CR, complete remission; PR, partial remission. Rapamycin stopped because of: *non-compliance, °inefficiency, ^side effects, §clinical trial. **(D)** Overall clinical benefit (Visual Analog Scale) according to physician's evaluation.

had received steroid treatment by the age of 20 (**Figure 2A**). Thirty-six patients received other immunosuppressive drugs, including azathioprine ($n = 1$), mycophenolate ($n = 3$), cyclosporine ($n = 5$), or rapamycin ($n = 27$); clinical benefit was reported in 28 of these patients. Rituximab was given to eight patients, with clinical benefit in all. **Figure 2B** illustrates the multiple lines of immunosuppressive treatments (steroids, immunosuppressive drugs, or rituximab), which had already been received by patients by the time of enrollment into the registry. Five patients underwent splenectomy (4 APDS1 and 1 APDS2) because of cytopenias or splenomegaly and 25 patients (12 APDS1 and 13 APDS2) underwent tonsillectomy (age range: 1–12 years), with clear benefit in only seven of them. The only available curative option is hematopoietic stem cell transplantation (HSCT) and the first experiences in this field have been published (9). Among the patients in the registry, 8/68 patients had undergone HSCT (7 APDS1 and 1 APDS2) by the time of registration (**Figure 2A**), with fatal outcome in one.

Rapamycin Therapy in APDS

Consistent with activation of mTOR signaling downstream of the activated PI3Kδ, patients with APDS may benefit from rapamycin (2). In the APDS2 cohort-paper (6), six patients had been treated with rapamycin, but the time of follow-up was too short to evaluate the response to treatment in four of them. Six of the patients in the reported APDS1 cohort (5) were treated with rapamycin for benign lymphoproliferation; five of them had a treatment response, but in one case, the therapy was stopped due to side effects. Additional case reports of rapamycin therapy have also been published (7, 10). In the ESID-APDS-registry cohort, rapamycin was the most frequently used immunosuppressive drug. We, therefore, decided to evaluate the experience with rapamycin (Sirolimus) in 26 patients (1 patient was not included because treatment was started and terminated before the diagnosis of APDS and the response to therapy was not well documented), 17 with APDS1, and 9 with APDS2. The main indications for treatment were lymphoproliferation, colitis, and/or cytopenia. Physicians were asked to judge the degree of severity of each manifestation as mild, moderate, or severe at the start of therapy, following 3–6 months of treatment and at the latest follow-up (average time of therapy monitoring: 1.6 years). Overall response judged by the physician visual analog scale was good in 10, moderate in 9, and poor in 7 (**Figure 2D**). Lymphoproliferation showed the best response (8 complete, 11 partial, 6 no remission), while bowel inflammation (3 complete, 3 partial, and 9 no remission) and cytopenia (3 complete, 2 partial, 9 no remission) responded less well, as shown in **Figure 2C**. Notably, of the eight patients who were on steroids at initiation of treatment with rapamycin (No. 1, 7, 9, 13, 19, 22, 23, 25), seven were able to stop steroids and one (No. 25) was able to reduce the dose. Two patients (No. 4, 5) stopped therapy because of poor compliance, in three cases (No. 6, 14, 15), the reason for cessation was lack of efficacy. Two patients (No. 7, 13) suffered from side effects (severe headaches, anorexia, renal toxicity) that led to the complete interruption of the treatment, whereas in three cases, the therapy was paused because of side effects (aphthous ulcers, liver toxicity, renal toxicity) but could be started again. Two patients (No. 3, 8) stopped despite efficacy because of enrollment in a clinical trial with PI3Kδ inhibitors. In two other individuals (No. 11, 12), treatment was interrupted after prolonged usage; in one patient (No. 20), this was due to the patient planning for pregnancy and, in another (No. 19), it followed the development of a lymphoma. Of note, three patients (No. 14, 18, 25) received also Rituximab during and one (No. 10) shortly before the treatment with rapamycin. One patient (No. 20) concomitantly received Adalimumab because of arthritis. Interestingly, some patients did not show any relevant alterations in the disease manifestations after 3–6 months of therapy but did show either improvement (No. 1, 8, 10, 18, 22, 23) or worsening (No. 6, 14, 19) after a longer period of observation on treatment (about 2 years).

DISCUSSION

We present an initial analysis of the prospective ESID-APDS registry, a longitudinal cohort study of patients with APDS1 and APDS2. This overview expands the known information regarding the clinical manifestations of the disease by adding the aspect of the evolution of the features over time. The emerging picture is the one of a PID characterized by the early occurrence of respiratory infections (mostly upper respiratory infections), followed by the development of chronic benign lymphoproliferation and subsequently other features of immune dysregulation, in particular, gastrointestinal manifestations and autoimmune cytopenias. We again noted the higher incidence of bronchiectasis in APDS1 compared with APDS2 patients; however, the numbers remain small and differences in CT uptake cannot be excluded as a confounder. However, this observation may stimulate future studies of the roles of the *PIK3CD* and *PIK3R1* genes and their proteins in the respiratory system. In the future, further analysis of the clinical evolution in this prospective cohort will allow better definition of long-term prognosis for this disease. In addition, the correlation of clinical features with the immunological abnormalities and their relationship with outcome parameters will help defining clinical and biological biomarkers of outcome.

The choice of treatment is a key issue in these patients who often present with severe concomitant manifestations not only of immunodeficiency but also of immune dysregulation. According to the registry, the combination of supportive therapy to prevent recurrent infections and the immunosuppressive treatment of immune dysregulation is often initiated early in life, with many patients undergoing multiple treatments. Rapamycin inhibits the biologically relevant downstream PI3K effector mTOR pathway, and it has been widely used with good efficacy in other PIDs, in particular, autoimmune lymphoproliferative syndrome (11, 12). Our interrogation of the ESID-APDS registry aligns with previous reports (7, 10) in suggesting that rapamycin reduces the severity of benign lymphoproliferative disease also in APDS. However, a less satisfactory response was documented regarding the non-lymphoproliferative manifestations, in particular, intestinal disease and cytopenias, which can be highly detrimental for the patients' quality of life. It is important to relate these registry results to the first results of targeted therapy with the PI3Kδ inhibitor leniolisib that have recently been published (13). In the first six patients, the drug showed an excellent control of the lymphoproliferation (6/6 patients) and in part also improved the cytopenias at the end of treatment (day 84). Three of the

six patients normalized their thrombocytopenia, one patient resolved his anemia, and three of four patients improved their lymphopenia, while there was no correction of the neutropenia observed in two patients; however, respiratory and gastrointestinal symptoms and outcomes were not reported in this study. Furthermore, our registry analysis highlighted that also colitis and skin disease can cause significant symptoms in these patients and should, therefore, be carefully evaluated in future clinical studies on novel therapies, particularly given previous reports of colitis associated with PI3K inhibitors (14). Longitudinal data capture on APDS patients in the ESID-APDS registry will be critical to observe the long-term benefits and/or side effects of these therapies, in particular, their effect on the incidence of lymphomas. It is noteworthy that one patient developed lymphoma while taking rapamycin. Another key question, where the registry will be helpful, is the question if and when to perform HSCT. The analysis of this question will profit from the principles established in the P-CID study, a prospective natural history study on profound combined immunodeficiency in which matched pairs of transplanted and non-transplanted patients with similar disease burden and immunological alterations are followed (15).

Finally, an attractive goal for the registry is to involve patients and their families directly in data acquisition. This could in the future allow collecting information about the quality-of-life of APDS patients, thus ameliorating the evaluation of the disease burden in all its complexity. In summary, thanks to the collaborative work of the participating centers, the ESID-APDS registry will comprise a valuable resource for physicians dealing with this disease and for shaping future research questions.

ETHICS STATEMENT

The study is carried out in accordance with the recommendations of Section 15 of the Code of Conduct of the General Medical Council of Baden-Württemberg, Germany. The protocol was approved by the Ethics committee of the University of Freiburg (IRB approval No. ESID registry: 493/14; IRB approval No. APDS registry: 458/15). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

MM collected analyzed and interpreted data and wrote the manuscript. HA, AA, ALA, OA, CB, SAB, FB, HB, MB, SOB, CC, ANDC, PC, MC, ANIC, FC, TC, LD, JE, SF, AF, MG, LH, MH, SJ, EK, AK, DK, BG, HL, NM, TM, FM, DM, AM, ON, BN, PO, AO, JP, CP, SP, JR, SS, ALS, ANS, SS, ASH, MS, PS, AUS, FS, WR, FT, JM, KW, AW, and PW repeatedly referred and registered patients. AN, GK and AU coordinated the registry. SR and RS provided the export data from the online-registry and gave informatic support. SK, ALC, and SE interpreted the data and wrote the manuscript. All the authors edited the manuscript.

ACKNOWLEDGMENTS

We highly appreciate the continuous support by our study nurse Henrike Ritterbusch. The registration of German patients is supported by pid-net (www.pid-net.org). We thank all the patients and families for their participation and trust.

FUNDING

This study was supported by the German Federal Ministry of Education and Research (BMBF 01E01303). The ESID-APDS registry is supported by the pharmaceutical companies Novartis, GlaxoSmithKline, and UCB UK.

REFERENCES

1. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
2. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
3. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923–8. doi:10.1172/JCI75746
4. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
5. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
6. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210–8.e9. doi:10.1016/j.jaci.2016.03.022
7. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kdelta syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27(6):640–4. doi:10.1111/pai.12585
8. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35(1):11–4. doi:10.1007/s10875-014-0109-1
9. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol* (2017) 139(3):1046–9. doi:10.1016/j.jaci.2016.09.040
10. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase delta syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
11. Klemann C, Esquivel M, Magerus-Chatinet A, Lorenz MR, Fuchs I, Neveux N, et al. Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome. *Haematologica* (2017) 102(2):e52–6. doi:10.3324/haematol.2016.153411
12. Teachey DT, Greiner R, Seif A, Attiyeh E, Bleesing J, Choi J, et al. Treatment with sirolimus results in complete responses in patients with autoimmune

lymphoproliferative syndrome. *Br J Haematol* (2009) 145(1):101–6. doi:10.1111/j.1365-2141.2009.07595.x
13. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective "activated PI3Kdelta syndrome"-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
14. Greenwell IB, Ip A, Cohen JB. PI3K inhibitors: understanding toxicity mechanisms and management. *Oncology (Williston Park)* (2017) 31(11):821–8.
15. Speckmann C, Doerken S, Aiuti A, Albert MH, Al-Herz W, Allende LM, et al. A prospective study on the natural history of patients with profound combined immunodeficiency: an interim analysis. *J Allergy Clin Immunol* (2017) 139(4):1302–10.e4. doi:10.1016/j.jaci.2016.07.040

Conflict of Interest Statement: The APDS registry is supported by the pharmaceutical companies Novartis, GlaxoSmithKline, and UCB UK, who have financed development and maintenance of the online level 3 documentation section for APDS as well as project management including ethics submission in all participating countries, data management, and quality controls. The financial support also allows some reimbursement of documentation activities for the participating centers. For those patients who have specifically agreed to this in the registry consent, anonymized data from the APDS Registry are available to industry partners for their purposes (e.g., designing a drug trial or data submission for regulatory approvals).

frontiers in Immunology

MINI REVIEW
published: 19 March 2018
doi: 10.3389/fimmu.2018.00575

Molecular Mechanisms of Human Disease Mediated by Oncogenic and Primary Immunodeficiency Mutations in Class IA Phosphoinositide 3-Kinases

*Gillian L. Dornan and John E. Burke**

Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC, Canada

The signaling lipid phosphatidylinositol 3,4,5, trisphosphate (PIP_3) is an essential mediator of many vital cellular processes, including growth, survival, and metabolism. PIP_3 is generated through the action of the class I phosphoinositide 3-kinases (PI3K), and their activity is tightly controlled through interactions with regulatory proteins and activating stimuli. The class IA PI3Ks are composed of three distinct p110 catalytic subunits (p110α, p110β, and p110δ), and they play different roles in specific tissues due to disparities in both expression and engagement downstream of cell-surface receptors. Disruption of PI3K regulation is a frequent driver of numerous human diseases. Activating mutations in the *PIK3CA* gene encoding the p110α catalytic subunit of class IA PI3K are frequently mutated in several cancer types, and mutations in the *PIK3CD* gene encoding the p110δ catalytic subunit have been identified in primary immunodeficiency patients. All class IA p110 subunits interact with p85 regulatory subunits, and mutations/deletions in different p85 regulatory subunits have been identified in both cancer and primary immunodeficiencies. In this review, we will summarize our current understanding for the molecular basis of how class IA PI3K catalytic activity is regulated by p85 regulatory subunits, and how activating mutations in the PI3K catalytic subunits *PIK3CA* and *PIK3CD* (p110α, p110δ) and regulatory subunits *PIK3R1* (p85α) mediate PI3K activation and human disease.

Keywords: primary immunodeficiency, oncogenic mutations, phosphoinositides, phosphoinositide 3-kinase, PIK3R2, PIK3R1, PIK3CA, PIK3CD

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Satoshi Okada, Hiroshima University, Japan
Anita Chandra, University of Cambridge, United Kingdom

***Correspondence:**
John E. Burke
jeburke@uvic.ca

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *16 January 2018*
Accepted: *07 March 2018*
Published: *19 March 2018*

Citation:
Dornan GL and Burke JE (2018) Molecular Mechanisms of Human Disease Mediated by Oncogenic and Primary Immunodeficiency Mutations in Class IA Phosphoinositide 3-Kinases. Front. Immunol. 9:575. doi: 10.3389/fimmu.2018.00575

INTRODUCTION

Phosphoinositide 3-kinases (PI3Ks) are essential mediators of signaling downstream of cell-surface receptors and play essential roles in numerous cellular processes, including growth, metabolism, and differentiation (1). PI3Ks generate the lipid phosphatidylinositol 3,4,5, trisphosphate (PIP_3), which recruits signaling proteins containing PIP_3 binding domains. Many signaling proteins are activated by PIP_3, including AGC family Ser/Thr kinases, TEK family tyrosine kinases, and modulators of Ras superfamily GTPases, specifically Guanine nucleotide exchange factors (GEFs), and GTPase activating proteins (2). One of the most well studied

PIP_3 effectors is the AGC protein kinase Akt, which plays key roles in regulating growth and metabolism (3).

The class IA PI3Ks are composed of three p110 catalytic subunits (p110α, p110β, and p110δ), which form an obligate constitutive heterodimer (4) with one of five p85-like regulatory subunits (p85α, p85β p55α, p50α, and p55γ). Class IA PI3Ks are activated downstream of receptor tyrosine kinases (RTKs) and other tyrosine phosphorylated receptors/adaptors, G-protein-coupled receptors (GPCRs), and Ras superfamily GTPases. The p110α and p110β catalytic subunits are ubiquitously expressed, while the p110δ and p110γ subunits share a more restricted immune cell-specific expression profile (5). Knockin genetic models and isoform-selective inhibitors have revealed the essential roles of specific PI3K isoforms, including p110α in insulin and growth factor signaling (6, 7), and p110δ and p110γ in mediating immune cell development and function (8–11).

Due to this fundamental role in a plethora of vital functions, the misregulation of PI3K signaling occurs in various human diseases (2). Underlying the importance of maintaining regulated levels of PI3K activity, disease can be caused by overactive and inactive PI3K signaling. The first clinically approved therapeutic Idelalisib specifically targeting p110δ was FDA approved in 2014 and has shown efficacy in the treatment of B cell-related malignancies (12–16). Even though p110δ inhibitors have shown promise as therapeutics, careful consideration of unexpected complications is critical, as long-term inhibition of p110δ signaling can lead to B cell genomic instability through an Activation-induced cytidine deaminase (AID)-dependent mechanism (17).

Mutations in both catalytic and regulatory subunits frequently activate lipid kinase activity through modification/disruption of inhibitory interfaces between the two subunits. Fundamental to understanding how mutations in different catalytic and regulatory subunits modify PI3K signaling in different cells/tissues is understanding how class IA PI3Ks are regulated by their p85 regulatory subunits, and how they are activated downstream of different activating stimuli. This review will specifically focus on the molecular mechanisms of how class IA PI3Ks are regulated, and how both oncogenic and primary immunodeficiency mutations/deletions in catalytic and regulatory subunits lead to disease.

CLASS IA PI3K REGULATION

The class IA regulatory subunits have three key roles: they stabilize the p110 catalytic subunit, they inhibit p110 catalytic activity, and they allow for the activation of activity downstream of proteins containing phosphorylated YXXM motifs through engagement of p85 SH2 domains (18, 19). While class IA catalytic subunits require a regulatory subunit for stability, the p85 subunits have been postulated to exist alone and can mediate cellular functions free of p110 (20, 21).

Both the class IA PI3K p110 catalytic subunit and p85 regulatory subunit are large, dynamic multi-domain proteins (**Figures 1A–C**). p110 is composed of an adaptor-binding domain (ABD), which interacts with p85, a Ras-binding domain (RBD), which mediates interaction with Ras superfamily GTPases, a C2 domain, a helical domain, and a bi-lobed kinase domain, composed of an N-lobe and a C-lobe. All class IA regulatory subunits contain two Src homology 2 domains [referred to as nSH2 and cSH2 to denote N-terminal and C-terminal] connected by a coiled-coil domain known as the inter SH2 (iSH2). The main interface holding the PI3K heterodimer together is the very tight interaction of the ABD of p110 with the iSH2 domain of p85 (22). Both p85α and p85β subunits also contain a Src homology 3 domain (SH3) and a bar cluster region homology domain (BH). A comparison of class IA PI3K domain organization compared with other SH2 containing protein kinases including Src family and Syk family kinases reveals the large size and complexity of the p110/p85 complex relative to other signaling kinases (**Figures 1C,D**).

Biochemical/biophysical studies have informed the molecular mechanism of how regulatory subunits bind and inhibit the different p110 catalytic subunits (18, 19, 22, 24, 25, 29–35). A number of inter- and intra-subunit interactions mediate inhibition of each of the class IA catalytic subunits (annotated on the domain schematic in **Figure 1B**). In all class IA PI3Ks, the ABD domain forms an intra-subunit inhibitory contact with the N-lobe of the kinase domain (32). How the ABD interacts with kinase domain is mediated by the ABD–RBD linker, which packs against the ABD. The C2 domain of p110 forms an inhibitory contact with the iSH2 domain of p85 regulatory subunits. Intriguingly, different p110 subunits have diverse capabilities to be inhibited by this interaction, with p110β being less inhibited by the C2–iSH2 interaction (36), compared with p110α and p110δ.

The nSH2 forms inhibitory interactions with the C2, helical, and C-lobe of all class IA p110 catalytic subunits (22, 24, 29, 30). The C-terminal SH2 domain, which interacts with the C-lobe of the kinase domain, only inhibits p110β (25) and p110δ (30). This interaction cannot occur in p110α due to a loop extension that sterically prevents this inhibitory interaction. Intriguingly, the nSH2 and cSH2 domains have different inhibitory interfaces with p110, with the nSH2 interacting with p110 through its pY binding site, and the cSH2-p110 interface not directly involving the pY binding site. Upon interaction with pYXXM motifs in phosphorylated receptors and their adaptors, the nSH2 and cSH2 interfaces with p110 are disrupted. Different regulation of class IA PI3Ks by their regulatory subunits has important functional implications for how they can be activated by different activating stimuli.

SIGNALING INPUTS

The ability of PI3K isoforms to mediate signaling in different tissues is a balance between differential PI3K expression and their unique ability to be activated by GPCRs, Ras superfamily GTPases, and phosphorylated receptors/adaptors. All class IA isoforms can be activated by proteins containing phosphorylated YXXM motifs, as this leads to SH2-mediated recruitment of regulatory subunits, and disruption of SH2 inhibitory contacts (22, 29, 30, 35) with the p110 catalytic subunits. p110α is more sensitive to activation downstream of a phosphopeptide derived from platelet-derived growth factor receptor than either p110β or p110δ *in vitro* (29), and this is likely due to the absence

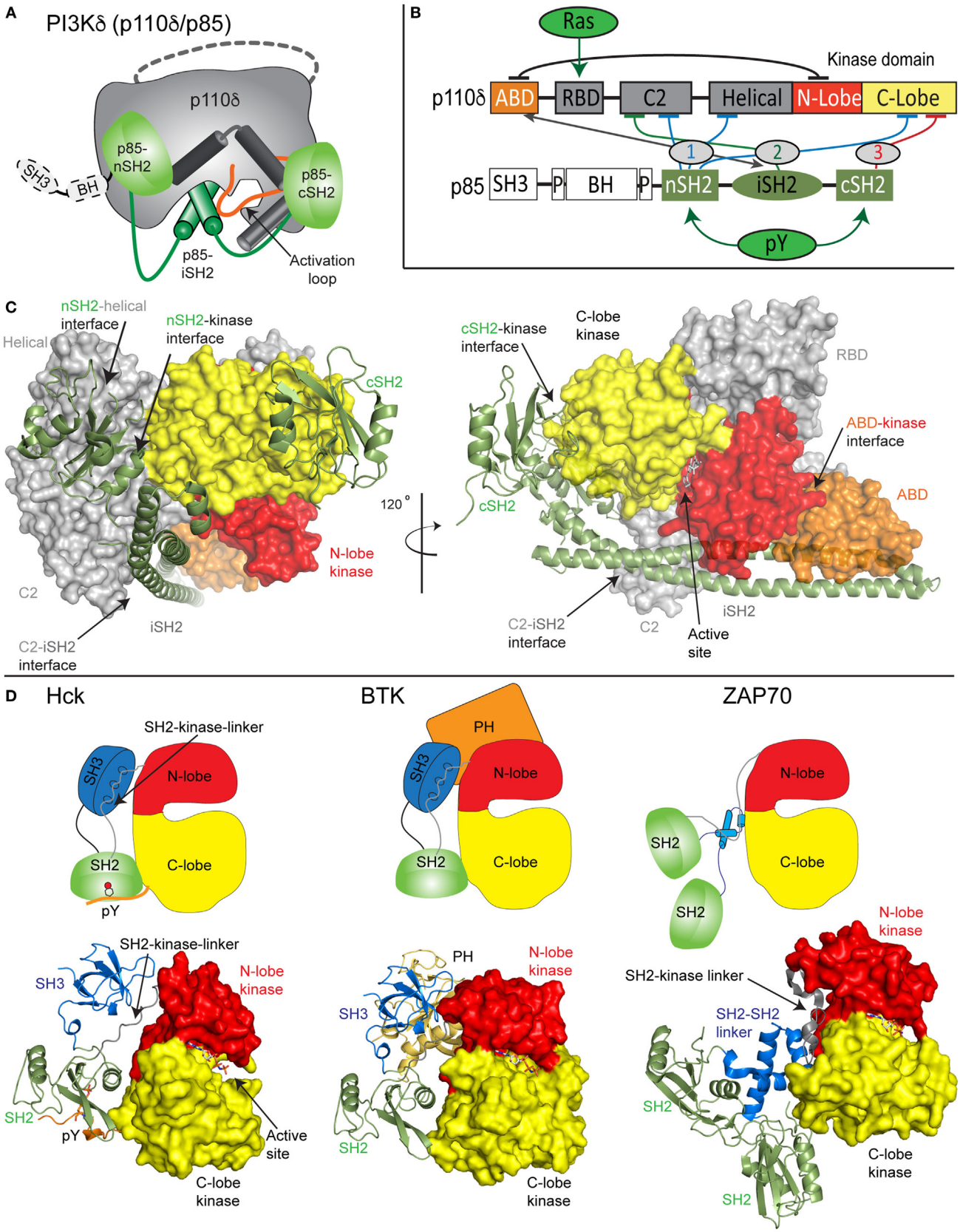


FIGURE 1 | Continued

FIGURE 1 | Model of class IA phosphoinositide 3-kinase (PI3K) complex of p110δ/p85α and comparison with other SH2-regulated kinases. **(A)** Cartoon model of the complex of p110δ/p85α, with key features annotated. **(B)** Domain schematic of p110δ and p85α with binding interfaces indicated by the double-sided arrow and inhibitory interfaces indicated by the numbered lines. Activators of PI3K [Ras and phosphorylated receptors (pY)], and their sites of interaction are indicated. The cSH2 domain of p85 only inhibits the p110β and p110δ isoforms and does not inhibit p110α. **(C)** Structural model of p110δ/p85α generated from multiple structures (23–25). The domains are colored according to the scheme shown in panel **(B)**. The p110 catalytic subunit is shown as a surface, and the p85 regulatory subunit shown in cartoon representation. Inhibitory intra- and inter-domain interfaces are annotated, and an inhibitor bound in the active site is shown in sticks. **(D)** Structures of the inhibited forms of SH2-regulated protein kinases involved in immune signaling, Hck (26), BTK (27), and ZAP70 (28) are shown along with cartoon representations indicating how SH2 domains inhibit kinase activity. This shows the various mechanisms of how SH2 domains can inhibit kinase activity, and the key differences in how p85 SH2 domains inhibit PI3K lipid kinase activity.

of the cSH2 inhibitory interface, which makes the cSH2 more accessible to interact with pYXXM motifs. *In vivo* evidence in support of free SH2 domains being more available to pYXXM motifs is that the E545K mutant of p110α, which disrupts the nSH2–helical interface (described in the following section), is more readily recruited to phosphorylated insulin receptor substrate proteins (37).

Class IA PI3Ks are activated downstream of the Ras superfamily of GTPases through interactions with the RBD domain present in p110 catalytic subunits (38, 39). The Ras superfamily is large and diverse, composed of five main families (Ras, Rho, Rab, Ran, and Arf) (40). The PI3K isoforms are differentially activated downstream of Ras superfamily members (39, 41), with p110α and p110δ being activated downstream of Ras family GTPases, and p110β being activated downstream of Rho family GTPases. Ras activates PI3K through enhanced membrane interaction, with Ras activation being strongly synergistic with activation downstream of phosphorylated receptors (42, 43). Mutant p110α deficient in its ability to be activated by Ras leads to decreased oncogenic transformation, tumor maintenance, and angiogenesis downstream of mutant Ras (44–46).

Class IA PI3Ks can synergize direct and indirect inputs downstream of specific upstream stimuli. p110β is unique in being activated downstream of phosphorylated receptors/adaptors, GPCRs, and Rho family GTPases (47). The ability of p110β to integrate signals from RTKs and GPCRs is critical in its signaling role in myeloid cells (48). p110α is sensitive to activation downstream of insulin receptors due to it being both directly and indirectly activated through RTK-mediated activation of Ras. The ability of different isoforms to be activated downstream of different upstream stimuli plays a key role in determining the capability for activating somatic point mutations to mediate human disease.

MUTATIONS OF *PIK3CA*, *PIK3CD*, AND *PIK3R1* IN CANCER, DEVELOPMENTAL DISORDERS, AND PRIMARY IMMUNODEFICIENCIES

Class IA PI3Ks in Cancer and Developmental Disorders

The importance of PI3K activity being properly regulated in human health is underscored by a vast array of human diseases caused by mutations in class IA PI3Ks (mutations in class I PI3Ks in immune disorders and developmental disorders are summarized in Table S1 in Supplementary Material). Mutations can arise in the germline *de novo* or be inherited in an autosomal dominant or recessive manner, and can also arise somatically in specific tissues. Somatic point mutation frequency in cancer in both *PIK3CA* (49) and *PIK3R1* (20, 50) is indicated in **Figures 2C,D**. Intriguingly, *de novo* germline and postzygotic, somatic mosaic mutations in similar locations in *PIK3CA* and *PIK3R2* (p85β) also lead to overgrowth and developmental disorder syndromes (51–56), revealing that the same mutant can lead to cancer and/or developmental disorders. There are two hotspot regions in *PIK3CA* located at the nSH2–helical interface (E542K and E545K) and the C-terminus of the kinase domain (H1047R) involved in membrane binding (**Figures 2B,C**). However, in addition, there are numerous rare mutations distributed throughout the primary sequence, primarily localized at the ABD–kinase interface, ABD–RBD linker, C2–iSH2 interface, and the regulatory arch of the kinase domain which is situated over the active site (**Figures 2A,B**). Rare mutations activate lipid kinase activity, induce oncogenic transformation (31, 57, 58), and are found in endometrial cancers (59).

Mutants located at the ABD–kinase, C2–iSH2, and nSH2–helical interfaces activate lipid kinase activity through disruption of these inhibitory contacts. Intriguingly, there appears to be allosteric long range coupling between these sites, as disruption of the C2–iSH2 interface also leads to disruption of the ABD–kinase interface (31). Mutations within the regulatory arch (a region composed of the two most C-terminal helices, kα10 and kα11, residues 1017–1049) appear to work through a separate mechanism, where conformational changes induced by these mutations drive increased membrane recruitment (31, 60). The regulatory arch lies directly over the active site of the enzyme (**Figure 2A**). Different mutations induce oncogenic transformation through different mechanisms, with the H1047R mutant requiring p85-mediated recruitment to RTKs, and no longer requiring Ras for transformation, while the E545K mutation still requires input from Ras, and no longer requires p85-mediated RTK activation (58). This is consistent with the putative mechanism of Ras activation, where Ras drives membrane recruitment, and H1047R evades this requirement due to enhanced membrane binding (42, 43).

Somatic cancer-associated point mutations in *PIK3R1* are similarly localized at regulatory interfaces (**Figures 2B–D**), with the most frequent mutation occurring at the C2–iSH2 interface (N564K/D). These mutants primarily activate PI3K signaling through p110α activation (50, 61, 62). Loss of p85α is also a driver of cancer as it acts as a tumor suppressor, and oncogenic transformation due to loss of p85α is also driven by p110α (63). Several deletions/truncations identified in *PIK3R1* also can

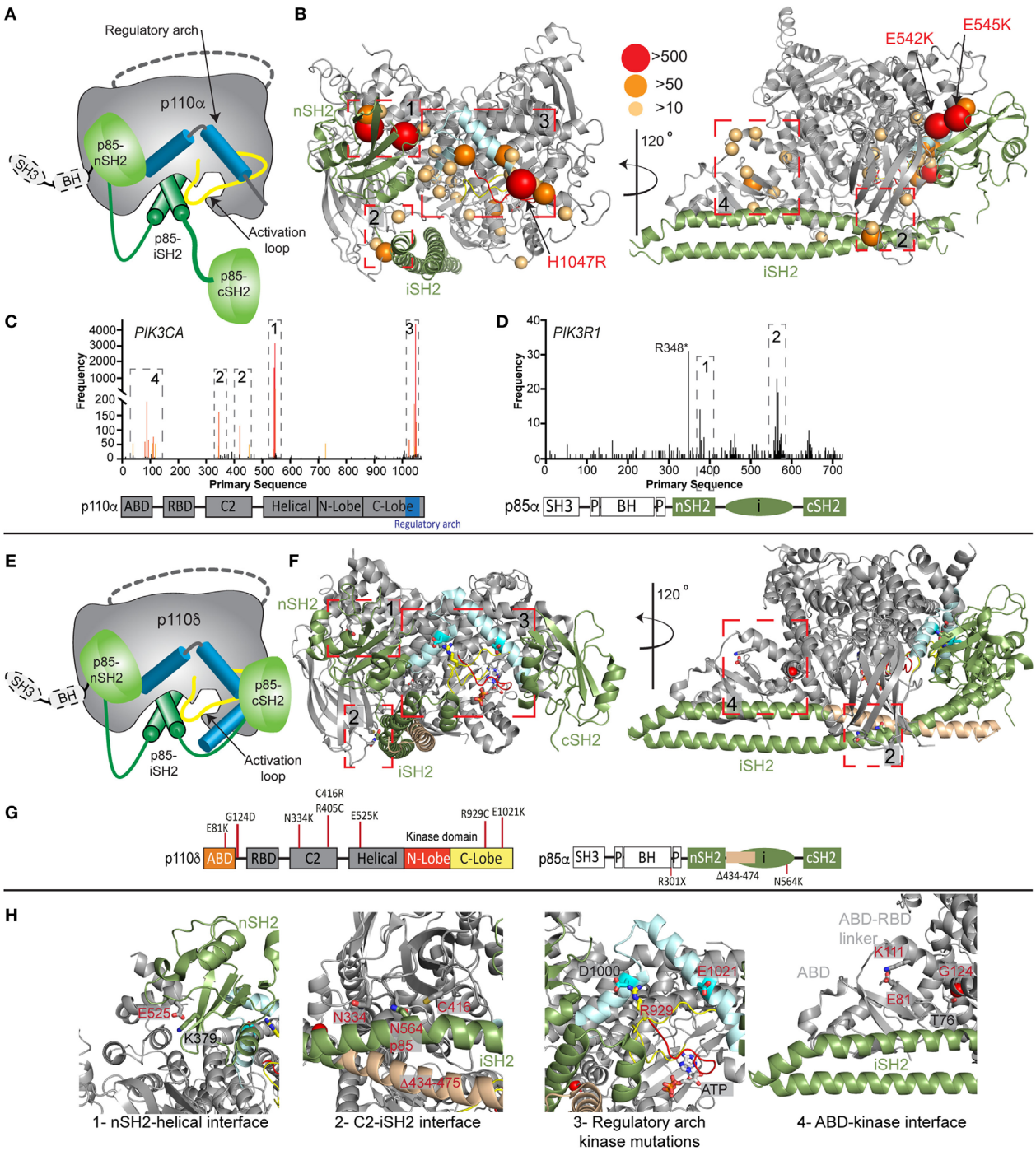


FIGURE 2 | Oncogenic and primary immunodeficiency mutations in *PIK3CA*, *PIK3CD*, and *PIK3R1*. **(A)** Cartoon schematic of the complex between p110α and p85α with key regulatory features annotated. **(B)** The locations of oncogenic mutations in *PIK3CA* are shown on a structural model of p110α and p85α (24), with the frequency of mutations annotated according to the legend [frequency derived from the Catalogue of Somatic Mutations in Cancer (COSMIC), http://cancer.sanger.ac.uk/cosmic]. The proteins are colored according to the cartoon in panel **(A)**. Regulatory interfaces [N-terminal SH2 domain (nSH2)–helical, C2–inter SH2 (iSH2), regulatory arch, and adaptor binding domain (ABD)–kinase] are boxed and numbered. Boxed regions 1–4 represent mutation hotspots in key regulatory regions. These are enlarged in panel **(H)** in the context of patient mutations in p110δ and p85α. **(C,D)** Frequency of somatic mutations in *PIK3CA* and *PIK3R1* shown on the primary sequence, with the domain schematic indicated below. The locations boxed on the structure in panel **(B)** are also indicated on primary sequence. **(E)** Cartoon schematic of the complex between p110δ and p85α with key regulatory features annotated. **(F)** The locations of primary immunodeficiency mutations in *PIK3R1* are shown on a structural model of p110δ and p85α (23). Boxed regions 1–4 represent mutation hotspots in key regulatory regions. These are enlarged in panel **(H)** in the context of patient mutations in p110δ and p85α. **(G)** Domain schematic of p110δ and p85α with locations of immune-linked mutations in *PIK3CD* and *PIK3R1* indicated. **(H)** Zoom in on molecular details of activating phosphoinositide 3-kinase (PI3K) delta syndrome mutations in p110δ and p85α, focused on the regulatory interfaces boxed in panel **(F)**, with all mutated residues and their interacting residues shown as sticks.

mediate oncogenic transformation through different mechanisms. Truncations at the C-terminus of the iSH2 domain can still interact with p110 subunits and disrupt inhibitory contacts (62), leading to increased PI3K activity. Intriguingly, oncogenic truncations also occur N-terminal to the iSH2 domain, and they are unable to bind p110 subunits. These truncations are proposed to function through modification of free p85 interactions with binding partners (20, 21, 64), including the antagonist of PI3K signaling, the phosphatase PTEN.

Mutations in *PIK3R1* leading to decreased PI3K signaling are also found in patients with developmental disorders, with autosomal-dominant or *de novo* mutations in the cSH2 (R649W, K653*, and Y657*) leading to insulin resistance, and dramatically decreased PI3K signaling (65–71). This condition is defined as SHORT syndrome (Short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly, and teething delay) and is caused by the inability of the cSH2 domain to interact with phosphorylated RTKs, as mutation of R649 disrupts the FLVR motif critical for SH2 binding to phosphorylated pYXXM motifs.

Class IA PI3Ks in Primary Immunodeficiencies

Activating, autosomal-dominant and *de novo* mutations in *PIK3CD* and *PIK3R1* have been discovered in patients with primary immunodeficiencies, and this condition is called activating PI3K delta syndrome (APDS), which is also referred to as PASLI (p110 delta activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency). Mutations in *PIK3CD*, referred to as APDS1, are found in similar locations to oncogenic mutations in p110α, with mutations discovered at the ABD (E81K), ABD–RBD linker (G124D), C2–iSH2 interface (N334K, R405C, and C416R), nSH2–helical interface (E525K and E525A), and at the C-terminus of the kinase domain (R929C, E1021K, and E1025G) (**Figures 2E–H**) (72–86). Biochemical experiments have revealed, similar to p110α mutations, that activation occurs due to disruption of p85-mediated regulatory inputs and conformational changes that promote membrane binding (83, 87). The most prevalent mutation in APDS1 is E1021K (similar location to H1047R in p110α); however, APDS mutations in p110δ are more frequently found distributed throughout the primary sequence compared with p110α (**Figures 2C,D,G**). In line with this observation, E1021K leads to a smaller increase in p110δ lipid kinase activity compared with H1047R p110α. It is likely that additional mutations in *PIK3CD* will be discovered that mimic previously discovered oncogenic mutations in *PIK3CA*, highlighting the need to sequence the entire *PIK3CD* gene in patients presenting with complex immunodeficiencies.

Mutations in *PIK3R1*, referred to as APDS2, have also been identified in a number of immunodeficiency patients, with the most frequent mutation resulting in a splice variant that removes exon 11 (resulting in a p85α with region 434–475 deleted, located at the N-terminus of the iSH2 domain) (88–92). *In vitro*, this deletion leads to increased activation of p110δ compared with p110α, and this is mediated through disruption of all p85 regulatory inputs for p110δ, and only partial disruption of p85 regulatory inputs for p110α (87). This mutant may decrease protein stability of p110 subunits, and there have been reports of these patients having symptoms consistent with both SHORT syndrome and APDS (92, 93). This may be due to increased p110δ signaling, and decreased p110α signaling caused by decreased stability of p110α. Activating point mutations in the iSH2 domain of *PIK3R1* at the C2–iSH2 interface (N564K) also cause APDS2 symptoms (86). This mutant is also found in solid tumors, and it appears in certain situations it can drive p110α-mediated oncogenesis or drive p110δ-mediated immunodeficiency. Loss of function mutations in *PIK3R1* also occur in immune disorders, with patients identified with autosomal recessive nonsense mutations in *PIK3R1* (W298*, R301*) leading to agammaglobulinemia, and severe defects in B-cell development (94, 95).

CONCLUSION

Tremendous advances in our understanding of PI3K structure, function, and regulation have occurred in the last decade. Detailed cellular and mice studies have revealed unexpected mechanisms of how PI3Ks are activated. The discovery of patients containing PI3K mutations in cancer, developmental disorders, and immunodeficiencies has revealed the key role of these enzymes in disease. PI3K-specific inhibitors have been developed, and the first PI3K inhibitor, selective for p110δ, has entered the clinic for treatment of blood cancers (14, 96), and other *PIK3CD*-specific inhibitors have showed efficacy in the treatment of APDS (97, 98). PI3K inhibitors may also be useful in targeting the tumor microenvironment (99), and in promoting tumor-specific immune responses (100). However, many PI3K inhibitors have failed in clinical trials for cancer, and there is still extensive work that needs to be done to understand PI3K signaling in human disease. For example, why do the same mutations occur in both cancer and immunodeficiencies, what are the other factors that predispose the same mutation toward a particular disease? Continued examination of PI3K signaling will be essential to fully understand its role in human disease and may reveal unexpected paths to novel therapeutic development.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing and editing of the manuscript.

FUNDING

JB is supported by funding from the Canadian Institutes of Health Research (New Investigator award and an Open Operating grant CRN-142393), the Cancer Research Society (Operating grant CRS-22641), and the Natural Sciences and Engineering Research Council of Canada (Discovery Grant NSERC-05218).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00575/full#supplementary-material.

REFERENCES

1. Burke JE, Williams RL. Synergy in activating class I PI3Ks. *Trends Biochem Sci* (2015) 40:88–100. doi:10.1016/j.tibs.2014.12.003
2. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell* (2017) 170:605–35. doi:10.1016/j.cell.2017.07.029
3. Manning BD, Toker A. AKT/PKB signaling: navigating the network. *Cell* (2017) 169:381–405. doi:10.1016/j.cell.2017.04.001
4. Geering B, Cutillas P, Nock G, Gharbi S, Vanhaesebroeck B. Class IA phosphoinositide 3-kinases are obligate p85-p110 heterodimers. *Proc Natl Acad Sci U S A* (2007) 104:7809–14. doi:10.1073/pnas.0700373104
5. Kok K, Geering B, Vanhaesebroeck B. Regulation of phosphoinositide 3-kinase expression in health and disease. *Trends Biochem Sci* (2009) 34:115–27. doi:10.1016/j.tibs.2009.01.003
6. Knight Z, Gonzalez B, Feldman M, Zunder E, Goldenberg D, Williams O, et al. A pharmacological map of the PI3-K family defines a role for p110-alpha in insulin signaling. *Cell* (2006) 125:733–47. doi:10.1016/j.cell.2006.03.035
7. Zhao JJ, Cheng H, Jia S, Wang L, Gjoerup OV, Mikami A, et al. The p110alpha isoform of PI3K is essential for proper growth factor signaling and oncogenic transformation. *Proc Natl Acad Sci U S A* (2006) 103:16296–300. doi:10.1073/pnas.0607899103
8. Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, et al. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* (2000) 287:1049–53. doi:10.1126/science.287.5455.1049
9. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kδ and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16:702–14. doi:10.1038/nri.2016.93
10. Okkenhaug K. Signaling by the phosphoinositide 3-kinase family in immune cells. *Annu Rev Immunol* (2013) 31:675–704. doi:10.1146/annurev-immunol-032712-095946
11. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* (2002) 297:1031–4. doi:10.1126/science.1073560
12. Brown JR, Byrd JC, Coutre SE, Benson DM, Flinn IW, Wagner-Johnston ND, et al. Idelalisib, an inhibitor of phosphatidylinositol 3 kinase p110δ, for relapsed/refractory chronic lymphocytic leukemia. *Blood* (2014) 123(22):3390–97. doi:10.1182/blood-2013-11-535047
13. Flinn IW, Kahl BS, Leonard JP, Furman RR, Brown JR, Byrd JC, et al. Idelalisib, a selective inhibitor of phosphatidylinositol 3-kinase-δ, as therapy for previously treated indolent non-Hodgkin lymphoma. *Blood* (2014) 123 (22):3406–13. doi:10.1182/blood-2013-11-538546
14. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med* (2014) 370:997–1007. doi:10.1056/NEJMoa1315226
15. Kahl BS, Spurgeon SE, Furman RR, Flinn IW, Coutre SE, Brown JR, et al. Results of a phase I study of idelalisib, a PI3Kδ inhibitor, in patients with relapsed or refractory mantle cell lymphoma (MCL). *Blood* (2014) 123(22): 3398–405. doi:10.1182/blood-2013-11-537555
16. Yang Q, Modi P, Newcomb T, Quéva C, Gandhi V. Idelalisib: first-in-class PI3K delta inhibitor for the treatment of chronic lymphocytic leukemia, small lymphocytic leukemia, and follicular lymphoma. *Clin Cancer Res* (2015) 21:1537–42. doi:10.1158/1078-0432.CCR-14-2034
17. Compagno M, Wang Q, Pighi C, Cheong T-C, Meng F-L, Poggio T, et al. Phosphatidylinositol 3-kinase δ blockade increases genomic instability in B cells. *Nature* (2017) 542:489–93. doi:10.1038/nature21406
18. Vadas O, Burke JE, Zhang X, Berndt A, Williams RL. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. *Sci Signal* (2011) 4:1–13. doi:10.1126/scisignal.2002165
19. Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. *Mol Cell Biol* (1998) 18:1379–87. doi:10.1128/MCB.18.3.1379
20. Cheung L, Hennessy B, Li J, Yu S, Myers A, Djordjevic B, et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discov* (2011) 1:170–85. doi:10.1158/2159-8290.CD-11-0039
21. Cheung LWT, Walkiewicz KW, Besong TMD, Guo H, Hawke DH, Arold ST, et al. Regulation of the PI3K pathway through a p85α monomer-homodimer equilibrium. *Elife* (2015) 4:e06866. doi:10.7554/eLife.06866
22. Miled N, Yan Y, Hon W-C, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science* (2007) 317:239–42. doi:10.1126/science.1135394
23. Heffron TP, Heald RA, Ndubaku C, Wei B, Augistin M, Do S, et al. The rational design of selective benzoxazepin inhibitors of the α-isoform of phosphoinositide 3-kinase culminating in the identification of (S)-2-((2-(1-isopropyl-1H-1,2,4-triazol-5-yl)-5,6-dihydrobenzo[f]imidazo[1,2-d][1,4]oxazepin-9-yl)oxy)propanamide (GDC-0326). *J Med Chem* (2016) 59:985–1002. doi:10.1021/acs.jmedchem.5b01483
24. Mandelker D, Gabelli SB, Schmidt-Kittler O, Zhu J, Cheong I, Huang C-H, et al. A frequent kinase domain mutation that changes the interaction between PI3Kalpha and the membrane. *Proc Natl Acad Sci U S A* (2009) 106:16996–7001. doi:10.1073/pnas.0908444106
25. Zhang X, Vadas O, Perisic O, Anderson KE, Clark J, Hawkins PT, et al. Structure of lipid kinase p110b/p85b elucidatesan unusual SH2-domain-mediated inhibitory mechanism. *Mol Cell* (2011) 41:567–78. doi:10.1016/j.molcel.2011.01.026
26. Sicheri F, Moarefi I, Kuriyan J. Crystal structure of the Src family tyrosine kinase Hck. *Nature* (1997) 385:602–9. doi:10.1038/385602a0
27. Wang Q, Vogan EM, Nocka LM, Rosen CE, Zorn JA, Harrison SC, et al. Autoinhibition of Bruton's tyrosine kinase (Btk) and activation by soluble inositol hexakisphosphate. *Elife* (2015) 4:1948. doi:10.7554/eLife.06074
28. Deindl S, Kadlecek TA, Brdicka T, Cao X, Weiss A, Kuriyan J. Structural basis for the inhibition of tyrosine kinase activity of ZAP-70. *Cell* (2007) 129:735–46. doi:10.1016/j.cell.2007.03.039
29. Burke JE, Williams RL. Dynamic steps in receptor tyrosine kinase mediated activation of class IA phosphoinositide 3-kinases (PI3K) captured by H/D exchange (HDX-MS). *Adv Biol Regul* (2013) 53:97–110. doi:10.1016/j.jbior.2012.09.005
30. Burke JE, Vadas O, Berndt A, Finegan T, Perisic O, Williams RL. Dynamics of the phosphoinositide 3-kinase p110δ interaction with p85α and membranes reveals aspects of regulation distinct from p110α. *Structure* (2011) 19:1127–37. doi:10.1016/j.str.2011.06.003
31. Burke JE, Perisic O, Masson GR, Vadas O, Williams RL. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110α (PIK3CA). *Proc Natl Acad Sci U S A* (2012) 109:15259–64. doi:10.1073/pnas.1205508109
32. Huang C, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu V, Kinzler K, et al. The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations. *Science* (2007) 318:1744–8. doi:10.1126/science.1150799
33. Vadas O, Burke JE. Probing the dynamic regulation of peripheral membrane proteins using hydrogen deuterium exchange-MS (HDX-MS). *Biochem Soc Trans* (2015) 43:773–86. doi:10.1042/BST20150065
34. Vadas O, Jenkins ML, Dornan GL, Burke JE. Using hydrogen-deuterium exchange mass spectrometry to examine protein-membrane interactions. *Methods Enzymol* (2017) 583:143–72. doi:10.1016/bs.mie.2016.09.008
35. Yu J, Wjasow C, Backer JM. Regulation of the p85/p110alpha phosphatidylinositol 3'-kinase. Distinct roles for the n-terminal and c-terminal SH2 domains. *J Biol Chem* (1998) 273:30199–203. doi:10.1074/jbc.273.46.30199
36. Dbouk HA, Pang H, Fiser A, Backer JM. A biochemical mechanism for the oncogenic potential of the p110{beta} catalytic subunit of phosphoinositide 3-kinase. *Proc Natl Acad Sci U S A* (2010) 107:19897–902. doi:10.1073/pnas.1008739107/-/DCSupplemental
37. Yang X, Turke AB, Qi J, Song Y, Rexer BN, Miller TW, et al. Using tandem mass spectrometry in targeted mode to identify activators of class IA PI3K in cancer. *Cancer Res* (2011) 71:5965–75. doi:10.1158/0008-5472.CAN-11-0445
38. Pacold ME, Suire S, Perisic O, Lara-Gonzalez S, Davis CT, Walker EH, et al. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. *Cell* (2000) 103:931–43. doi:10.1016/S0092-8674(00)00196-3
39. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, et al. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* (1994) 370:527–32. doi:10.1038/370527a0

40. Cherfils J, Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol Rev* (2013) 93:269–309. doi:10.1152/physrev.00003.2012
41. Fritsch R, de Krijger I, Fritsch K, George R, Reason B, Kumar MS, et al. RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell* (2013) 153:1050–63. doi:10.1016/j.cell.2013.04.031
42. Buckles TC, Ziemba BP, Masson GR, Williams RL, Falke JJ. Single-molecule study reveals how receptor and ras synergistically activate PI3Kα and PIP3 signaling. *Biophys J* (2017) 113:2396–405. doi:10.1016/j.bpj.2017.09.018
43. Siempelkamp BD, Rathinaswamy MK, Jenkins ML, Burke JE. Molecular mechanism of activation of class IA phosphoinositide 3-kinases (PI3Ks) by membrane-localized HRas. *J Biol Chem* (2017) 292:12256–66. doi:10.1074/jbc.M117.789263
44. Castellano E, Sheridan C, Thin MZ, Nye E, Spencer-Dene B, Diefenbacher ME, et al. Requirement for interaction of PI3-kinase p110α with RAS in lung tumor maintenance. *Cancer Cell* (2013) 24:617–30. doi:10.1016/j.ccr.2013.09.012
45. Gupta S, Ramjaun AR, Haiko P, Wang Y, Warne PH, Nicke B, et al. Binding of ras to phosphoinositide 3-kinase p110alpha is required for ras-driven tumorigenesis in mice. *Cell* (2007) 129:957–68. doi:10.1016/j.cell.2007.03.051
46. Murillo MM, Zelenay S, Nye E, Castellano E, Lassailly F, Stamp G, et al. RAS interaction with PI3K p110α is required for tumor-induced angiogenesis. *J Clin Invest* (2014) 124:3601–11. doi:10.1172/JCI74134
47. Dbouk HA, Vadas O, Shymanets A, Burke JE, Salamon RS, Khalil BD, et al. G protein-coupled receptor-mediated activation of p110β by Gβγ is required for cellular transformation and invasiveness. *Sci Signal* (2012) 5:ra89. doi:10.1126/scisignal.2003264
48. Houslay DM, Anderson KE, Chessa T, Kulkarni S, Fritsch R, Downward J, et al. Coincident signals from GPCRs and receptor tyrosine kinases are uniquely transduced by PI3Kβ in myeloid cells. *Sci Signal* (2016) 9:ra82–82. doi:10.1126/scisignal.aae0453
49. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* (2004) 304:554. doi:10.1126/science.1096502
50. Urick ME, Rudd ML, Godwin AK, Sgroi D, Merino M, Bell DW. PIK3R1 (p85α) is somatically mutated at high frequency in primary endometrial cancer. *Cancer Res* (2011) 71:4061–7. doi:10.1158/0008-5472.CAN-11-0549
51. Lindhurst MJ, Parker VER, Payne F, Sapp JC, Rudge S, Harris J, et al. Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA. *Nat Genet* (2012) 44:928–33. doi:10.1038/ng.2332
52. Mirzaa GM, Conti V, Timms AE, Smyser CD, Ahmed S, Carter M, et al. Characterisation of mutations of the phosphoinositide-3-kinase regulatory subunit, PIK3R2, in perisylvian polymicrogyria: a next-generation sequencing study. *Lancet Neurol* (2015) 14:1182–95. doi:10.1016/S1474-4422(15)00278-1
53. Nakamura K, Kato M, Tohyama J, Shiohama T, Hayasaka K, Nishiyama K, et al. AKT3 and PIK3R2 mutations in two patients with megalencephaly-related syndromes: MCAP and MPPH. *Clin Genet* (2014) 85:396–8. doi:10.1111/cge.12188
54. Orloff MS, He X, Peterson C, Chen F, Chen J-L, Mester JL, et al. Germline PIK3CA and AKT1 mutations in Cowden and Cowden-like syndromes. *Am J Hum Genet* (2013) 92:76–80. doi:10.1016/j.ajhg.2012.10.021
55. Rivière J-B, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, et al. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nat Genet* (2012) 44:934–40. doi:10.1038/ng.2331
56. Terrone G, Voisin N, Abdullah Alfaiz A, Cappuccio G, Vitiello G, Guex N, et al. De novo PIK3R2 variant causes polymicrogyria, corpus callosum hyperplasia and focal cortical dysplasia. *Eur J Hum Genet* (2016) 24:1359–62. doi:10.1038/ejhg.2016.7
57. Gymnopoulos M, Elsliger M-A, Vogt PK. Rare cancer-specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci U S A* (2007) 104:5569–74. doi:10.1073/pnas.0701005104
58. Zhao L, Vogt P. Helical domain and kinase domain mutations in p110-alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A* (2008) 105:2652–7. doi:10.1073/pnas.0712169105
59. Rudd ML, Price JC, Fogoros S, Godwin AK, Sgroi DC, Merino MJ, et al. A unique spectrum of somatic PIK3CA (p110alpha) mutations within primary endometrial carcinomas. *Clin Cancer Res* (2011) 17:1331–40. doi:10.1158/1078-0432.CCR-10-0540
60. Hon WC, Berndt A, Williams RL. Regulation of lipid binding underlies the activation mechanism of class IA PI3-kinases. *Oncogene* (2012) 31:3655–66. doi:10.1038/onc.2011.532
61. Jaiswal BS, Janakiraman V, Kljavin NM, Chaudhuri S, Stern HM, Wang W, et al. Somatic mutations in p85a promote tumorigenesis through class IA PI3K activation. *Cancer Cell* (2009) 16:463–74. doi:10.1016/j.ccr.2009.10.016
62. Jimenez C, Jones DR, Rodriguez-Viciana P, Gonzalez-Garcia A, Leonardo E, Wennström S, et al. Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *EMBO J* (1998) 17:743–53. doi:10.1093/emboj/17.3.743
63. Thorpe LM, Spangle JM, Ohlson CE, Cheng H, Roberts TM, Cantley LC, et al. PI3K-p110α mediates the oncogenic activity induced by loss of the novel tumor suppressor PI3K-p85α. *Proc Natl Acad Sci U S A* (2017) 114:7095–100. doi:10.1073/pnas.1704706114
64. Cheung LWT, Yu S, Zhang D, Li J, Ng PKS, Panupinthu N, et al. Naturally occurring neomorphic PIK3R1 mutations activate the MAPK pathway, dictating therapeutic response to MAPK pathway inhibitors. *Cancer Cell* (2014) 26:479–94. doi:10.1016/j.ccell.2014.08.017
65. Bárcena C, Quesada V, De Sandre-Giovannoli A, Puente DA, Fernández-Toral J, Sigaudy S, et al. Exome sequencing identifies a novel mutation in PIK3R1 as the cause of SHORT syndrome. *BMC Med Genet* (2014) 15:51. doi:10.1186/1471-2350-15-51
66. Chudasama KK, Winnay J, Johansson S, Claudi T, König R, Haldorsen I, et al. SHORT syndrome with partial lipodystrophy due to impaired phosphatidylinositol 3 kinase signaling. *Am J Hum Genet* (2013) 93:150–7. doi:10.1016/j.ajhg.2013.05.023
67. Dyment DA, Smith AC, Alcantara D, Schwartzentruber JA, Basel-Vanagaite L, Curry CJ, et al. Mutations in PIK3R1 cause SHORT syndrome. *Am J Hum Genet* (2013) 93:158–66. doi:10.1016/j.ajhg.2013.06.005
68. Huang-Doran I, Tomlinson P, Payne F, Gast A, Sleigh A, Bottomley W, et al. Insulin resistance uncoupled from dyslipidemia due to C-terminal PIK3R1 mutations. *JCI Insight* (2016) 1:e88766. doi:10.1172/jci.insight.88766
69. Klatka M, Rysz I, Kozyra K, Polak A, Kołłataj W. SHORT syndrome in a two-year-old girl – case report. *Ital J Pediatr* (2017) 43:44. doi:10.1186/s13052-017-0362-z
70. Schroeder C, Riess A, Bonin M, Bauer P, Riess O, Döbler-Neumann M, et al. PIK3R1 mutations in SHORT syndrome. *Clin Genet* (2014) 86:292–4. doi:10.1111/cge.12263
71. Thauvin-Robinet C, Auclair M, Duplomb L, Caron-Debarle M, Avila M, St-Onge J, et al. PIK3R1 mutations cause syndromic insulin resistance with lipoatrophy. *Am J Hum Genet* (2013) 93:141–9. doi:10.1016/j.ajhg.2013.05.019
72. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi:10.1126/science.1243292
73. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2016) 139:597–606.e4. doi:10.1016/j.jaci.2016.06.021
74. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34:272–6. doi:10.1007/s10875-014-0012-9
75. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2017) 139:1032–5.e6. doi:10.1016/j.jaci.2016.08.028
76. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183:221–9. doi:10.1111/cei.12706
77. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of

primary sclerosing cholangitis. *J Clin Immunol* (2015) 35:11–4. doi:10.1007/s10875-014-0109-1
78. Heurtier L, Lamrini H, Chentout L, Deau M-C, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110δ cause activated PI3K-δ syndrome 1 (APDS1). *Haematologica* (2017) 102:e278–81. doi:10.3324/haematol.2017.167601
79. Liu H, Tang XL, Liu JR, Li HM, Zhao SY. [Clinical and genetic analysis for activated PI3K-δ syndrome by PIK3CD gene mutation]. *Zhonghua Er Ke Za Zhi* (2016) 54:698–702. doi:10.3760/cma.j.issn.0578-1310.2016.09.013
80. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
81. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29–31. doi:10.1016/j.clim.2017.05.020
82. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kδ syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
83. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110δ cause activated PI3Kδ syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140:1152–6.e10. doi:10.1016/j.jaci.2017.03.026
84. Teranishi H, Ishimura M, Koga Y, Eguchi K, Sonoda M, Kobayashi T, et al. Activated phosphoinositide 3-kinase δ syndrome presenting with gut-associated T-cell lymphoproliferative disease. *Rinsho Ketsueki* (2017) 58:20–5. doi:10.11406/rinketsu.58.20
85. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh T-W, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138:1672–80.e10. doi:10.1016/j.jaci.2016.03.055
86. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Schölvinck L, Kalina T, et al. Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
87. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kδ regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114:1982–7. doi:10.1073/pnas.1617244114
88. Deau M-C, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124:3923–8. doi:10.1172/JCI75746
89. Hauck F, Magg T, Krolo A, Bilic I, Hirschmugl T, Laass M, et al. Variant PIK3R1 hypermorphic mutation and clinical phenotypes in a family with short statures, mild immunodeficiency and lymphoma. *Klin Padiatr* (2017) 229:113–7. doi:10.1055/s-0043-104218
90. Kuhlen M, Hönscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
91. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211:2537–47. doi:10.1084/jem.20141759
92. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36:462–71. doi:10.1007/s10875-016-0281-6
93. Bravo García-Morato M, García-Miñaúr S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, López-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
94. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang Y-D, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85α subunit of PI3K. *J Exp Med* (2012) 209:463–70. doi:10.1084/jem.20112533
95. Tang P, Upton JEM, Barton-Forbes MA, Salvadori MI, Clynick MP, Price AK, et al. Autosomal recessive agammaglobulinemia due to a homozygous mutation in PIK3R1. *J Clin Immunol* (2017) 62:1034–8. doi:10.1007/s10875-017-0462-y
96. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* (2014) 370:1008–18. doi:10.1056/NEJMoa1314583
97. Hoegenauer K, Soldermann N, Zécri F, Strang RS, Graveleau N, Wolf RM, et al. Discovery of CDZ173 (Leniolisib), representing a structurally novel class of PI3K delta-selective inhibitors. *ACS Med Chem Lett* (2017) 8:975–80. doi:10.1021/acsmedchemlett.7b00293
98. Rao VK, Webster S, Dalm VASH, Sediva A, van Hagen PM, Holland S, et al. Effective "activated PI3Kδ syndrome-"targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
99. Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, et al. PI3Kγ is a molecular switch that controls immune suppression. *Nature* (2016) 539:437–42. doi:10.1038/nature19834
100. Ali K, Soond DR, Piñeiro R, Hagemann T, Pearce W, Lim EL, et al. Inactivation of PI(3)K p110δ breaks regulatory T-cell-mediated immune tolerance to cancer. *Nature* (2014) 510(7505):407–11. doi:10.1038/nature13444

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 05 April 2018
doi: 10.3389/fimmu.2018.00568

Enhanced AKT Phosphorylation of Circulating B Cells in Patients With Activated PI3Kδ Syndrome

Takaki Asano[1], Satoshi Okada[1], Miyuki Tsumura[1], Tzu-Wen Yeh[2], Kanako Mitsui-Sekinaka[3], Yuki Tsujita[3], Youjiro Ichinose[4], Akira Shimada[5], Kunio Hashimoto[6], Taizo Wada[7], Kohsuke Imai[8], Osamu Ohara[9], Tomohiro Morio[2], Shigeaki Nonoyama[3] and Masao Kobayashi[1]*

[1] Department of Pediatrics, Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima, Japan, [2] Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, [3] Department of Pediatrics, National Defense Medical College, Tokorozawa, Japan, [4] Department of Pediatrics, Ako Central Hospital, Ako, Japan, [5] Department of Pediatrics, Okayama University Graduate School of Medicine, Okayama, Japan, [6] Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [7] Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Kanazawa, Japan, [8] Department of Community Pediatrics, Perinatal and Maternal Medicine, Tokyo Medical and Dental University (TMDU), Tokyo, Japan, [9] Department of Technology Development, Kazusa DNA Research Institute, Kisarazu, Japan

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Michel Massaad, American University of Beirut Medical Center, Lebanon
Silvia Clara Giliani, University of Brescia, Italy
Vanessa L. Bryant, Walter and Eliza Hall Institute of Medical Research, Australia

***Correspondence:**
Satoshi Okada
saok969@gmail.com

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: 30 November 2017
Accepted: 06 March 2018
Published: 05 April 2018

Citation:
Asano T, Okada S, Tsumura M, Yeh T-W, Mitsui-Sekinaka K, Tsujita Y, Ichinose Y, Shimada A, Hashimoto K, Wada T, Imai K, Ohara O, Morio T, Nonoyama S and Kobayashi M (2018) Enhanced AKT Phosphorylation of Circulating B Cells in Patients With Activated PI3Kδ Syndrome. Front. Immunol. 9:568. doi: 10.3389/fimmu.2018.00568

Activated PI3Kδ syndrome (APDS) is a primary immunodeficiency characterized by recurrent respiratory tract infections, lymphoproliferation, and defective IgG production. Heterozygous mutations in *PIK3CD*, *PIK3R1*, or *PTEN*, which are related to the hyperactive phosphoinositide 3-kinase (PI3K) signaling, were recently presented to cause APDS1 or APDS2 (APDSs), or APDS-like (APDS-L) disorder. In this study, we examined the AKT phosphorylation of peripheral blood lymphocytes and monocytes in patients with APDSs and APDS-L by using flow cytometry. $CD19^+$ B cells of peripheral blood in APDS2 patients showed the enhanced phosphorylation of AKT at Ser473 (pAKT) without any specific stimulation. The enhanced pAKT in $CD19^+$ B cells was normalized by the addition of a p110δ inhibitor. In contrast, $CD3^+$ T cells and $CD14^+$ monocytes did not show the enhanced pAKT in the absence of stimulation. These findings were similarly observed in patients with APDS1 and APDS-L. Among $CD19^+$ B cells, enhanced pAKT was prominently detected in $CD10^+$ immature B cells compared with $CD10^-$ mature B cells. Enhanced pAKT was not observed in B cells of healthy controls, patients with common variable immunodeficiency, and hyper IgM syndrome due to CD40L deficiency. These results suggest that the enhanced pAKT in circulating B cells may be useful for the discrimination of APDS1, APDS2, and APDS-L from other antibody deficiencies.

Keywords: activated PI3 kinase delta syndrome, AKT phosphorylation, catalytic subunit p110δ of phosphatidylinositol 3-kinase, flow cytometry, immunodeficiency, regulatory subunit p85α of phosphatidylinositol 3-kinase

Abbreviations: APDS, activated PI3Kδ syndrome; APDS1, activated PI3Kδ syndrome 1; APDS2, activated PI3Kδ syndrome 2; APDS-L, activated PI3Kδ syndrome like immunodeficiency; APDSs, activated PI3Kδ syndrome 1 and activated PI3Kδ syndrome 2; AUC, area under the curve; cDNA, complementary DNA; CI, confidence interval; CMV, cytomegalovirus; CVID, common variable immunodeficiency; ΔMFI, difference in mean fluorescence intensity; EZR, Easy R; EBV, Epstein Barr virus; GOF, gain of function; HIGM, hyper IgM syndrome; IVIG, intravenous immunoglobulin; LOF, loss of function; MFI, mean fluorescence intensity; mTOR, mechanistic target of rapamycin; NK, natural killer; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI3K, phosphoinositide 3-kinase; PID, primary immunodeficiency syndrome; PIP2, phosphatidylinositol 3, 4- triphosphate; PIP3, phosphatidylinositol 3, 4, 5- triphosphate; PHTS, PTEN Hamartoma Tumor Syndromes; PTEN, phosphatase and tensin homolog; ROC, receiver operating characteristic; Ser473, Serine473; pAKT, AKT phosphorylation.

INTRODUCTION

Activated PI3Kδ syndrome (APDS) is a primary immunodeficiency (PID) characterized by recurrent respiratory tract infections, chronic Epstein Barr virus and cytomegalovirus infections, lymphoproliferation, increased lymphoma susceptibility, and poor antibody production (1–4). Heterozygous gain-of-function mutations in *PIK3CD*, which encodes the catalytic subunit p110δ of phosphoinositide 3-kinase (PI3K), have been identified in patients with APDS1 (1, 3). Subsequent studies have demonstrated that a heterozygous mutation in *PIK3R1* encoding p85α, a regulatory subunit of PI3K, is responsible for APDS2: a PID with similar clinical manifestations to APDS1 (2, 4). Moreover, a patient with a heterozygous loss-of-function mutation in *PTEN*, which encodes phosphatase and tensin homolog and is associated with PTEN hamartoma tumor syndrome (PHTS), was recently reported to develop APDS-like immunodeficiency (APDS-L) with incomplete penetrance (5, 6).

Phosphoinositide 3-kinases convert phosphatidylinositol 3,4-triphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) and are involved in cellular functions including proliferation, differentiation, survival, and trafficking (7, 8). Both p110δ and p85α belong to class IA PI3Ks and have an essential role in the differentiation, development, and functions of several distinct stages of B- and T-lymphocytes (7, 8). They also have an important role in the antibody maturation process by regulating immunoglobulin class-switch recombination and plasma cell differentiation (9). When stimulated, PIP3 recruits AKT to the plasma membrane where AKT is activated *via* phosphorylation by PDK1 and mTORC2 (7, 8). In contrast, PTEN antagonizes PI3Ks by catalytic dephosphorylation of PIP3 to PIP2 (10). The recent identification of APDS1 and APDS2 (APDSs) and APDS-L revealed that the hyperactive PI3K/AKT signaling affects the immune system in humans, leading to the development of PID.

Here, we investigated four unrelated Japanese patients with APDS2 caused by a heterozygous mutation in *PIK3R1*. Activated T cells from the patients showed enhanced phosphorylation of Ser473 of AKT (pAKT) consistent with findings in previous reports (2, 4). We next investigated the status of pAKT using peripheral blood mononuclear cells (PBMCs) isolated from patients and analyzed by flow cytometry. We observed that circulating $CD19^+$ B cells from APDS2 patients, but not other cell populations, showed enhanced pAKT. This finding was similarly detected in circulating $CD19^+$ B cells from patients with APDSs or APDS-L, but not from healthy controls, common variable immunodeficiency (CVID) patients, or hyper IgM syndrome (HIGM) patients due to CD40L deficiency. Therefore, enhanced pAKT signaling in circulating $CD19^+$ B cells was considered a specific finding in patients with APDSs or APDS-L. Furthermore, by focusing on $CD10^+CD19^+$ immature B cells, this method allowed us to distinguish APDSs and APDS-L patients from healthy controls and patients with CVID or HIGM (CVID/HIGM). This flow-cytometry-based assay of PI3K activity enabled the discrimination analysis of identified mutations in *PIK3CD*, *PIK3R1*, or *PTEN*. It may also serve as a rapid diagnostic method to discriminate APDSs and APDS-L patients from other PID.

MATERIALS AND METHODS

Cases

We investigated four unrelated Japanese patients with APDS2 who were involved in a previous international survey (P13, P14, P19, and P26 in the previous report) (11). The detailed clinical manifestations of those patients are available in Materials and Methods in Supplementary Material. All of the patients carried heterozygous germline mutations in *PIK3R1* (Figure S1 in Supplementary Material). The identification of *PIK3R1* mutation was performed by a candidate gene approach in P4. For the other three patients, the mutations were identified by whole exome sequencing and were confirmed by Sanger sequencing. The identified mutations were 1425 + 2 T > A (P1), 1300−1 G > C (P2), 1425 + 1 G > C (P3), and 1425 + 1 G > T (P4). The *PIK3R1* mutations identified in P3 and P4 were previously shown to be pathogenic mutations (2, 4).

We included four CVID patients, aged 33 (P5), 36 (P6), 17 (P7), and 30 (P8) years, whose genetic causes have not been identified (detailed in Materials and Methods in Supplementary Material). The absence of pathogenic mutations in *PIK3CD*, *PIK3R1*, and *PTEN* was confirmed in those patients. We also included one HIGM patient due to CD40L deficiency (P9) who was 41 years old (detailed in Materials and Methods in Supplementary Material).

Immunoblot Analysis

$CD3^+$ T cells and $CD19^+$ B cells were separated from PBMCs using the IMag™ Cell Separation System (BD Biosciences, San Jose, CA, USA). The separated cells were then subjected to immunoblot analysis using the following antibodies: anti-AKT antibody (Cell Signaling Technology, Danvers, MA, USA), anti-phospho-AKT (Ser473) antibody (Cell Signaling Technology), and ß-actin (SIGMA-ALDRICH, Saint Louis, MO, USA).

Preparation of Activated T Cells

Activated T cells were derived from PBMCs according to a previous report (2). Briefly, PBMCs were cultured with 1×10^6 cells per mL in RPMI 1640 GlutaMax supplemented with 10% human AB serum, penicillin and streptomycin, PMA (1 μmol/L), and ionomycin (20 ng/mL) for 2 days. The cells were then separated by Lymphoprep density-gradient centrifugation and washed twice with RPMI 1640 GlutaMax. Then, they were cultured in RPMI 1640 GlutaMax supplemented with 10% human AB serum and IL-2 (100 IU/mL) for 16–24 h.

B-Cell Stimulation

For B-cell stimulation, PBMCs were purified by Lymphoprep density-gradient centrifugation and incubated at 1×10^6 cells per mL in RPMI 1640 GlutaMax supplemented with 10% human AB serum, penicillin, and streptomycin. The cells were stimulated with CD40L (1 μg/mL) and IL-4 (20 ng/mL) for 30 min. They were then harvested and subjected to flow-cytometry analysis of AKT phosphorylation.

Flow-Cytometry Analysis of AKT Phosphorylation

Peripheral blood mononuclear cells from APDS1 (four patients), APDS2 (four patients), APDS-L (four patients), CVID (four patients), HIGM (one patient), and 24 adult healthy controls were subjected to flow-cytometry analysis. We assessed pAKT at Ser473 by flow cytometry as follows. PBMCs were suspended at a density of 1×10^6 cells/μL in serum-free RPMI with or without 10 μM of 110δ inhibitor (IC87114) in the presence of FITC-conjugated anti-CD19 (HIB19) (BD Biosciences). The cells were incubated for 20 min at 37°C and washed twice. They were fixed and permeabilized according to the BD Phosflow protocol (protocol III). They were then stained and subjected to flow cytometry. The following antibodies were used for staining: Alexa Fluor 647-conjugated anti-phospho AKT (Ser473) (D9E) (Cell Signaling Technology), FITC-conjugated anti-CD19 (BD Biosciences), PE-conjugated anti-CD3 (UCHT1) (BD Biosciences), PE-conjugated anti-CD14 (Mφ97) (BD Biosciences), or FITC-conjugated anti-CD56 (C5.9) (SIGMA-ALDRICH), PE-conjugated CD16 (3G8) (BD Biosciences), and PerCP-Cy 5.5-conjugated anti-CD10 (HI10a) (BD Biosciences). Negative selection of B cells from PBMCs was performed using Pan B-Cell Isolation Kit, human (Miltenyi Biotec Inc., Auburn, CA, USA).

Statistical Analysis

Receiver operating characteristic (ROC) curves were created with Easy R (EZR) software available online (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html). EZR is statistical software and is based on R and R commander. EZR enables the application of statistical functions (12). Statistical hypotheses were tested using a two-tailed *t*-test. A p value < 0.05 was considered significant.

RESULTS

Mutation Analysis of Patients With APDS2

We found a splice site mutation at the + 2 position following exon 10 (**Figure 1A**). Amplification of cDNA showed an aberrant, faster migrating band suggesting a deletion (**Figure 1B**). Sanger sequencing demonstrated a deletion of exon 10 in the patient (**Figure 1C**) but not in his mother (**Figure 1D**). Thus, we confirmed that the identified *PIK3R1* mutations in P1, as well as the other two mutations, are pathogenic mutations, leading to the skipping of exon 10 with a deletion of amino acid residues 434–475 of p85α (**Figure 1B**). The former diagnosis of four patients with APDS2 was CVID (P1), HIGM (P2 and P4), and IgG subclass deficiency (P3). The identified mutations in PIK3R1 were *de novo* in Family B, C, and D, since we found no

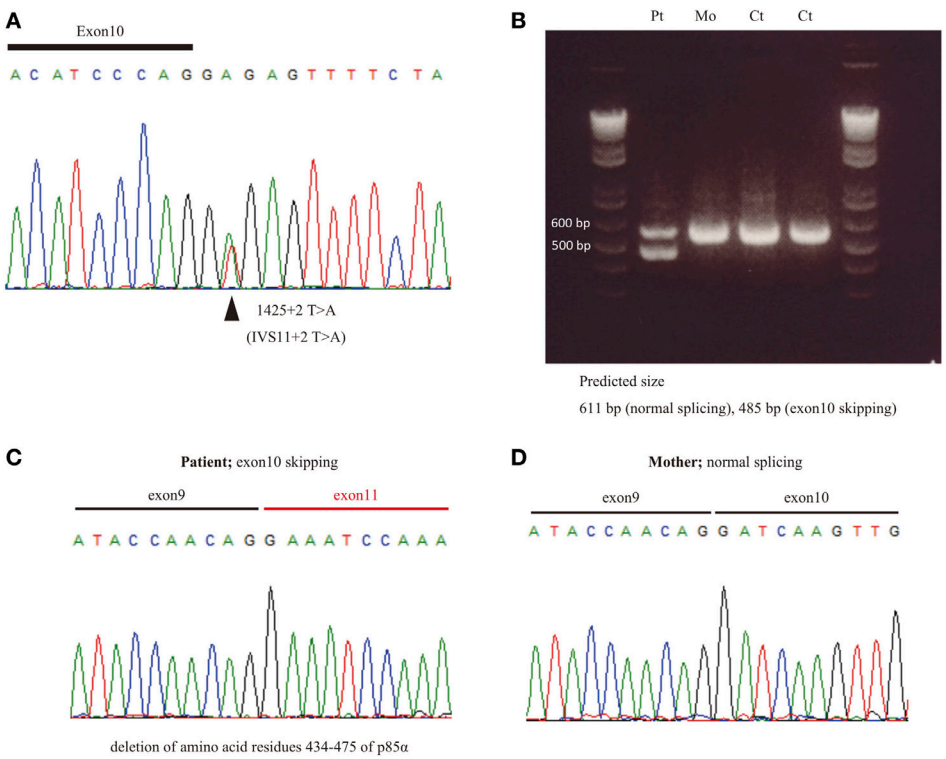


FIGURE 1 | The effect of splice site mutation identified in P1. **(A)** A germline heterozygous mutation, **(B)** 1425 + 2 T > A, in *PIK3R1* was identified by whole exome sequencing and confirmed by Sanger sequencing. **(C)** mRNA was extracted from peripheral blood mononuclear cells from P1 and his mother. Complementary DNA was then synthesized to assess the significance of the nucleotide substitution, 1425 + 2 T > A, on splicing. **(C,D)** The RT-PCR fragment was cloned into a pGEM-T easy vector. Loss of exon 10 in patient **(C)** but not mother **(D)** was revealed by Sanger sequencing.

asymptomatic carrier in a familial study. In order to determine the mechanism of disease in patients with APDS2, we focused on pAKT function associated with PI3K signaling.

Circulating CD19⁺ B Cells From Patients With APDSs or APDS-L Showing Enhanced pAKT Signaling

Enhanced pAKT signaling associated with hyperactive PI3K signaling is a common finding in patients with APDSs (1–4). We first assessed the status of pAKT in fresh (non-cultured) PBMCs from APDS2 patients by flow cytometry. There was no obvious difference in the level of pAKT in CD3⁺ T cells, CD16⁺CD56⁺ natural killer (NK) cells, and CD14⁺ monocytes between APDS2 patients and healthy controls (**Figure 2A**). In P1's CD14⁺ monocytes, pAKT was slightly enhanced in the absence of p110δ inhibitor treatment compared with untreated, but this difference was non-significant. In contrast, CD19⁺ B cells from the APDS2 patient (P1) had significantly higher levels of pAKT compared with those from healthy controls. By treating them with a p110δ inhibitor, the enhancement of pAKT observed in CD19⁺ B cells was normalized. In order to exclude the possibility of stimulation of B cells by staining them with anti-CD19 antibody, we confirmed this finding by analyzing circulating B cells separated by negative selection (**Figure 2B**).

We measured the mean fluorescence intensity (MFI) of pAKT in the presence or absence of p110δ inhibitor treatment by flow cytometry. We then evaluated enhanced pAKT signaling by

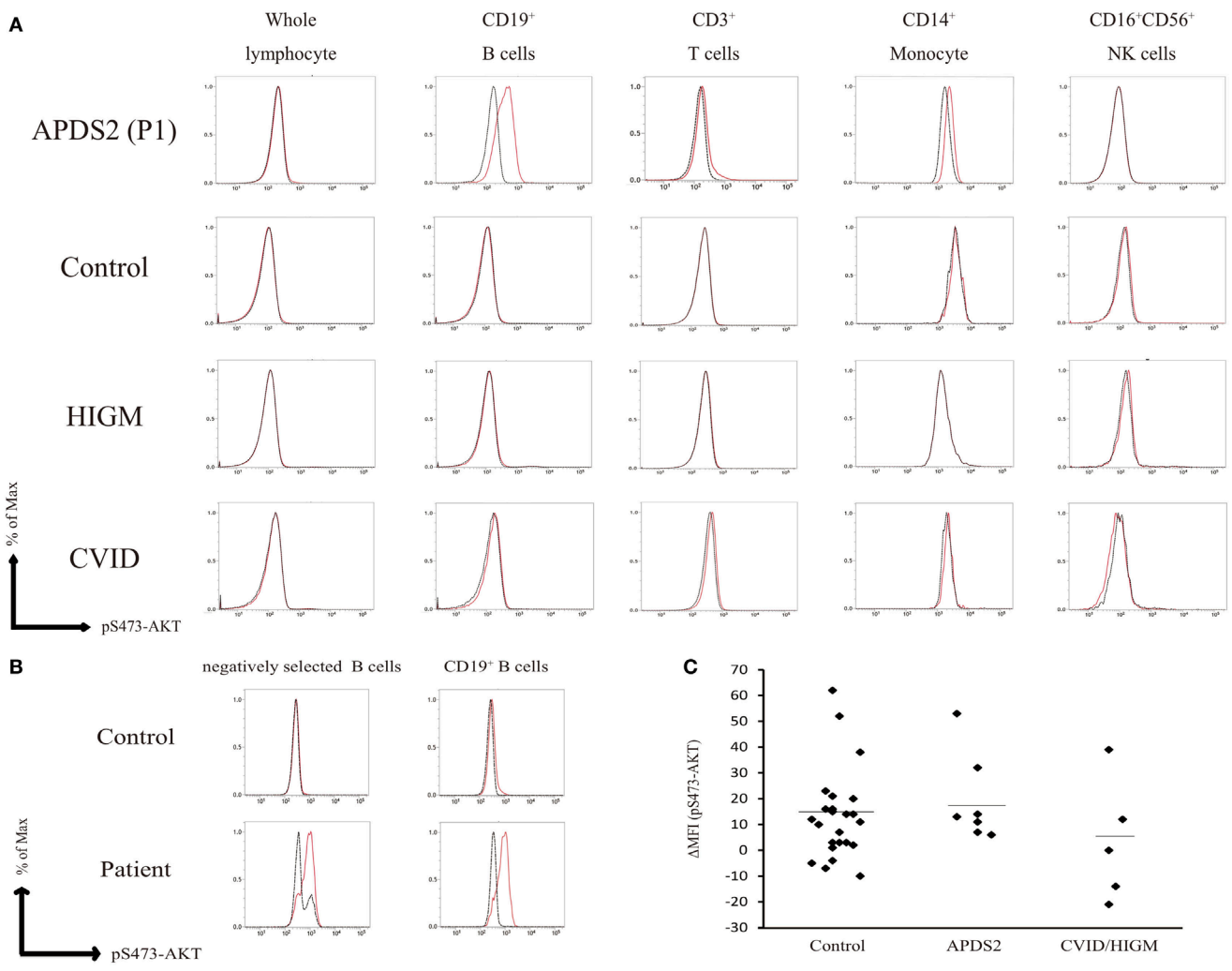


FIGURE 2 | Flow-cytometry-based AKT phosphorylation (pAKT) assay of PMBCs. **(A)** The level of pAKT in the presence or absence of p110δ inhibitor treatment was assessed by flow cytometry in total peripheral blood mononuclear cells (PBMCs), CD3⁺ T cells, CD14⁺ monocytes, and CD16⁺CD56⁺ natural killer cells from activated PI3Kδ syndrome 2 (APDS2) (P1), common variable immunodeficiency (CVID) (P8), or hyper IgM syndrome (HIGM) (P9). The number of events analyzed was >5,000. Red bold line: no treatment and black dotted line: p110δ inhibitor treatment. **(B)** The level of pAKT in negatively selected B cells from PBMCs from P1 and one healthy control was compared with pAKT in CD19⁺ B cells. Red solid line: no treatment and black dotted line: p110δ inhibitor treatment. **(C)** A summary of difference in mean fluorescence intensity of whole lymphocytes derived from 24 adult healthy controls or all of the patients with APDS2 or CVID/HIGM. There is no statistical significant among this three groups (control vs. CVID; $p = 0.24$, APDS2 vs. CVID; $p = 0.13$, and control vs. APDS2; $p = 0.52$).

calculating the difference in MFI (ΔMFI) of pAKT in CD19$^+$ B cells as the difference between MFI (the absence of p110δ inhibitor) and MFI (the presence of p110δ inhibitor). CD19$^+$ B cells from APDS2 patients had significantly higher ΔMFI of pAKT than those from healthy controls or CVID/HIGM patients, although ΔMFI of pAKT in whole lymphocytes was almost at the same levels among all individuals (**Figures 2C** and **3D**). The protein expression of total AKT in CD19$^+$ B cells from an APDS2 patient (P1) was equivalent to that in healthy controls (**Figure 3A**). The result was consistent with the previous studies showing the normal AKT protein expression in APDSs and APDS-L patients (1–4, 6). The enhancement of pAKT in CD19$^+$ B cells was confirmed by immunoblotting (**Figure 3B**; Figure S2 in Supplementary Material). In contrast, there was no difference in the level of pAKT in CD3$^+$ T cells (**Figure 3B**; Figure S2 in Supplementary Material).

We next tested the hypothesis that enhanced pAKT signaling in CD19$^+$ B cells is a common finding of patients with hyperactive PI3K signaling. We analyzed PBMCs from patients with APDS1 or APDS-L carrying a heterozygous mutation in *PIK3CD* or *PTEN*, respectively. As expected, CD19$^+$ B cells from APDS1 and APDS-L showed significantly higher levels of pAKT compared with healthy controls and CVID/HIGM patients (**Figure 3C**). As above, the enhancement of pAKT observed in these patients was normalized by treatment with a p110δ inhibitor. Similar to the results obtained from patients with APDS2, the level of pAKT was normal in CD3$^+$ T cells, CD16$^+$CD56$^+$ NK cells, and CD14$^+$ monocytes from patients with APDS1 or APDS-L (data not shown). However, CD19$^+$ B cells and other cell populations from patients with CVID and HIGM had normal levels of pAKT expression (**Figure 2A**). Curiously, CD19$^+$ B cells from APDS2 patients had the highest ΔMFI of pAKT among all APDSs patients

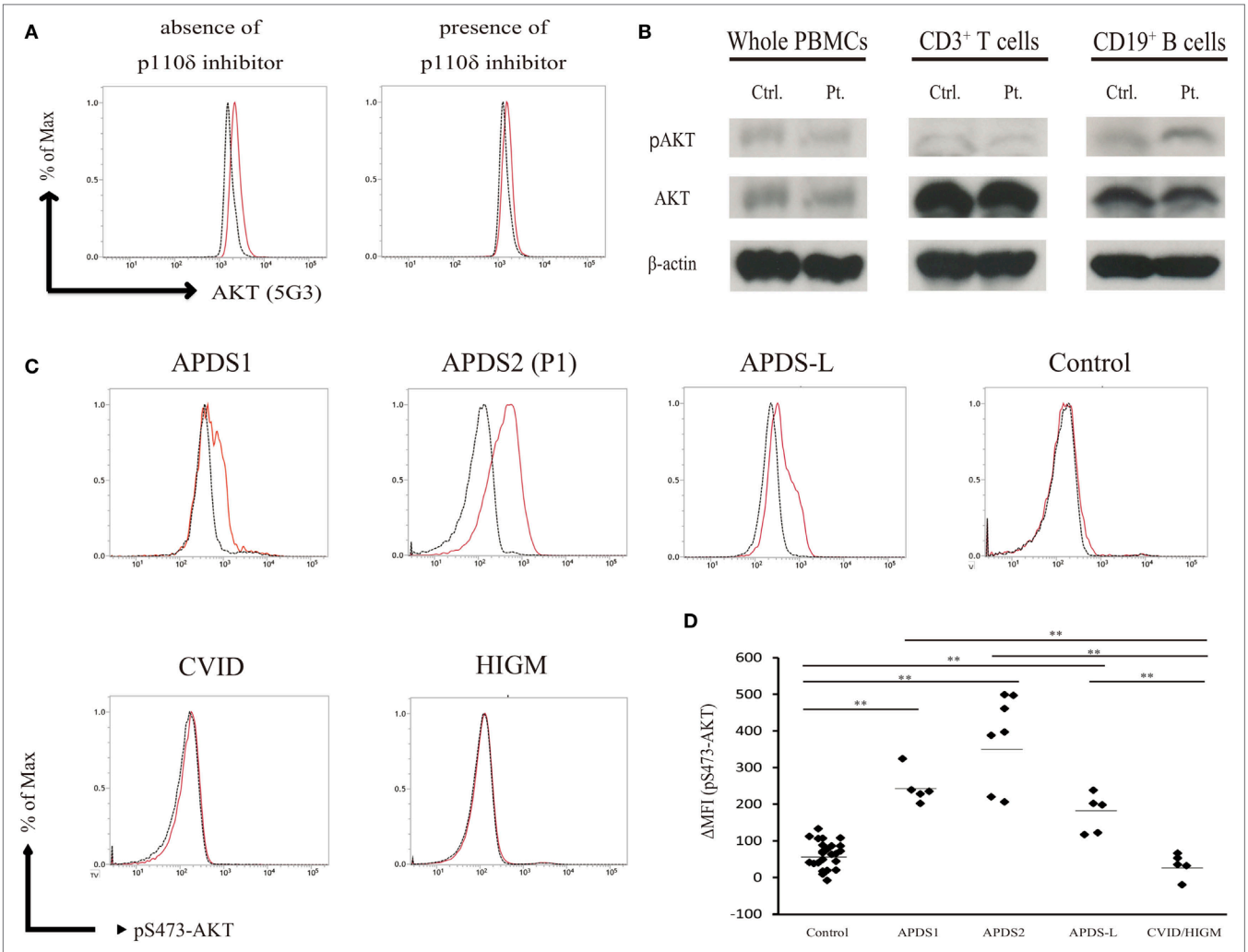


FIGURE 3 | Enhanced AKT phosphorylation (pAKT) in CD19$^+$ B cells from patients with activated PI3Kδ syndromes (APDSs) or APDS-like (APDS-L). **(A)** Total AKT expression in CD19$^+$ B cells from APDS2 patients (P1) was measured by flow-cytometry. Red bold line: activated PI3Kδ syndrome 2 (APDS2) patient and black dotted line: healthy control. **(B)** The phosphorylation and protein expression of AKT was investigated by immunoblotting. **(C)** pAKT (pS473) in the presence of p110δ inhibitor was analyzed by flow cytometry in CD19$^+$ B cells from healthy controls and patients with APDS1, APDS2, or APDS-L, CVID/HIGM. The number of events analyzed was >5,000. **(D)** A summary of difference in mean fluorescence intensity of CD19$^+$ B cells derived from healthy controls or patients with APDS1 (four patients), APDS2 (P1, P2, P3, and P4), APDS-L (four patients), or CVID/HIGM (P5, P6, P7, P8, and P9). *$p < 0.05$ and **$p < 0.01$.

(**Figure 3D**), followed by elevated ΔMFI of pAKT in APDS-L patients observed as significantly higher than healthy controls and CVID/HIGM patients (**Figure 3D**). In addition, higher levels of pAKT were also observed in $CD19^+$ B cells from cryopreserved PBMCs (Figures S3A,B in Supplementary Material). Therefore, the enhancement of pAKT in $CD19^+$ B cells was considered to be a specific finding among patients with APDSs and APDS-L.

Enhanced pAKT Signaling in Activated T Cells From APDS2 Patients

The previous study showed the enhancement of pAKT signaling in activated T cells from APDSs (1–4). We next investigated pAKT levels in activated T cells derived from PBMCs by flow cytometry. $CD3^+CD4^+$ and $CD3^+CD8^+$-activated T cells from P1 showed enhanced pAKT compared with those from healthy controls (**Figure 4A**). The enhancement of pAKT observed in P1 was normalized by treating cells with a p110δ inhibitor. The result was consistent with previous studies that investigated activated T cells from patients with APDSs (1–4). Thus, we confirmed the enhancement of pAKT in activated T cells from APDS2 patients by flow cytometry. Next, we stimulated B cells with CD40L and IL-4 and investigated the level of pAKT. Following stimulation with CD40L and IL-4, we observed the enhancement of pAKT in $CD19^+$ B cells from APDS2 patient and healthy control (**Figure 4B**). However, this difference became less striking after CD40L and IL-4 stimulation.

The Enhancement of pAKT in Patients With APDSs Pronounced in $CD10^+CD19^+$ Immature B Cells

Phosphoinositide 3-kinase signaling has important roles in differentiation, development, and functions in several distinct stages of B and T cells (7, 8). Patients with APDS1, APDS2, or APDS-L had increased numbers of transitional B cells in the peripheral blood, possibly reflecting the pivotal role of PI3K signaling in the differentiation of B cells (1–5). In our study, APDS2 patients had increased numbers of transitional B cells in peripheral blood consistent with previous studies (Figure S4 in Supplementary Material). We investigated pAKT levels in $CD19^+$ B cells by dividing them into three developmental stages: (i) total $CD19^+$ B cells, (ii) $CD10^-CD19^+$ mature B cells, and (iii) $CD10^+CD19^+$ immature B cells (corresponding to transitional B cells) by flow cytometry (Figure S5 in Supplementary Material). $CD19^+$ B cells at all three developmental stages from patients with APDS1, APDS2, or APDS-L had higher levels of pAKT compared with healthy controls. Surprisingly, the enhancement of pAKT was most pronounced in $CD10^+CD19^+$ immature B-cell populations from APDS1, APDS2, or APDS-L patients (**Figure 5**). This finding was confirmed by $CD10^+$ negatively selected B cells, controlling for the potential B-cell activation by anti-CD19 antibody staining (Figure S6 in Supplementary Material).

Establishment of a Flow-Cytometry-Based Rapid Discrimination Assay Based on the Enhancement of PI3K Signaling

We found that $CD19^+$ B cells from APDSs or APDS-L patients had significantly higher levels of pAKT than healthy controls and patients with CVID or HIGM. The higher level of pAKT observed in these patients was normalized by treating the cells with a p110δ inhibitor. This discovery led us to the idea that the detection of enhanced pAKT signaling in $CD19^+$ B cells is a useful diagnostic tool for the rapid discrimination study of suspected APDS patients. $CD19^+$ B cells from patients with APDS2 had the highest ΔMFI of pAKT among all APDSs or APDS-L patients. In contrast, elevated ΔMFI of pAKT in $CD19^+$ B cells was modest in APDS-L patients (**Figure 3D**). We next analyzed the ΔMFI of pAKT in $CD10^-CD19^+$ and $CD10^+CD19^+$ B cells. As expected, the ΔMFI of pAKT was high in both B-cell populations from APDSs or APDS-L patients when compared with those from healthy controls (**Figures 5C–E**). The high ΔMFI of pAKT was emphasized in the analysis of $CD10^+CD19^+$ immature B cells (**Figure 5E**). Moreover, if we focused on $CD10^+CD19^+$ immature B cells, there was no overlap in the value of ΔMFI between APDSs and APDS-L patients and the other populations including healthy controls and CVID/HIGM patients. This finding strongly suggests that the flow-cytometry-based assay to measure the ΔMFI of pAKT can be used as a discrimination assay to detect the enhancement of PI3K activity in patients with APDSs and APDS-L (flow chart is shown in Figure S7 in Supplementary Material).

Cutoff Value of ΔMFI of pAKT Segregating APDSs and APDS-L Patients

We created an ROC curve based on the results of the ΔMFI of pAKT obtained from the analysis of $CD19^+$ B cells. We used EZR for statistical analysis and set up the cutoff value of ΔMFI of pAKT as 117. This cutoff value allows the segregation of APDSs and APDS-L patients from healthy controls or CVID patients with 100% sensitivity and 96.0% specificity (Figure S8 in Supplementary Material). The area under the curve was 0.996 (95% confidence interval 0.986−1.000) (12).

DISCUSSION

Here, we investigated four Japanese cases with APDS2 carrying a heterozygous mutation in *PIK3R1*. Elkaim et al. recently summarized the clinical and immunological aspects of 36 genetically diagnosed APDS2 patients and revealed that recurrent upper respiratory tract infections (100%), pneumonitis (71%), and chronic lymphoproliferation (89%) were the most common clinical features (11). Malignant diseases were identified in 28% of patients, most of them were B-cell lymphomas. Laboratory findings showed that patients with APDS2 had decreased serum IgA and IgG levels (87%), increased IgM levels (58%), B-cell lymphopenia (88%), and an increased frequency of transitional B cells (93%) (11). All four patients developed recurrent upper or lower respiratory tract infections and showed decreased serum IgG, which required intravenous immunoglobulin replacement therapy. Elevated serum IgM was observed in two patients, and one patient developed malignant lymphoma. Therefore, the four Japanese patients in the current study were considered to be typical cases of APDS2.

The former diagnoses of the four patients in the current study were HIGM (P2 and P4), CVID (P1), and IgG subclass deficiency

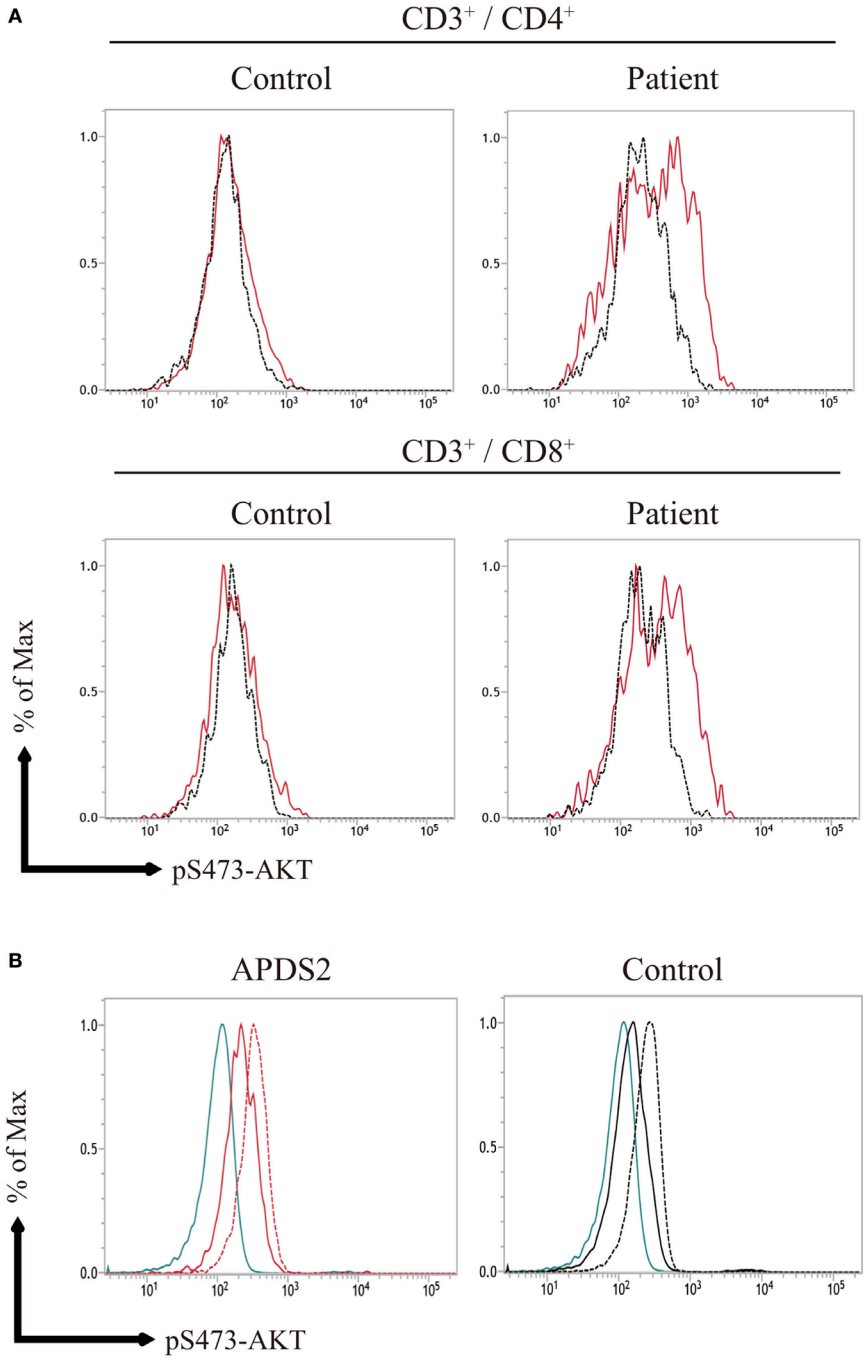


FIGURE 4 | **(A)** AKT phosphorylation (pAKT) expression in activated T cells, **(B)** pAKT expression CD19+ B cells stimulated with CD40L and IL-4. **(A)** The level of pAKT in T cells was assessed by flow cytometry in activated PI3Kδ syndrome 2 patient (P1) and healthy controls (representative result from two controls is shown). The total number of events analyzed was 2,000. Red bold line: no treatment and black dotted line: p110δ inhibitor treatment. **(B)** pAKT expression in CD19+ B cells from (P1) and healthy control following stimulation with CD40L and IL-4. Blue line: isotype control, black and red solid line: no treatment, and black and red dot line: CD40L and IL-4 treatment.

(P3). Based on the phenotypic diversity and similarity in clinical and laboratory findings, considerable numbers of patients with APDS2, as well as patients with APDS1 (13), have been historically diagnosed as HIGM or CVID. From the first identification of APDS1 associated with hyperactive PI3K signaling, a definitive diagnosis of APDSs is performed by the identification of mutations in *PIK3CD* or *PIK3R1*. In addition to these genetic tests, the detection of enhanced pAKT signaling in T-cell blasts by

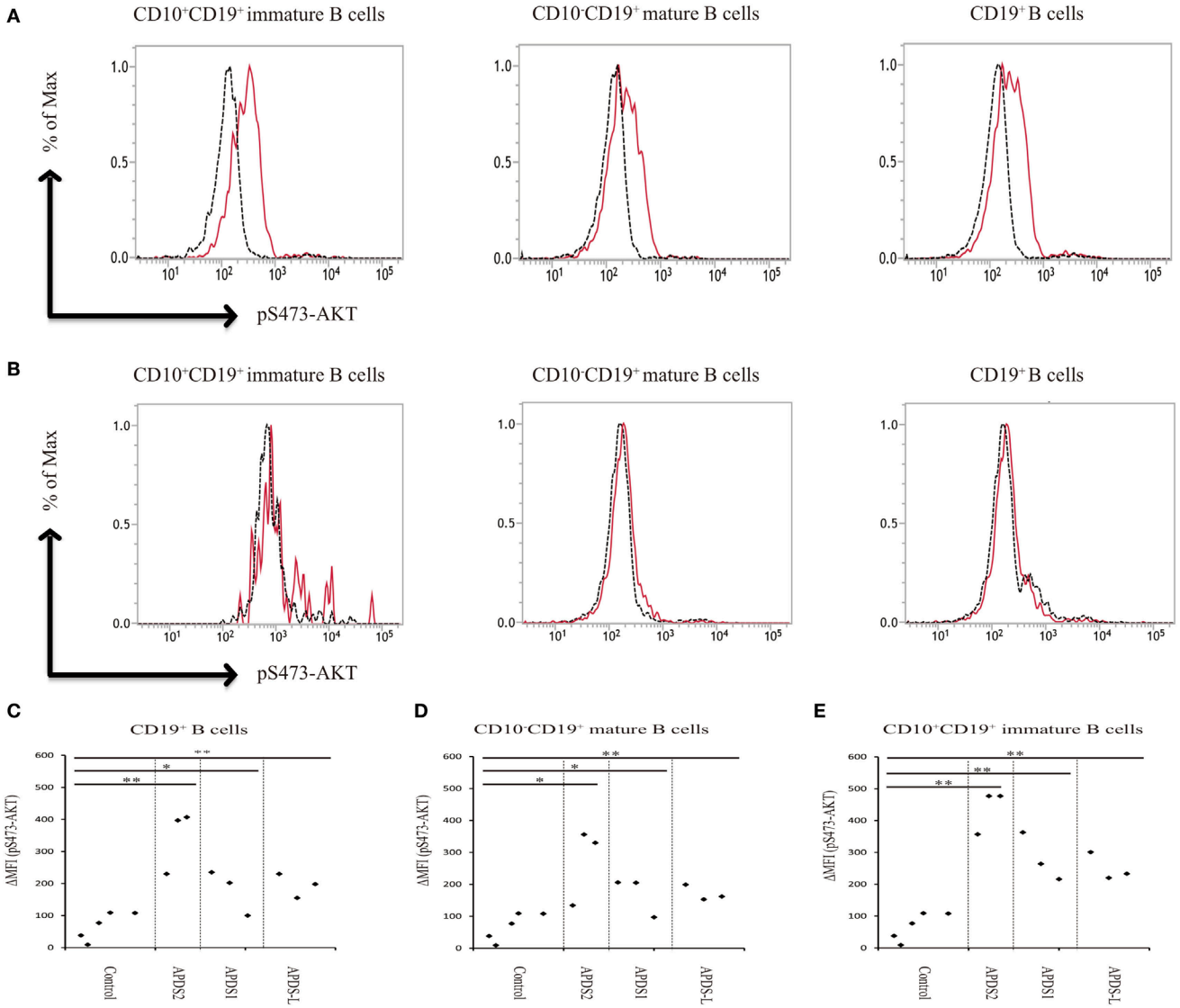


FIGURE 5 | Enhanced AKT phosphorylation (pAKT) in activated PI3Kδ syndrome 2 (APDS2) patients was pronounced in a flow-cytometry assay of $CD10^+CD19^+$ B cells. **(A,B)** The enhancement of pAKT observed in $CD19^+$ B cells was further investigated by separating them into $CD10^+CD19^+$ immature B cells and $CD10^-CD19^+$ mature B cells from P2 (representative histogram of three experiments) **(A)** and healthy controls **(B)**. The number of events analyzed was >5,000. As an exception, $CD10^+CD19^+$ immature B cells analyzed in healthy control were 800 events. Red solid line: no treatment and black dotted line: p110δ inhibitor treatment. **(C,D,E)** A summary of difference in mean fluorescence intensity of $CD19^+$ B cells **(C)**, $CD10^-CD19^+$ mature B cells **(D)**, and $CD10^+CD19^+$ immature B cells **(E)** from healthy controls or patients with APDS1 (three patients), APDS2 (P1, P2, and P4), or APDS-like (APDS-L) (three patients). $^*p < 0.05$ and $^{**}p < 0.01$.

functional assays has been used to confirm hyperactive PI3K signaling. However, this assay method is not suitable for rapid diagnosis, because it requires the cultivation of T cells. In the current study, we observed significantly higher levels of pAKT in $CD19^+$ B cells, but not $CD3^+$ T cells, $CD16^+CD56^+$ NK cells, or $CD14^+$ monocytes isolated from fresh PBMCs from APDSs and APDS-L patients. The enhancement of pAKT in $CD19^+$ B cells was pronounced in $CD10^+CD19^+$ immature B cells. Moreover, when we focused on this $CD10^+CD19^+$ B-cell population, there was no overlap in the value of ΔMFI of pAKT between APDSs or APDS-L patients and the other populations, including healthy controls and patients with CVID or HIGM. We also made a similar observation using cryopreserved PBMCs from patients with APDSs or APDS-L. This finding allowed us to perform the rapid detection of hyperactive PI3K signaling without culturing patient cells. Although further studies are required to optimize and evaluate this flow-cytometry-based assay system, this assay system has a potential to enable a rapid diagnosis of APDSs and APDS-L

In this study, the enhanced ΔMFI of pAKT in $CD19^+$ B cells was a common finding among patients with APDSs or APDS-L. This observation was further enhanced if we focused

TABLE 1 | Clinical features of patients with APDSs, APDS-L, or CVID.

Clinical feature	APDS1 (%)	APDS2 (%)	APDS-L (%)	CVID (%)
Pneumonia	85	71	50	32–77
Lymphoproliferation	75	89	44	N.D.
Splenomegaly	58	43	N.D.	15–30
Enteropathy	25	24	N.D.	9
Granuloma	0	N.D.	N.D.	8–9
Meningitis/encephalitis	1.9	N.D.	N.D	3–4
Autoimmunity	42	17	N.D.	22–29
Malignancy	13	28	22	3–8
Neurodevelopmental delay	19	31	50	N.D.
IVIG therapy	77	89	19	80
Reference	(20)	(11)	(5, 6)	(20)

on $CD10^+CD19^+$ immature B cells. These observations may reflect the importance of PI3K signaling in class-switch recombination in B cells (9, 14), and be related to abnormalities in the developmental stages of B cells, such as abnormalities of the germinal center structure (11, 15–17), increased circulating transitional B cells, and decreased class switching B cells in patients with APDSs (1–4). Curiously, the ΔMFI of pAKT was the highest in $CD19^+$ B cells from APDS2 patients and was the lowest in $CD19^+$ B cells from APDS-L patients. It is interesting to speculate possible molecular mechanism underlie this observation. The p85α is known to enhance enzymatic activity of PTEN (18). Although PTEN protein expression is normal in APDS2 patients (2), its enzymatic activity might be affected by functional impairment of p85α. Therefore, impairment of p85α may enhance pAKT by losing its inhibitory role of p110δ and its enhancing effect against PTEN enzymatic activity.

Relatively mild enhancement of pAKT in APDS-L patients might explain the clinical observation that only a part of patients with PHTS, caused by heterozygous mutations in PTEN, present with antibody deficiency (5). Indeed, the clinical penetrance of APDS-L is not high in patients with PHTS (19). There are considerable overlaps in the clinical manifestations between APDS1 and APDS2 (**Table 1**) (1–4, 11, 20); however, there are also some differences in these two disorders. Indeed, patients with APDS2 have a higher susceptibility to lymphoma than patients with APDS1 (11, 20, 21) (Okano et al., under revision). The clinical penetrance of APDS1 is quite high, but not complete, possibly explaining the existence of asymptomatic carriers or cases with mild symptoms that only show recurrent respiratory infections and diagnosed as APDS1 by familial studies that identified a proband case (20). However, to date no asymptomatic carriers have been reported in patients with APDS2. Although we require large cohort studies to make strong conclusions, these clinical observations might be partially explained by the difference in elevated ΔMFI of pAKT. Further studies are necessary to understand the role of elevated ΔMFI of pAKT in $CD19^+$ B cells on the immunological manifestations among patients with APDSs and APDS-L. The selective effect of pAKT in B cells (transitional B cells in particular), which may provide a detailed pathological mechanism of APDSs and APDS-L, remains to be explained.

Recently, molecular targeting therapy using an mTOR inhibitor was also effective for the treatment of lymphoproliferation in patients with APDSs (1, 3, 11). Therefore, the prompt and appropriate diagnosis of APDSs definitely benefits patients by providing a therapeutic choice of target therapy. The flow-cytometry-based rapid assay of PI3K activity described here has the potential to provide a rapid diagnosis of APDSs and APDS-L.

CONCLUDING REMARKS

The flow-cytometry-based rapid assay of PI3K activity described here provides a rapid discrimination assay of identified mutations in *PIK3CD*, *PIK3R1*, and *PTEN*, and might also be a potential diagnostic tool for patients with APDSs or APDS-L.

ETHICS STATEMENT

We obtained written informed consent for genomic analysis and blood-sample-based functional studies of the patients, parents, and siblings in accordance with the Declaration of Helsinki. The genetic analysis and blood-sample-based functional studies were approved by the Institutional Review Board of Hiroshima University.

AUTHOR CONTRIBUTIONS

Patient workup: TA, SO, KM-S, YT, YI, AS, KH, TW, KI, TM, SN, and MK. Flow-cytometry analysis: TA and MT. Drafting the manuscript: TA, SO, and KM. Final approval of the version to be published: TA, SO, MT, T-WY, KM-S, YT, YI, AS, KH, TW, KI, OO, TM, SN, and MK. Agreement to be accountable for all aspects of the work: TA, SO, MT, T-WY, KM-S, YT, YI, AS, KH, TW, KI, OO, TM, SN, and MK.

ACKNOWLEDGMENTS

We thank Michael Ciancanelli for proofreading.

FUNDING

This study was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (16H05355 and 16K15528 to SO, 17H04233 to SN), the Ministry of Health, Labour and Welfare, Japan (17933299 to SN), and Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development, AMED.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00568/full#supplementary-material.

REFERENCES

1. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
2. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124(9):3923–8. doi:10.1172/JCI75746
3. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
4. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
5. Driessen GJ, IJspeert H, Wentink M, Yntema HG, van Hagen PM, van Strien A, et al. Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency. *J Allergy Clin Immunol* (2016) 138(6):1744–7.e5. doi:10.1016/j.jaci.2016.07.010
6. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138(6):1672–80.e10. doi:10.1016/j.jaci.2016.03.055
7. Koyasu S. The role of PI3K in immune cells. *Nat Immunol* (2003) 4(4):313–9. doi:10.1038/ni0403-313
8. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* (2003) 3(4):317–30. doi:10.1038/nri1056
9. Omori SA, Cato MH, Anzelon-Mills A, Puri KD, Shapiro-Shelef M, Calame K, et al. Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling. *Immunity* (2006) 25(4):545–57. doi:10.1016/j.immuni.2006.08.015
10. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* (1998) 273(22):13375–8. doi:10.1074/jbc.273.22.13375
11. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138(1):210–8.e9. doi:10.1016/j.jaci.2016.03.022
12. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* (2013) 48(3):452–8. doi:10.1038/bmt.2012.244
13. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hulsdunker J, Eskandarian Z, et al. Activating PI3Kdelta mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183(2):221–9. doi:10.1111/cei.12706
14. Dengler HS, Baracho GV, Omori SA, Bruckner S, Arden KC, Castrillon DH, et al. Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation. *Nat Immunol* (2008) 9(12):1388–98. doi:10.1038/ni.1667
15. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159(1):33–6. doi:10.1016/j.clim.2015.04.014
16. Sander S, Chu VT, Yasuda T, Franklin A, Graf R, Calado DP, et al. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. *Immunity* (2015) 43(6):1075–86. doi:10.1016/j.immuni.2015.10.021
17. Di Fonte R, Baronio M, Plebani A, Lougaris V, Fousteri G. Reduced germinal center follicular helper T cells but normal follicular regulatory T cells in the tonsils of a patient with a mutation in the PI3KR1 gene. *Clin Immunol* (2016) 164:43–4. doi:10.1016/j.clim.2016.01.016
18. Chagpar RB, Links PH, Pastor MC, Furber LA, Hawrysh AD, Chamberlain MD, et al. Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* (2010) 107(12):5471–6. doi:10.1073/pnas.0908899107
19. Chen HH, Handel N, Ngeow J, Muller J, Huhn M, Yang HT, et al. Immune dysregulation in patients with PTEN hamartoma tumor syndrome: analysis of FOXP3 regulatory T cells. *J Allergy Clin Immunol* (2016) 139(2):607–20.e15. doi:10.1016/j.jaci.2016.03.059
20. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2016) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
21. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol* (2014) 134(1):233–6. doi:10.1016/j.jaci.2014.02.020

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 09 May 2018
doi: 10.3389/fimmu.2018.00966

"Immune TOR-opathies," a Novel Disease Entity in Clinical Immunology

*Sophie Jung[1,2,3], Laura Gámez-Díaz[3†], Michele Proietti[3†] and Bodo Grimbacher[3]**

[1] CNRS, UPR 3572 (I2CT), Institut de Biologie Moléculaire et Cellulaire (IBMC), Strasbourg, France, [2] Hôpitaux Universitaires de Strasbourg, Pôle de Médecine et de Chirurgie Bucco-Dentaires, Strasbourg - Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France, [3] Center for Chronic Immunodeficiency (CCI), Medical Center – Faculty of Medicine, University of Freiburg, Freiburg, Germany

Primary immunodeficiencies (PIDs) represent a group of mostly monogenic disorders caused by loss- or gain-of-function mutations in over 340 known genes that lead to abnormalities in the development and/or the function of the immune system. However, mutations in different genes can affect the same cell-signaling pathway and result in overlapping clinical phenotypes. In particular, mutations in the genes encoding for members of the phosphoinositide3-kinase (PI3K)/AKT/mTOR/S6 kinase (S6K) signaling cascade or for molecules interacting with this pathway have been associated with different PIDs that are often characterized by the coexistence of both immune deficiency and autoimmunity. The serine/threonine kinase mechanistic/mammalian target of rapamycin (mTOR), which acts downstream of PI3K and AKT, is emerging as a key regulator of immune responses. It integrates a variety of signals from the microenvironment to control cell growth, proliferation, and metabolism. mTOR plays therefore a central role in the regulation of immune cells' differentiation and functions. Here, we review the different PIDs that share an impairment of the PI3K/AKT/mTOR/S6K pathway and we propose to name them "immune TOR-opathies" by analogy with a group of neurological disorders that has been originally defined by PB Crino and that are due to aberrant mTOR signaling (1). A better understanding of the role played by this complex intracellular cascade in the pathophysiology of "immune TOR-opathies" is crucial to develop targeted therapies.

Keywords: AKT, immune dysregulation, kinase, mTOR, PI3k, primary immunodeficiency, S6K

OPEN ACCESS

Edited by:
Carrie L. Lucas, Yale University, United States

Reviewed by:
David A. Fruman, University of California, Irvine, United States
David Andrew Fulcher, Australian National University, Australia

***Correspondence:**
Bodo Grimbacher
bodo.grimbacher@uniklinik-freiburg.de

†These authors have contributed equally to this work.

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *19 January 2018*
Accepted: *18 April 2018*
Published: *09 May 2018*

Citation:
Jung S, Gámez-Díaz L, Proietti M and Grimbacher B (2018) "Immune TOR-opathies," a Novel Disease Entity in Clinical Immunology. Front. Immunol. 9:966. doi: 10.3389/fimmu.2018.00966

INTRODUCTION

Primary immunodeficiencies (PIDs) comprise more than 350 inherited disorders that affect the development and/or the functions of the components of the immune system (2, 3). They are individually rare but collectively, they are "more common than thought" (4), particularly due to the rapid increase in the number of newly described disorders and of causative genes that have been identified. In fact, the study of PIDs has frequently contributed to the discovery of new genes that are pivotal in immune cell development, effector functions, or in the maintenance of immune homeostasis (5). Susceptibility to severe and recurrent infections is a constant clinical manifestation in PID patients. However, an overlap between immune deficiency (infections and/or malignancies) and immune dysregulation (autoimmunity, autoinflammation, and/or allergy) is often observed in certain types of PIDs (2, 3, 6). Although PIDs are mostly inherited as monogenic disorders, disease penetrance, as well as disease expressivity, may result from interactions between

genetic, epigenetic, and/or environmental factors. This contributes to the wide phenotypic diversity, even between individuals with an identical mutation in the same gene (2, 3, 7). The International Union of Immunological Societies (IUIS) PID expert committee regularly publishes a classification based on shared pathogenesis and/or clinical phenotypes with the latest update in 2017 (2, 3).

The serine/threonine kinase mechanistic/mammalian target of rapamycin (mTOR) plays a central role within the phosphoinositide3-kinase (PI3K)/AKT/mTOR/S6 kinase (S6K) signaling pathway. It acts as a downstream effector of AKT in two structural and functional distinct protein complexes named mTOR complex 1 and 2 (mTORC1 and mTORC2, respectively) (8). mTOR integrates the different cues from the microenvironment to control cell growth, proliferation, and metabolism, thereby exerting crucial functions in the regulation of immune homeostasis (8, 9).

Defects in the genes encoding for the different members of the PI3K/AKT/mTOR/S6K cascade or for molecules interacting with this pathway are frequently associated with immune dysfunction. We therefore propose here to cluster the different PIDs that share an impairment of the PI3K/AKT/mTOR/S6K pathway. Considering the central role of mTOR in the signaling cascade, this subgroup of PIDs will be referred hereafter as "immune TOR-opathies." The term "mTOR-opathies" was initially coined in 2007 by PB Crino to define a wide spectrum of neurological disorders due to abnormal mTOR signaling that are characterized by focal malformations of cortical development, epilepsy, and neurobehavioral disabilities (1, 10).

In this review, we describe the PI3K/AKT/mTOR/S6K signaling cascade, focusing on the genetic and molecular defects of the different "immune TOR-opathies," and on the impact of this pivotal pathway in the development of immune deficiency and immune dysregulation, a hallmark of "immune TOR-opathies."

PI3K/AKT/mTOR/S6K SIGNALING PATHWAY PLAYS A CRUCIAL ROLE IN IMMUNE HOMEOSTASIS

S6 kinase activation involves a complex signaling cascade that connects a number of critical kinases, including PI3Ks, AKT (also called PKB for protein kinase B), and mTOR (11) (**Figure 1**). The PI3K/AKT/mTOR/S6K pathway plays a major role in the control of cell proliferation (increase in number), cell growth (increase in size), survival, and metabolism (12). It is therefore crucial in the regulation of immune responses, as well as in the promotion of B cells, T cells, and myeloid cells differentiation, activation, and function (9).

Among the different classes of PI3Ks, class IA molecules have the most important function in immune cells (13). Those heterodimeric proteins are formed by the association of a catalytic subunit of approximately 110 kDa (p110α, p110β, or p110δ encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD* respectively), and a Src-homology 2 (SH2) domain-containing regulatory subunit (p85, p50, and p55α encoded by *PIK3R1*; p85β encoded by *PIK3R2*; and p55γ encoded by *PIK3R3*). The catalytic subunits p110α and p110β are widely expressed, whereas the expression of p110δ is restricted to leukocytes (13, 14). The regulatory subunit controls the cellular location and the activity of the enzyme by recruiting the catalytic subunit to membrane-associated proteins that have been phosphorylated on YXXM motifs by tyrosine kinases (12, 13). In immune cells, class IA PI3Ks can be activated *via* multiple surface tyrosine-kinase-associated receptors, including the T- and B-cell receptors (TCR and BCR, respectively), toll-like receptors (TLRs), as well as various co-receptors [CD19, inducible T-cell costimulator (ICOS), CD28, PD-1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)], and cytokine receptors (IL-1, IL-2, IL-4, IL-12, and IFN-γ) that contain YXXM motifs in their cytoplasmic domain (12). After activation, class I PI3Ks catalyze the conversion of phosphatidylinositol-(4,5)-bisphosphate [$PI(4,5)P_2$ or PIP_2] to phosphatidylinositol-(3,4,5)-trisphosphate [$PI(3,4,5)P_3$ or PIP_3] (12). PIP_3 acts as binding sites for various intracellular enzymes harboring pleckstrin-homology (PH) domains, in particular for the serine/threonine kinase AKT, which is then recruited at the inner leaflet of the cell membrane to be phosphorylated. The activity of AKT is positively regulated by the binding of PIP_3 to its PH domain, but also by the phosphorylation at position Thr308 by phosphoinositide-dependent kinase-1 (PDK1) and at position Ser473 by mTORC2 (15) (**Figure 1**). Once AKT is activated, it inhibits the tuberous sclerosis heterodimeric complex (TSC1/TSC2 complex), inducing the release of the GTP-binding protein Ras homolog enriched

Abbreviations: AMPK, AMP-activated protein kinase; APDS, activated PI3Kδ syndrome; ASCT2, sodium-dependent neutral amino acid transporter type 2; BAD, Bcl-2-associated death promoter; BCL10, B-cell lymphoma/leukemia 10; BCR, B-cell receptor; BDCP, BEACH domain-containing protein; BEACH, Beige and Chediak-Higashi; BENTA, B cell expansion with NF-κB and T cell anergy; BTK, Bruton's tyrosine kinase; CARD11, caspase recruitment domain-containing protein 11; CARMIL2, capping protein regulator and myosin 1 linker 2; CBM, CARD11-BCL10-MALT1; CID, combined immunodeficiency; CMV, cytomegalovirus; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CR2, complement receptor 2; CVID, common variable immunodeficiency; CWS, Cowden syndrome; DEPTOR, DEP domain-containing mTOR interacting protein; EBV, Epstein-Barr virus; 4E-BP1, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; FOXO, Forkhead box protein O; GOF, gain-of-function; ICOS, inducible T-cell costimulator; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; IUIS, International Union of Immunological Societies; KO, knockout; LOF, loss-of-function; LPS, lipopolysaccharide; LRBA, lipopolysaccharide-responsive beige-like anchor protein; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; mLST8/GβL, mammalian lethal with SEC13 protein 8/G protein β subunit-like; mSIN, stress-activated map kinase-interacting protein 1; mTOR, mechanistic/mammalian target of rapamycin; mTORC, mTOR complex; NF-κB, nuclear factor-kappa B; PASLI, p110δ activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency; PDK1, phosphoinositide-dependent kinase-1; PH, pleckstrin-homology; PHTS, *PTEN* hamartoma tumor syndrome; PHLPP, PH domain leucine-rich repeat protein phosphatase; PID, primary immunodeficiency; PIP_2, phosphatidylinositol-(4,5)-bisphosphate; PIP_3, phosphatidylinositol-(3,4,5)-trisphosphate; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PRAS40, proline-rich AKT substrate 40 kDa; PROTOR, protein observed with Rictor; PTEN, phosphatase and tensin homolog; RAPTOR, regulatory-associated protein of mTOR; RHEB, Ras homolog enriched in brain; RICTOR, rapamycin-insensitive companion of mTOR; RLTPR, RGD, leucine-rich repeat, tropomodulin and proline-rich-containing protein; S6K, S6 kinase; SH2, Src-homology 2; TCR, T-cell receptor; T_{FH}, follicular helper T cells; TH, T helper; TLR, toll-like receptor; Tregs, regulatory T cells; VZV, varicella zoster virus.

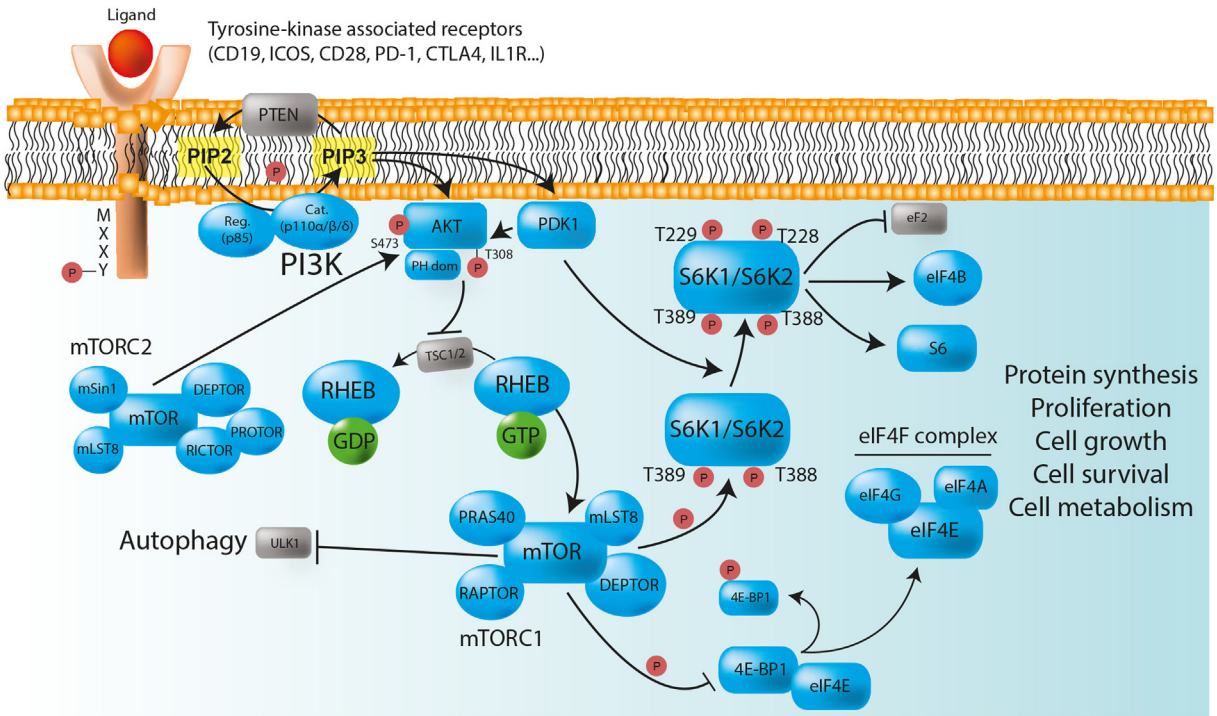


FIGURE 1 | The PI3K/AKT/mTOR/S6K pathway plays a major role in the control of immune cell homeostasis. Class IA PI3Ks are heterodimeric molecules composed of a p110 catalytic subunit (p110α, p110β, or p110δ) and a p85 regulatory subunit. In immune cells, class IA PI3Ks can be activated *via* multiple surface tyrosine kinase-associated receptors [e.g., BCR, TCR, TLR, CD19, ICOS, PD-1, and CTLA-4] that bear YXXM motifs in their cytoplasmic domain. In the absence of ligand binding, the TSC1/TSC2 complex negatively regulates mTORC1, and therefore protein synthesis, by converting RHEB into its inactive GDP-bound state. After receptor activation, phosphorylated YXXM motifs provide binding sites for the p85 regulatory subunit that brings the p110 catalytic subunit to the membrane, where it converts PIP_2 to PIP_3. PIP_3 serves as plasma membrane docking sites for PH-domain containing proteins, such as AKT, and its upstream activator PDK1. The activity of AKT is also positively regulated by mTORC2. Once phosphorylated, AKT inhibits the TSC1/TSC2 complex, and allows the release of GTP-bound RHEB, thereby enabling mTORC1 activation. Activated mTORC1 triggers biosynthetic pathways (protein synthesis) essential for cell proliferation, survival, and metabolism through S6Ks and 4E-BP1 phosphorylation, while inhibiting ULK1, and therefore autophagy. S6K phosphorylate numerous substrate, including ribosomal protein S6, eukaryotic translation initiation factor eIF4B, and eukaryotic elongation factor 2 (eEF2) kinase. The phosphorylation of 4E-BP1 prevents its binding to the cap-binding protein eIF4E, allowing it to participate in the formation of the eIF4F complex, which is composed of the DEAD-box RNA helicase eIF4A, the cap-binding protein eIF4E, and the large "scaffold" protein eIF4G, and which is required for the initiation of cap-dependent translation. PTEN is a negative regulator of PI3K/AKT/mTOR/S6K signaling pathway that dephosphorylates PIP_3 back to PIP_2. Red circles: phosphorylation; normal arrows: activation; blunt arrows: inhibition.

in brain (RHEB) from the inhibition by TSC2, therefore enabling the activation of mTORC1 (16) (**Figure 1**).

The serine/threonine kinase mTOR was identified while investigating the mechanism of action of rapamycin (also known as sirolimus), an immunosuppressive drug inhibiting mTOR enzymatic activity that is currently used to prevent organ transplant rejection and to treat lymphoproliferative diseases (17, 18). mTOR associates with distinct sets of proteins to form the intracellular signaling complexes mTORC1 and mTORC2 (8). Both complexes contain mammalian lethal with SEC13 protein 8/G protein β subunit-like (mLST8/GβL) and DEP domain-containing mTOR interacting protein (DEPTOR). In contrast, the partners regulatory-associated protein of mTOR (RAPTOR) and proline-rich AKT substrate 40 kDa (PRAS40) define the mTORC1 network, whereas rapamycin-insensitive companion of mTOR (RICTOR), stress-activated map kinase-interacting protein 1 (mSIN1), and protein observed with Rictor (PROTOR) are specific to the mTORC2 complex (8, 19, 20) (**Figure 1**). The major function of mTORC1 is to sense nutrients and mitogenic signals (8, 19, 20). Thus, when conditions are favorable, mTORC1 triggers biosynthetic pathways essential for cell growth and proliferation, mainly through direct phosphorylation of ribosomal S6K and eukaryotic translation initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) (8, 19, 20). mTORC1 also inhibits the serine/threonine kinase ULK1, thereby suppressing autophagy, a conserved catabolic process by which double-membrane vesicles (autophagosomes) engulf cytoplasmic contents for lysosomal degradation. Autophagy allows the recycling of cellular components and the generation of nutrients under metabolic stress, promoting cell survival (8, 21, 22). It is also implicated in more complex functions and participates in the regulation of immunity (23). Overall, the phosphorylation of S6Ks and 4E-BP1, along with the suppression of autophagy by active mTORC1, are essential for cell growth (24). Conversely, in case of starvation, AMP-activated protein kinase (AMPK) inactivates mTORC1 and phosphorylates the active sites of ULK1, therefore, enabling autophagy initiation (8, 21, 22). mTORC2 plays various

roles in cell survival, metabolism, proliferation, and cytoskeleton organization *via* the phosphorylation of AKT on Ser473 (mTORC2-dependent), leading to the phosphorylation, sequestration, and further inhibition of Forkhead box protein O (FOXO) (9). Negative regulators controlling PI3K/AKT/mTOR/S6K pathway include the phosphatase and tensin homolog (PTEN) that dephosphorylates PIP_3 back to PIP_2, thereby downregulating AKT signaling (25) (**Figure 1**).

Together with 4E-BP1, ribosomal S6Ks represent the best characterized substrates of mTORC1 (11, 26, 27). Like AKT, S6K1 (isoforms p70- and p85-S6K1), and S6K2 (isoforms p54- and p60-S6K2) belong to the AGC serine/threonine kinases family (26). The S6K activation begins with the phosphorylation of serine residues in the C-terminal domain that expose the internal region of the protein, allowing mTOR to phosphorylate Thr389 in S6K1 and Thr388 in S6K2. Indeed, S6K activation absolutely requires mTORC1-mediated phosphorylation (28). The subsequent phosphorylation by PDK1 at Thr229 in S6K1 and at Thr228 in S6K2 leads to their full activation (26) (**Figure 1**). S6K proteins originally gained their name due to their ability to phosphorylate ribosomal protein S6, a component of the 40S ribosome subunit, and their preferred phosphorylation motif has been characterized as RXRXXS/T (26). S6K1 and S6K2 have many functional similarities. They regulate several cellular and molecular processes, including transcription, protein synthesis, metabolism, cell proliferation, and survival (11, 26, 28). Although S6K1 has been more extensively studied, some distinct functions of S6K2 have been described (29). For instance, it has been shown that S6K2 plays a role in Th17 differentiation through the regulation of the transcription factor RORγ (30) despite a more recent study suggesting that this function may be context-specific (31). Ribosomal protein S6 was the first discovered substrate of S6Ks. It promotes biosynthetic pathways that are important for cell growth (27, 28), but the functional significance of its phosphorylation still remains not fully understood (28). However, the analysis of the phosphorylation status of p70-S6K1 (at Thr 389) and its substrate ribosomal protein S6 (at Ser240/244; S6K dependent) is widely and routinely used as a readout of mTORC1 activity (32, 33), in particular in lymphocytes populations, where other mTOR signaling markers are more difficult to monitor. A number of other S6K1 substrates have been involved in the regulation of protein synthesis at levels of initiation (eIF4B: eukaryotic translation initiation factor 4B), and elongation (eEF2: eukaryotic elongation factor 2), but also in RNA splicing (CBC: cap binding complex; SKAR: S6K1 Aly/REF-like target) (**Figure 1**). In addition, S6K1 plays a role in cell survival by blocking apoptosis through phosphorylation of the pro-apoptotic protein Bcl-2-associated death promoter (BAD), thereby preventing its interaction with BCL-X or BCL-2 (11, 26, 28). Some evidences also indicate that S6K1 may participate in cytoskeleton dynamics, in particular in F-actin reorganization (34).

Studies in animal models have suggested that reduced PI3K/AKT/mTOR/S6K signaling (hypoactivation) can lead to immune deficiency, whereas uncontrolled PI3K/AKT/mTOR/S6K signaling (hyperactivation) is associated with autoimmunity and hematological malignancies (12). Nevertheless, this simplistic dichotomous model does not reflect the highly complex regulation of this pathway. Indeed, several human PIDs that are associated with a hyperactivation of the PI3K/AKT/mTOR/S6K pathway have features of both immunodeficiency and immune dysregulation, suggesting a tight and dynamic modulation of the signaling cascade for optimal immune cell function.

mTOR plays a central role in the regulation of immune responses evidenced in numerous studies showing that mTOR or mTORC1 inhibition can have both positive and negative effects on lymphocytes, in particular on T-cell development and functions [reviewed in Ref. (9)]. The mTOR hypomorphic mouse, which is a model of mTORC1/mTORC2 inhibition [murine *Mtor* knockout (KO) is lethal and there are no reported cases of human loss-of-function (LOF) mutations in *MTOR*] is characterized by an immunodeficient phenotype with impaired development, proliferation, and migration of lymphocytes, as well as abnormal antibody production (35). Reduced mTOR expression results in decreased phosphorylation of the mTORC1 target p70-S6K1 and of the mTORC2 target AKT (phosphorylation at Ser473) in fibroblasts and TCR stimulated T cells. However, despite reduction of p70-S6K1 phosphorylation in murine B cells activates with lipopolysaccharide (LPS), mTORC2 activity is increased, suggesting that AKT regulation may be cell-type specific (35). In addition, PI3K/AKT/mTOR pathway seems to play differing roles during the differentiation and function of regulatory T cells (Tregs). Tissue tolerance is associated with the upregulation of enzymes that consume many of the essential amino acids (36). These starvation conditions lead to mTOR inhibition, promoting the expression of FoxP3 in naïve T cells, and therefore the generation of $CD4^+$ $FoxP3^+$ Tregs (37). In fact, continued TCR signaling and constitutive PI3K/AKT/mTOR activity antagonizes Foxp3 induction (9, 37, 38). However, under mTOR inhibitory conditions, Tregs are not optimally functional, requiring mTOR re-activation or inflammatory conditions to acquire their full suppressive potential. Alternate cycles of mTOR activity may therefore be needed for optimal functional induction of Tregs (37, 39). The mTOR downstream effectors S6Ks are essential in controlling the cell size and proliferation of certain cell types such as hepatocytes (40, 41). However, in contrast to mTOR, the functions of S6K1 and S6K2 in lymphocytes still remain controversial (33). Simultaneous deletion of *S6K1* and *S6K2* genes in a murine model was associated with a severe reduction in viability due to perinatal lethality, but single *S6K1* or *S6K2* KO mice did not exhibit obvious immune defects (although no detailed immunological study was performed) (41, 42). In addition, it has been shown *in vitro* using *S6K1/S6K2* double KO T and B cells that S6K activity is dispensable for lymphocytes growth and proliferation after antigen receptor engagement (33). Germline deletion of *Rps6* that encodes for ribosomal protein S6 is embryonically lethal (43) and T cell-specific deletion of *Rps6* abolishes thymic T-cell development (44). By contrast, the role of S6 phosphorylation is not well understood. Knockin mice in which all serine residues of S6 protein have been mutated to alanine to prevent phosphorylation by S6Ks are viable (45) and show normal T-cell activation and differentiation (46). All these

data clearly demonstrate the complexity of PI3K/AKT/mTOR/S6K pathway regulation.

GAIN-OF-FUNCTION (GOF) MUTATIONS IN THE GENES ENCODING CLASS I PI3K CAUSE ACTIVATED PI3Kδ SYNDROME (APDS)

Hyperactivation of the PI3K/AKT/mTOR/S6K signaling pathway in immune cells can be the consequence of heterozygous GOF mutations in the genes encoding for PI3Kδ that cause an immune dysregulation disorder called activated PI3Kδ syndrome [APDS; also known as "p110δ activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency" (PASLI)] (47). Molecularly, APDS encompasses two different disorders: APDS1 and APDS2. APDS1 (or PASLI-CD) is the consequence of mutations in the *PIK3CD* gene encoding for p110δ, the catalytic subunit of PI3Kδ that result in single-amino-acid substitutions leading to p110δ overactivation. APDS2 (or PASLI-R1) results from mutations in the *PIK3R1* gene encoding for p85α, the regulatory subunit of PI3Kδ. These mutations impair the binding of p85α to its cognate partner p110δ that is, therefore, inefficiently inhibited (47–51). Up to date, more than 150 APDS patients have been reported (48–68). They display features of both immune deficiency and immune dysregulation, and all of them present with early-onset, as well as severe and recurrent sino-pulmonary infections, mostly by encapsulated bacteria (47, 58). Benign lymphoproliferation (hepatosplenomegaly, lymphadenopathy, focal nodular lymphoid hyperplasia), various autoimmune manifestations, and B cell lymphomas are also frequently observed (47, 54, 55, 58, 61). Growth retardation is, however, commonly associated with APDS2, but not APDS1 (58, 66).

Most APDS patients have elevated transitional B cells, reduced class-switched memory B cells, variable immunoglobulin levels (mainly reduced IgG and increased IgM levels, hypogammaglobulinemia, or in some cases agammaglobulinemia) associated with a poor vaccine response, and an impaired *in vitro* B cell isotype switching (47, 51, 64, 69). Abnormalities in B lymphocytes from APDS patients recapitulate the defects of class-switch recombination that are observed in B lymphocytes from PTEN-deficient mice (70). Although APDS was initially described as a common variable immunodeficiency (CVID)-like disease, affected patients also suffer from recurrent herpes virus infections (i.e., EBV, CMV, and VZV), indicating an impaired T cell function (47, 52, 54, 56–58, 65). In addition, the majority of APDS patients show a progressive $CD4^+$ T cell lymphopenia with a decreased frequency of $CD4^+$ naïve T cells [in contrast to the lethal $CD4^+$ T cell hyperplasia that is described in mice with a T cell-specific deletion of *PTEN* (71)], but an excessive accumulation of terminally differentiated, senescent $CD8^+$ effector T cells (64). Considering the T cell abnormalities, APDS may be classified as combined immunodeficiency (CID) rather than as CVID-like disease.

In T cells, PI3Kδ is activated downstream of CD28, leading to enhanced AKT and mTOR signaling, which blocks autophagy but stimulates T cell proliferation and terminal differentiation through the phosphorylation of S6K (12). Activated AKT also mediates the phosphorylation and subsequent degradation of FOXO transcription factors that regulate T cell expansion and memory T cell differentiation (72). The analysis of PI3K signaling in T cells from APDS patients showed a constitutive hyperphosphorylation of both AKT (on Thr308: PI3K/PDK1 dependent and on Ser473: mTORC2 dependent) and S6 (on Ser235/236 and Ser240/244: mTORC1 dependent) (50–52, 64, 65, 67). The general overactivation of the PI3K/mTOR/S6K signaling pathway promotes the switch to an anabolic cellular state with increased aerobic glycolysis that is required for the expansion of effector T cells (73). Downregulation of mTOR signaling and reversion to a catabolic cellular state by autophagy induction, are, however, crucial for memory T cell formation and prolonged survival (73). In APDS patients, the constant maintenance of aerobic glycosis restrains the function and survival of memory $CD8^+$ T cells, leading to an abundance of senescent effector and short-lived effector memory $CD8^+$ T cells that exhibit a poor recall response *in vitro* and could account for the defective antiviral immunity *in vivo* (64, 65, 74). Similarly, high AKT and S6 phosphorylation levels were observed in transformed EBV-B cells, peripheral blood mononuclear cells, and isolated B cells (total B cells and isolated B cell subsets) from APDS patients at basal state and after B cell stimulation (48, 51, 52, 65). However, the link between the increased PI3K/mTOR/S6K signaling in B cells and the observed B cell phenotype is still a focus of research.

The insights into the pathophysiology of APDS allowed refining the therapeutic approaches. Indeed, it has been shown that *in vitro* treatment of unstimulated T cell blasts with the mTOR inhibitor rapamycin (sirolimus) leads to a decrease of S6 hyperphosphorylation (64). More notably, the administration of rapamycin was found to improve the clinical and immunological phenotype of two APDS patients with a reduction of hepatosplenomegaly and lymphadenopathy, as well as a normalization of T cell subpopulations (64, 67). However, PI3Kδ regulates additional pathways to mTOR (such as FOXO for example) and mTOR is also controlled by PI3K-independent pathways (13). Therefore, selective inhibitors of the PI3Kδ subunit, which have already shown remarkable success in certain hematologic malignancies, should be considered as future therapeutic options in APDS patients. Both *in vitro* and *in vivo* data support the specific inhibition of PI3Kδ as a promising therapy. Indeed, the selective p110δ inhibitor IC87114 is able to dampen the activity of the mutated PI3Kδ *in vitro* in APDS1 patients' T cells (52), and both p110δ (APDS1) and p85α (APDS2) are strongly inhibited *in vitro* by the PI3Kδ-specific inhibitor idelalisib (GS-1101 or CAL-101), which is currently approved by the US-Food and Drug Administration for the treatment of chronic lymphocytic leukemia (50, 75). In addition, the first clinical trial (#NCT02435173) that has been conducted by Novartis with the PI3Kδ-specific inhibitor leniolisib (CDZ173) in six APDS patients produced encouraging results (76). Oral administration of leniolisib during 12 weeks was well tolerated and was associated with an improvement of both laboratory and clinical parameters (reduction of peripheral transitional B cells, naive B cells, and senescent T cells; decrease of IgM and inflammatory cytokines levels; reduction of splenomegaly and lymphadenopathy) (76). Another clinical trial for an inhaled PI3Kδ inhibitor, sponsored by GlaxoSmithKline, is currently ongoing in patients with APDS (#NCT02593539) (47).

LOF MUTATIONS IN *PTEN* LEAD TO AN ACTIVATED PIK3δ SYNDROME-LIKE DEFICIENCY (APDS-LIKE)

PTEN encodes a lipid and protein phosphatase that dephosphorylates PIP_3 back to PIP_2 (77), thereby inhibiting the PI3K/mTOR/AKT/S6K signaling cascade (25). Impairment of PTEN activity is associated with an overabundance of PIP_3 and a constitutive downstream activation of AKT, leading to cellular proliferation and overgrowth (78).

A complete disruption of *Pten* in mouse results in early embryonic death (79), whereas *Pten* heterozygous mutant mice display hyperplastic-dysplastic features, develop spontaneously tumors (80), and present a lethal polyclonal autoimmune disorder with a phenotype that is reminiscent of *Fas*-deficient mice (81). Mice carrying a B cell-specific deletion of *Pten* show abnormal B cell differentiation and function, with increased numbers of marginal zone and B1-a B cells in the spleen, a production of serum autoantibodies, an impaired response to T-dependent and T-independent immunizations, as well as a defect in immunoglobulin class-switch recombination (70, 82, 83).

In humans, heterozygous germline mutations in *PTEN* may cause different autosomal dominant disorders including Cowden syndrome (CWS; OMIM 158350), Bannayan–Riley–Ruvalcaba syndrome (OMIM 153480), and Proteus syndrome (OMIM 176920), which are characterized by the development of multiple benign hamartoma and malignant tumors (84–86). The term *PTEN* hamartoma tumor syndrome (PHTS) is therefore used to describe any patient with a germline *PTEN* mutation regardless of the phenotype (78). Browning et al. reported a case of CWS associated with CID (87). In line with this observation, recent studies indicated that heterozygous LOF mutations in *PTEN* lead to immunodeficiency and immune dysregulation, with a clinical and immunological presentation that resembles APDS phenotype, including recurrent infections, organomegaly, and $CD4^+$ T cell lymphopenia (68, 88). However, immunodeficiency seems to occur only in some, but not all, patients with *PTEN* LOF mutations (68). Similarly to patients with heterozygous GOF mutations in *PIK3CD*, *PTEN* mutations are associated with an aberrant hyperactivation of the PI3K/AKT/mTOR/S6K pathway with increased phosphorylation of AKT, mTOR, and S6 in T cells (68, 87). Driessen et al. further studied, in a cohort of nine PHTS patients, the impact of germline *PTEN* mutations on the peripheral B cell development and the humoral immune response (89). They observed decreased counts of switched memory B cells associated with a dysregulated T-dependent B cell response, abnormalities in class-switch recombination, and decreased somatic hypermutation, resulting in hypogammaglobulinemia in about one-third of the patients (89). In mice, it has been shown that the level of activation-induced cytidine deaminase, the main regulator of somatic hypermutation and class-switch recombination, is regulated by the PI3K/AKT signaling cascade (70, 83, 90). This could explain, at least in part, the dysregulated humoral immune response observed in human PTEN deficiency (89).

Surprisingly, despite PTEN dysfunction, PHTS patients display a normal frequency and phenotype of $CD4^+$ $FoxP3^+$ Tregs, as well as a normal activation of the downstream signaling pathway with similar percentages of S6-phosphorylated Tregs in PHTS patients and controls subjects (88). In this cell subset, the enzyme PH domain leucine-rich repeat protein phosphatase (PHLPP), located downstream of PTEN and highly expressed in normal Tregs, provides a complementary phosphatase activity that is important for limiting PI3K hyperactivation (88). *PTEN* haploinsufficiency leads to APDS-like immune dysregulation, but the compensatory activity of the phosphatase PHLPP may help to maintain checkpoint control at the immunological synapse in human Tregs (88), possibly preventing the development of autoimmune manifestations.

LIPOPOLYSACCHARIDE-RESPONSIVE BEIGE-LIKE ANCHOR PROTEIN (LRBA) DEFICIENCY IS ASSOCIATED WITH IMPAIRED mTOR/S6K SIGNALING IN T CELLS

Lipopolysaccharide-responsive beige-like anchor protein (LRBA) belongs to the Beige and Chediak-Higashi (BEACH) domain-containing protein (BDCP) family together with eight other human proteins (91, 92). Although the exact functions of BDCPs remain unclear, they are considered to act as scaffolding molecules forming multiprotein complexes involved in vesicle trafficking and receptor signaling (92). Biallelic mutations in *LRBA* cause a PID and immune dysregulation disorder known as LRBA deficiency (93). LRBA-deficient patients show an early-onset broad spectrum of clinical and immunological manifestations, including recurrent infections, organomegaly, inflammatory bowel-like disease, hypogammaglobulinemia, and autoimmunity (94, 95). Several LRBA-deficient patients present with an immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like syndrome, indicating Treg cells impairment, that might contribute to the development of the various autoimmune manifestations (96). In fact, nearly two-thirds of LRBA-deficient patients have reduced Tregs frequency (95) with decreased expression of the canonical Treg markers (FOXP3, CD25, Helios, CTLA-4) and impaired Treg cell-mediated suppression (96). Additional perturbations observed in the T cell compartment such as increased proportion of circulating follicular helper T cells (T_{FH}) and decreased proportion of circulating follicular Tregs suggest an ineffective regulation of autoantibodies' production (96). Although the frequency of recent thymic emigrants seems to be normal, conventional T cells and Tregs from LRBA-deficient patients exhibit an increased apoptosis (96). In mice, Treg-specific disruption of mTORC1 (through the deletion of *Raptor*) leads to a profound loss of Treg suppressive activity with early development of a lethal autoimmunity and lymphoproliferation (39). Mechanistically, mTORC1 signaling promotes the cholesterol/lipid metabolism that is crucial for cell proliferation and for CTLA-4 upregulation, thereby establishing functional Treg competency (39). CTLA-4 belongs to the T cell co-stimulatory molecule family, including CD28, ICOS, and PD1. It is a critical negative regulator of T cell proliferation that serves as a "checkpoint" of immune responses (97). Interestingly, the role of LRBA in CTLA-4 post-transcriptional regulation is currently

the only proven cellular function for LRBA (98). Specifically, LRBA binds through its BEACH domain to the cytoplasmic tail of CTLA-4, allowing its vesicular transport to the plasma membrane of Tregs, and activated conventional T cells (98). CTLA-4 is then able to remove, *via* transendocytosis, the CD80 and CD86 co-stimulatory molecules from the cell surface of antigen-presenting cells, thereby controlling T cell activation (99). However, when LRBA is absent, the adaptor protein AP-1 binds to CTLA-4, leading to its lysosomal degradation (98). Decreased CTLA-4 expression might therefore contribute to the high frequency of autoimmune manifestations observed in patients with LRBA deficiency (94, 95). Indeed, patients with heterozygous LOF mutations in *CTLA-4* develop an immune dysregulation syndrome with an LRBA-deficiency-like clinical phenotype (100–102) known since 2014 as CTLA-4 deficiency. Surprisingly, CTLA-4 was assessed to bind to PI3K with the same avidity as CD28, possibly leading to the activation of PDK1 that phosphorylates AKT at position Thr308 (103, 104), thereby activating mTORC1 signaling cascade. Moreover, in T cells, CTLA-4 dependent activation of PI3K and AKT was shown to sustain T cell anergy without cell death (105). However, the intracellular signaling capacity of CTLA-4 was recently questioned (106). In contrast, it has been reported that activated LRBA-deficient $CD4^+$ and $CD8^+$ T cell subsets show an impaired mTORC1 and mTORC2 activity with a reduced phosphorylation of downstream mTORC1 (S6 and 4E-BP1) and mTORC2 (AKT at position Ser473) substrates (96). Therefore, the PI3K/mTOR/S6K signaling pathway should also be investigated in patients with CTLA-4 deficiency.

Besides Tregs dysfunction, patients with LRBA deficiency present defects in the B cell compartment with reduced numbers of switched memory B cells and plasmablasts, impaired immunoglobulin secretion, low proliferative responses, and a high susceptibility to apoptosis (95, 96). In addition, LRBA-deficient B cells show an impairment of the autophagic flux with an abnormal accumulation of autophagosomes (93). Pengo et al. have shown that autophagy is required for plasma cell homeostasis and long-lived humoral immunity by limiting endoplasmic reticulum stress and immunoglobulin synthesis, while sustaining energy metabolism and plasma cell viability (107). The impaired B cell differentiation and hypogammaglobulinemia observed in LRBA-deficient individuals may therefore be attributable to an increased B cell apoptosis and a reduced plasma cell survival due to defective autophagy. In fact, autophagy is also essential for the survival of memory B cells, and for the maintenance of protective antibody responses required to control viral infections in mice (108). In addition, the accumulation of apoptotic cells may trigger as well the development of autoimmunity (109). mTOR plays a key role at the interface of the pathways controlling cell growth and autophagy. Under nutrient starvation, reduced growth factor signaling, or stress conditions, mTOR is inhibited, and autophagy is therefore promoted. Conversely, the activation of the PI3K/AKT pathway negatively regulates autophagy induction (22). It has been previously suggested that LRBA might act as a scaffold protein, coordinating the assembly and activation of mTOR complexes or of protein networks involved in the autophagic process, as well as the recruitment of downstream molecules (96). Future studies addressing mTOR/S6K signaling in the B cell compartment of LRBA-deficient patients may help to further clarify the links between LRBA, autophagy, and B cell homeostasis.

MUTATIONS AFFECTING THE CARD11-BCL10-MALT1 (CBM) SIGNALOSOME COMPLEX ARE RESPONSIBLE FOR NOVEL PID PHENOTYPES WITH AN ABNORMAL ACTIVATION OF THE mTOR/S6K SIGNALING PATHWAY

Upon TCR and CD28 activation, the adapter protein caspase recruitment domain-containing protein 11 (CARD11, also called CARMA1), which is specifically expressed in hematopoietic cells, becomes phosphorylated by protein kinase C and other kinases including AKT (110). Phosphorylated CARD11 recruits B-cell lymphoma/leukemia 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) to form a scaffold called the CBM (CARD11-BCL10-MALT1) signalosome complex that is necessary for optimal activation of the canonical nuclear factorp-κB (NF-κB) pathway (111). Recently, it has been shown that CARD11 and the paracaspase MALT1, but not BCL10, are also required for an optimal activation of the mTOR/S6K pathway in T cells in response to TCR and CD28 co-receptor stimulation (112).

LOF autosomal recessive mutations in *CARD11*, *MALT1*, and *BCL10* are the cause of a new group of CIDs characterized by recurrent sinopulmonary infections, dysregulated B cell development, and abnormal T cell proliferation despite normal lymphocytes counts, due to a defective canonical NF-κB activation after antigen receptor stimulation (113–118). However, these recently described disorders have a distinct phenotype from other known PIDs affecting the NF-κB axis (113). In addition, there are notable differences between the clinical presentation of CARD11, MALT1, and BCL10 deficiencies (113). For instance, CARD11-deficient patients display variable immunoglobulin levels and Tregs numbers, a predominance of *Pneumocystis jirovecii* infections, but no gastrointestinal inflammation, whereas BCL10 deficiency has an impact on lymphocytes (low memory T cells) and fibroblasts but not on myeloid cells. CARD11 and BCL10 deficiencies are both characterized by the lack of autoimmune manifestations despite reduced Treg numbers, possibly reflecting the individual nuanced and independent functions of the CBM proteins (113). CARD11-deficient and *MALT1*-knockdown cells are characterized by a reduced phosphorylation of S6K and S6, emphasizing the role of CARD11 and MALT1 in the mTOR/S6K signaling pathway (112). In addition, the metabolic reprogramming and the proliferation of $CD4^+$ T cells that are also mTORC1 dependent are impaired after MALT1 inhibition (112).

Very recently, Ma et al. have described rare heterozygous hypomorphic *CARD11* mutations in eight individuals from four unrelated families with severe atopic dermatitis (119). The phenotype also included variable cutaneous and respiratory infections (88%), eosinophilia (86%), B cell lymphopenia (29%) with low IgM, but normal or elevated IgA (43%), and hyper-IgE

(71%) (119). Transfection of mutant CARD11 constructs into T cell lines demonstrated both LOF and a dominant-negative effect on mTORC1 (indicated by reduced S6 phosphorylation), but also on NF-κB signaling, at basal state and after antigen-receptor-induced stimulation. Similarly, mTORC1 activity was also attenuated in T cells, and to a lesser extent in B cells, from patients with heterozygous hypomorphic mutations in *CARD11*, whereas AKT phosphorylation on Ser473 (mTORC2-dependent) was normal (119). mTOR activity is known to be crucial for T helper (T_H) cell differentiation (120). Patients' T cells were characterized by an impaired T_H1 cytokine production (low IFN-γ) and a T_H2-skewed phenotype, consistent with their atopic predisposition. The reduced CARD11-dependent mTORC1 activation could contribute to impaired T_H1 differentiation in these patients, allowing mTORC2-dependent T_H2 response to dominate (119). The role of CARD11 in the regulation of mTORC1 activation depends on its ability to facilitate TCR-induced upregulation, but also on its capacity to activate sodium-dependent neutral amino acid transporter type 2 (ASCT2, also known as SLC1A5), an essential amino acid transporter required for extracellular glutamine import during T cell activation (121). Indeed, T cells from patients with germline hypomorphic *CARD11* mutations showed reduced ASCT2 upregulation after TCR activation (119). However, the addition of exogenous glutamine in T cell culture medium was able to boost mTORC1 activation with increased S6 phosphorylation, and to partially correct the T_H1 cell defect including proliferation and IFN-γ production (119). Further studies are required to evaluate whether glutamine supplementation, a very simple therapeutic intervention, could ameliorate atopic dermatitis in patients with *CARD11* mutations (119). This clearly illustrates that a fine comprehension of the mechanisms regulating the mTOR/S6K signaling pathway is an essential prerequisite for a proper improvement of the patients' therapeutic management.

Germline heterozygous GOF mutations in *CARD11* have been linked to a novel congenital B cell lymphoproliferative disorder called BENTA for "B cell Expansion with NF-κB and T cell Anergy" (122, 123). Five different GOF *CARD11* mutations in 16 patients have been described so far (74, 122–124). They abrogate the requirement for antigen receptor engagement in CARD11 activation, resulting in spontaneous CBM signalosome formation, and constitutive NF-κB activation that is responsible for an excessive accumulation of both immature transitional B cells, and polyclonal mature naive B cells (122, 125). BENTA patients develop massive B cell lymphocytosis early in life accompanied by splenomegaly and lymphadenopathy, but without obvious signs of autoimmunity (122, 123). Moreover, GOF *CARD11* mutations can potentially predispose to B cell malignancies (74, 122, 126). Despite excessive B cell accumulation, BENTA disease is associated with an underlying immunodeficiency characterized by low frequencies of circulating memory and class-switched B cells, poor humoral response to T cell-independent polysaccharide-based vaccines, impaired plasma cell differentiation, and low IgM as well as variable IgA/IgG secretion. Recurrent sinopulmonary infections are common, and opportunistic viral infections have been noted in some patients (74). Although circulating T cells are present at normal numbers, they are hyporesponsive upon *in vitro* stimulation, suggesting that they may be anergic (74, 113, 122–124). GOF mutations in *CARD11* affect B and T cells differently, promoting proliferation and survival of B lymphocytes *versus* anergy in T lymphocytes, but the underlying mechanisms remain poorly understood (74). Similarly to LOF *CARD11* mutations, BENTA-associated mutations may therefore perturb other CARD11-dependent downstream signaling cascades including the mTOR/S6K pathway (74). However, to our knowledge, there are currently no published data on mTOR and S6K phosphorylation in the context of BENTA disease.

FUTURE STUDIES SHOULD EXPLORE mTOR/S6K SIGNALING PATHWAY IN T CELLS FROM CARMIL2-DEFICIENT PATIENTS

Biallelic LOF mutations in the gene encoding for the cell membrane-cytoskeleton-associated protein RLTPR (RGD, leucine-rich repeat, tropomodulin and proline-rich-containing protein), also known as CARMIL2 (capping protein regulator and myosin 1 linker 2), have been shown to be responsible for a novel PID disorder characterized by cutaneous and pulmonary allergy, by various bacterial, fungal, and mycobacterial infections, as well as by EBV lymphoproliferation (EBV^+ smooth muscle tumors) (127, 128). In addition to its involvement in cell polarity and migration (129), CARMIL2 plays an important role in T cells by acting as a scaffold protein, bridging CD28 to CARD11 and therefore to the NF-κB signaling axis (130). Mutations in *CARMIL2* prevent the association of CARMIL2 with CARD11 (130). CARMIL2-deficient T cells have a perturbed cytoskeletal organization leading to abnormalities in T cell polarity and migration, but also an impaired CD28-mediated co-signaling with a defective activation of the canonical NF-κB pathway (127, 128, 130). CARMIL2-deficient patients have a normal production of T_H2 cytokines, but a reduced secretion of T_H1, as well as T_H17 effector cytokines, and therefore the strong decrease in Treg numbers does not result in the development of autoimmunity (127, 130). This phenotype is reminiscent of CARD11-deficient patients (119). Considering the newly described role of CARD11 in the mTOR/S6K pathway activation following TCR and CD28 stimulation, future studies should also address this signaling cascade in T cells from CARMIL2-deficient patients.

MUTATIONS IN GENES ENCODING FOR THE CD19-COMPLEX COULD BE ASSOCIATED WITH A DISTURBED PI3K/mTOR/S6K SIGNALING

CD19 is a B cell lineage-specific transmembrane protein expressed from the pro B cell stage until plasma cell differentiation (131). It forms the CD19-complex together with CD21, CD81, and CD225 on the membrane of mature B cells. This complex is recruited to the BCR after ligation by complement (C3d) opsonized antigen *via* the complement receptor 2 (CR2, also known as CD21). This

increases the BCR-mediated signal into B cells, as the BCR itself only delivers a weak tonic signal. CD19, with its many tyrosine residues, amplifies this signal to properly activate B cells (131–133). Biallelic mutations in *CD19*, leading to loss of CD19 membrane expression, to concomitant reduction of CD21 levels, and hence B cell activation, have been described in CVID patients (134–137). Affected patients have recurrent bacterial infections, hypogammaglobulinemia, decreased memory B cell numbers, defective antibody response after vaccination, as well as impaired somatic hypermutation, class-switch recombination, and immunoglobulin repertoire selection (134–138). As expected, they show neither T cell defects nor signs of lymphoproliferation (134–137). However, autoimmune manifestations (thrombocytopenia, glomerulonephritis) and autoantibody production have been reported (134, 135, 137, 139). Since CD81 is required for CD19 expression on the plasma membrane, patients with CD81 deficiency display a phenotype that is highly similar to CD19-deficient patients (140, 141). Upon BCR ligation, CD19 is rapidly phosphorylated at multiple tyrosine residues, leading to the recruitment of various downstream signaling intermediates. A prominent feature of CD19 signaling is the binding of the p85α regulatory subunit and the subsequent activation of class IA PI3K, thereby promoting AKT phosphorylation (132). In the absence of CD19, AKT activity is reduced in B cells (142). However, CD19 amplifies not only BCR signaling, but also plays a crucial role in the regulation of TLR9 responses in human B cells (143). It recruits PI3K and mediates AKT as well as Bruton's tyrosine kinase (BTK) phosphorylation after ligation of nucleic acids, controlling both early B cell activation and proliferation (143). In fact, although AKT phosphorylation at position Ser473 is still induced after BCR triggering in CD19-deficient B cells, it is strongly reduced after CpG stimulation. In addition, inhibition of PI3K and AKT results in TLR9-induced B cell activation defects that are similar to those observed in CD19-deficient B cells (143). Therefore, CD19 deficiency may also be associated with abnormal mTOR/S6K signaling in B cells, but no data are currently available in the literature. However, since the phenotype of p85α-deficient mice is much more severe than the one of CD19-deficient mice, other signaling components might compensate for the loss of CD19 (142, 144–146).

PI3K/mTOR/S6K SIGNALING SHOULD BE INVESTIGATED IN ICOS-DEFICIENT PATIENTS

Inducible T-cell costimulator (ICOS, also known as CD278) is another member of the CD28 T cell co-stimulatory molecules family (147). CD28 is expressed in resting and activated T cells, whereas ICOS expression is induced only upon T cell activation. Like CD28, ICOS delivers a positive signal that sustains T cell responses, and it is crucial for cell proliferation as well as cytokine production (148). CD28 and ICOS share a common signaling pathway, including PI3K recruitment (149, 150). In addition, ICOS plays an essential role in T_{FH} differentiation as well as in germinal center formation, and hence in isotype switching and in the development of memory B cells (151, 152). ICOS deficiency was the first monogenic defect reported to cause CVID (153). To date, homozygous mutations (deletions) in *ICOS* have been identified in 16 patients, resulting in the absence of ICOS protein on T cells (153–158). ICOS deficiency was initially considered as a "predominantly antibody deficiency" by the IUIS PID expert committee (159), but following published patients with more complex phenotypes [reviewed by Ref. (154)], allowed a reclassification of the disease as a CID (2, 3). Besides hypogammaglobulinemia (93% of the cases) associated with an increased susceptibility to bacterial infections, more than two-thirds of the patients presented with autoimmunity and immune dysregulation (mainly enteropathy and psoriasis). Viral and opportunistic infections were frequently observed, and two patients developed malignancies (154). ICOS deficiency is associated with several immunological abnormalities including decreased numbers of switched memory B cells and circulating $CXCR5^+$ T_{FH} that coincide with an impaired germinal center formation (151, 154). B cell counts seem to decline progressively during the course of the disease, possibly as a consequence of a bone marrow production failure. IL-17 levels are markedly decreased in all patients who have been assessed for cytokine production, but without being associated with an increased susceptibility to *Candida* infection (154). ICOS is responsible for a greater PI3K activity than CD28, leading to a strong subsequent phosphorylation of AKT (150, 160). It bears a unique YMFM motif in its cytoplasmic tail that binds to the p85α regulatory subunit of PI3K (149).

In addition, ICOS interaction with its ligand ICOSL induces the recruitment of the PI3K regulatory subunit p50α at the synapse of T cell/antigen-presenting cells conjugates (160). ICOS deficiency should therefore be associated with impaired PI3K signaling. The activity of PI3K, as well as of downstream effector signaling molecules including mTOR and S6K, should be explored in T cells from ICOS-deficient individuals.

Regarding CD28, no PID has been associated so far with mutations in the gene encoding for this other T cell co-stimulatory receptor.

CONCLUSION

There are several lines of evidence that link the PI3K/AKT/mTOR/S6K signaling pathway to PIDs. Further studies are nevertheless required to characterize more deeply the crosstalk between the PI3K/AKT/mTOR/S6K cascade and other signaling molecules, as well as the disease-specific defects. Understanding the genetics and mechanisms behind the "immune TOR-opathies" is crucial to improve the management of the patients. The use of inhibitors such as mTOR and PI3K inhibitors that specifically target this signaling pathway and could restore properly the immune function represent very promising therapeutic approaches. Selective PI3K inhibitors should be considered as future treatment options, in particular in APDS patients, with encouraging preliminary results in ongoing clinical trials.

AUTHOR CONTRIBUTIONS

SJ, LG-D, and BG wrote the review. MP prepared the figure. All authors concur with the submission.

ACKNOWLEDGMENTS

We would like to thank Dr. F. Gros for proofreading of the manuscript.

FUNDING

SJ is supported by grants from Hôpitaux Universitaires de Strasbourg (HUS), Université de Strasbourg (UdS), Centre National de la Recherche Scientifique (CNRS), and by EU-funded (ERDF, European Regional Development Fund) project INTERREG V Upper Rhine programma "RARENET." BG, LG-D, and MP are supported by the German Ministry for Education and Research (BMBF, Bundesministerium für Bildung und Forschung) with the grants IFB/CCI:01EO1303 and E:med/SysInflame:01ZX1306F, by the German Center for Infection Research (DZIF, Deutsches Zentrum für Infektionsforschung): 8000805-3, and by the German Research Society (DFG, Deutsche Forschungsgemeinschaft) with the grants GR 1617/8-1 and SFB1160-IMPATH.

REFERENCES

1. Crino PB. Focal brain malformations: a spectrum of disorders along the mTOR cascade. *Novartis Found Symp* (2007) 288:260–72; discussion 272–81.
2. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. *J Clin Immunol* (2018) 38:96–128. doi:10.1007/s10875-017-0464-9
3. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* (2018) 38:129–43. doi:10.1007/s10875-017-0465-8
4. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova J-L, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. *J Clin Immunol* (2013) 33:1–7. doi:10.1007/s10875-012-9751-7
5. Fischer A. Human primary immunodeficiency diseases: a perspective. *Nat Immunol* (2004) 5:23–30. doi:10.1038/ni1023
6. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* (2010) 125:S182–94. doi:10.1016/j.jaci.2009.07.053
7. Maródi L, Notarangelo LD. Immunological and genetic bases of new primary immunodeficiencies. *Nat Rev Immunol* (2007) 7:851–61. doi:10.1038/nri2195
8. Huang K, Fingar DC. Growing knowledge of the mTOR signaling network. *Semin Cell Dev Biol* (2014) 36:79–90. doi:10.1016/j.semcdb.2014.09.011
9. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol* (2012) 30:39–68. doi:10.1146/annurev-immunol-020711-075024
10. Crino PB. mTOR: a pathogenic signaling pathway in developmental brain malformations. *Trends Mol Med* (2011) 17:734–42. doi:10.1016/j.molmed.2011.07.008
11. Ismail HMS. Downstream the mTOR: S6 kinases between divergence and redundancy. *J Biochem Pharmacol Res* (2013) 1(2):94–105.
12. Okkenhaug K, Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* (2003) 3:317–30. doi:10.1038/nri1056
13. Okkenhaug K. Signalling by the phosphoinositide 3-kinase family in immune cells. *Annu Rev Immunol* (2013) 31:675–704. doi:10.1146/annurev-immunol-032712-095946
14. Chantry D, Vojtek A, Kashishian A, Holtzman DA, Wood C, Gray PW, et al. p110delta, a novel phosphatidylinositol 3-kinase catalytic subunit that associates with p85 and is expressed predominantly in leukocytes. *J Biol Chem* (1997) 272:19236–41. doi:10.1074/jbc.272.31.19236
15. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* (2007) 129:1261–74. doi:10.1016/j.cell.2007.06.009
16. Inoki K, Li Y, Xu T, Guan K-L. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes Dev* (2003) 17:1829–34. doi:10.1101/gad.1110003
17. Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, et al. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* (2002) 110:177–89. doi:10.1016/S0092-8674(02)00833-4
18. Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* (1991) 253:905–9. doi:10.1126/science.1715094
19. Kim D-H, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* (2002) 110:163–75. doi:10.1016/S0092-8674(02)00808-5
20. Sarbassov DD, Ali SM, Kim D-H, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* (2004) 14:1296–302. doi:10.1016/j.cub.2004.06.054
21. Kim J, Kundu M, Viollet B, Guan K-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* (2011) 13:132–41. doi:10.1038/ncb2152
22. Jung CH, Ro S-H, Cao J, Otto NM, Kim D-H. mTOR regulation of autophagy. *FEBS Lett* (2010) 584:1287–95. doi:10.1016/j.febslet.2010.01.017
23. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* (2011) 469:323–35. doi:10.1038/nature09782
24. Limon JJ, Fruman DA. Akt and mTOR in B cell activation and differentiation. *Front Immunol* (2012) 3:228. doi:10.3389/fimmu.2012.00228
25. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* (1998) 95:29–39. doi:10.1016/S0092-8674(00)81780-8
26. Tavares MR, Pavan ICB, Amaral CL, Meneguello L, Luchessi AD, Simabuco FM. The S6K protein family in health and disease. *Life Sci* (2015) 131:1–10. doi:10.1016/j.lfs.2015.03.001
27. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* (2012) 149:274–93. doi:10.1016/j.cell.2012.03.017
28. Magnuson B, Ekim B, Fingar DC. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochem J* (2012) 441:1–21. doi:10.1042/BJ20110892
29. Pardo OE, Seckl MJ. S6K2: the neglected S6 kinase family member. *Front Oncol* (2013) 3:191. doi:10.3389/fonc.2013.00191
30. Kurebayashi Y, Nagai S, Ikejiri A, Ohtani M, Ichiyama K, Baba Y, et al. PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1 expression and nuclear translocation of RORγ. *Cell Rep* (2012) 1:360–73. doi:10.1016/j.celrep.2012.02.007
31. Pai C, Walsh CM, Fruman DA. Context-specific function of S6K2 in Th cell differentiation. *J Immunol* (2016) 197:3049–58. doi:10.4049/jimmunol.1600167
32. Ikenoue T, Hong S, Inoki K. Monitoring mammalian target of rapamycin (mTOR) activity. *Methods Enzymol* (2009) 452:165–80. doi:10.1016/S0076-6879(08)03611-2
33. So L, Lee J, Palafox M, Mallya S, Woxland CG, Arguello M, et al. The 4E-BP-eIF4E axis promotes rapamycin-sensitive growth and proliferation in lymphocytes. *Sci Signal* (2016) 9:ra57. doi:10.1126/scisignal.aad8463
34. Ip CKM, Cheung ANY, Ngan HYS, Wong AST. p70 S6 kinase in the control of actin cytoskeleton dynamics and directed migration of ovarian cancer cells. *Oncogene* (2011) 30:2420–32. doi:10.1038/onc.2010.615
35. Zhang S, Readinger JA, DuBois W, Janka-Junttila M, Robinson R, Pruitt M, et al. Constitutive reductions in mTOR alter cell size, immune cell development, and antibody production. *Blood* (2011) 117:1228–38. doi:10.1182/blood-2010-05-287821
36. Cobbold SP, Adams E, Farquhar CA, Nolan KF, Howie D, Lui KO, et al. Infectious tolerance via the consumption of essential amino acids and mTOR signaling. *Proc Natl Acad Sci U S A* (2009) 106:12055–60. doi:10.1073/pnas.0903919106
37. Cobbold SP. The mTOR pathway and integrating immune regulation. *Immunology* (2013) 140:391–8. doi:10.1111/imm.12162

38. Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. *Proc Natl Acad Sci U S A* (2008) 105:7797–802. doi:10.1073/pnas.0800928105
39. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish Treg cell function. *Nature* (2013) 499:485–90. doi:10.1038/nature12297
40. Espeillac C, Mitchell C, Celton-Morizur S, Chauvin C, Koka V, Gillet C, et al. S6 kinase 1 is required for rapamycin-sensitive liver proliferation after mouse hepatectomy. *J Clin Invest* (2011) 121:2821–32. doi:10.1172/JCI44203
41. Shima H, Pende M, Chen Y, Fumagalli S, Thomas G, Kozma SC. Disruption of the p70(s6k)/p85(s6k) gene reveals a small mouse phenotype and a new functional S6 kinase. *EMBO J* (1998) 17:6649–59. doi:10.1093/emboj/17.22.6649
42. Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, et al. S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway. *Mol Cell Biol* (2004) 24:3112–24. doi:10.1128/MCB.24.8.3112-3124.2004
43. Panić L, Tamarut S, Sticker-Jantscheff M, Barkić M, Solter D, Uzelac M, et al. Ribosomal protein S6 gene haploinsufficiency is associated with activation of a p53-dependent checkpoint during gastrulation. *Mol Cell Biol* (2006) 26:8880–91. doi:10.1128/MCB.00751-06
44. Sulic S, Panic L, Barkic M, Mercep M, Uzelac M, Volarevic S. Inactivation of S6 ribosomal protein gene in T lymphocytes activates a p53-dependent checkpoint response. *Genes Dev* (2005) 19:3070–82. doi:10.1101/gad.359305
45. Ruvinsky I, Katz M, Dreazen A, Gielchinsky Y, Saada A, Freedman N, et al. Mice deficient in ribosomal protein S6 phosphorylation suffer from muscle weakness that reflects a growth defect and energy deficit. *PLoS One* (2009) 4:e5618. doi:10.1371/journal.pone.0005618
46. Salmond RJ, Brownlie RJ, Meyuhas O, Zamoyska R. Mechanistic target of rapamycin complex 1/S6 kinase 1 signals influence T cell activation independently of ribosomal protein S6 phosphorylation. *J Immunol* (2015) 195:4615–22. doi:10.4049/jimmunol.1501473
47. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kδ and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16:702–14. doi:10.1038/nri.2016.93
48. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36(5):462–71. doi:10.1007/s10875-016-0281-6
49. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29–31. doi:10.1016/j.clim.2017.05.020
50. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110δ cause activated PI3Kδ syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017) 140(4):1152–6.e10. doi:10.1016/j.jaci.2017.03.026
51. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Schölvinck L, Kalina T, et al. Genetic defects in PI3Kδ affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
52. Angulo I, Vadas O, Garçon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi:10.1126/science.1243292
53. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. A case of APDS patient: defects in maturation and function and decreased in vitro anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2015) 178:20–8. doi:10.1016/j.clim.2015.12.008
54. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139:597–606.e4. doi:10.1016/j.jaci.2016.06.021
55. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34:272–6. doi:10.1007/s10875-014-0012-9
56. Deau M-C, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124:3923–8. doi:10.1172/JCI75746
57. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183:221–9. doi:10.1111/cei.12706
58. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138:210–8.e9. doi:10.1016/j.jaci.2016.03.022
59. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35:11–4. doi:10.1007/s10875-014-0109-1
60. Jou S-T, Chien Y-H, Yang Y-H, Wang T-C, Shyur S-D, Chou C-C, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33:361–9. doi:10.1111/j.1744-313X.2006.00627.x
61. Kracker S, Curtis J, Ibrahim MAA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase δ syndrome. *J Allergy Clin Immunol* (2014) 134:233–6. doi:10.1016/j.jaci.2014.02.020
62. Kuhlen M, Hönscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
63. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159:33–6. doi:10.1016/j.clim.2015.04.014
64. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
65. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211:2537–47. doi:10.1084/jem.20141759
66. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kδ syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27:640–4. doi:10.1111/pai.12585
67. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase δ syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
68. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh T-W, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138:1672–80.e10. doi:10.1016/j.jaci.2016.03.055
69. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang Y-D, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85α subunit of PI3K. *J Exp Med* (2012) 209:463–70. doi:10.1084/jem.20112533
70. Suzuki A, Kaisho T, Ohishi M, Tsukio-Yamaguchi M, Tsubata T, Koni PA, et al. Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. *J Exp Med* (2003) 197:657–67. doi:10.1084/jem.20021101
71. Suzuki A, Yamaguchi MT, Ohteki T, Sasaki T, Kaisho T, Kimura Y, et al. T cell-specific loss of Pten leads to defects in central and peripheral tolerance. *Immunity* (2001) 14:523–34. doi:10.1016/S1074-7613(01)00134-0
72. Sullivan JA, Kim EH, Plisch EH, Peng SL, Suresh M. FOXO3 regulates CD8 T cell memory by T cell-intrinsic mechanisms. *PLoS Pathog* (2012) 8:e1002533. doi:10.1371/journal.ppat.1002533
73. Van der Windt GJW, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* (2012) 249:27–42. doi:10.1111/j.1600-065X.2012.01150.x

74. Arjunaraja S, Snow AL. Gain-of-function mutations and immunodeficiency: at a loss for proper tuning of lymphocyte signaling. *Curr Opin Allergy Clin Immunol* (2015) 15:533–8. doi:10.1097/ACI.0000000000000217
75. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kδ regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114:1982–7. doi:10.1073/pnas.1617244114
76. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective "activated PI3Kδ syndrome"-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130:2307–16. doi:10.1182/blood-2017-08-801191
77. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* (1998) 273:13375–8. doi:10.1074/jbc.273.22.13375
78. Mester J, Eng C. When overgrowth bumps into cancer: the PTEN-opathies. *Am J Med Genet C Semin Med Genet* (2013) 163:114–21. doi:10.1002/ajmg.c.31364
79. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, et al. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. *Curr Biol* (1998) 8:1169–78. doi:10.1016/S0960-9822(07)00488-5
80. Cristofano AD, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. *Nat Genet* (1998) 19: 348–55. doi:10.1038/1235
81. Cristofano AD, Kotsi P, Peng YF, Cordon-Cardo C, Elkon KB, Pandolfi PP. Impaired Fas response and autoimmunity in Pten$^{+/-}$ mice. *Science* (1999) 285:2122–5. doi:10.1126/science.285.5436.2122
82. Anzelon AN, Wu H, Rickert RC. Pten inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. *Nat Immunol* (2003) 4:287–94. doi:10.1038/ni892
83. Omori SA, Cato MH, Anzelon-Mills A, Puri KD, Shapiro-Shelef M, Calame K, et al. Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling. *Immunity* (2006) 25:545–57. doi:10.1016/j.immuni.2006.08.015
84. Liaw D, Marsh DJ, Li J, Dahia PLM, Wang SI, Zheng Z, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* (1997) 16:64–7. doi:10.1038/ng0597-64
85. Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, et al. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nat Genet* (1997) 16:333–4. doi:10.1038/ng0897-333
86. Eng C, Murday V, Seal S, Mohammed S, Hodgson SV, Chaudary MA, et al. Cowden syndrome and Lhermitte-Duclos disease in a family: a single genetic syndrome with pleiotropy? *J Med Genet* (1994) 31:458–61. doi:10.1136/jmg.31.6.458
87. Browning MJ, Chandra A, Carbonaro V, Okkenhaug K, Barwell J. Cowden's syndrome with immunodeficiency. *J Med Genet* (2015) 52:856–9. doi:10.1136/jmedgenet-2015-103266
88. Chen HH, Händel N, Ngeow J, Muller J, Hühn M, Yang H-T, et al. Immune dysregulation in patients with PTEN hamartoma tumor syndrome: analysis of FOXP3 regulatory T cells. *J Allergy Clin Immunol* (2016) 139(2):607–20. e15. doi:10.1016/j.jaci.2016.03.059
89. Driessen GJ, IJspeert H, Wentink M, Yntema HG, van Hagen PM, van Strien A, et al. Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency. *J Allergy Clin Immunol* (2016) 138:1744–7.e5. doi:10.1016/j.jaci.2016.07.010
90. Werner M, Hobeika E, Jumaa H. Role of PI3K in the generation and survival of B cells. *Immunol Rev* (2010) 237:55–71. doi:10.1111/j.1600-065X.2010.00934.x
91. Wang JW, Howson J, Haller E, Kerr WG. Identification of a novel lipopolysaccharide-inducible gene with key features of both A kinase anchor proteins and chs1/beige proteins. *J Immunol* (2001) 166:4586–95. doi:10.4049/jimmunol.166.7.4586
92. Cullinane AR, Schäffer AA, Huizing M. The BEACH is hot: a LYST of emerging roles for BEACH-domain containing proteins in human disease. *Traffic* (2013) 14:749–66. doi:10.1111/tra.12069
93. Lopez-Herrera G, Tampella G, Pan-Hammarström Q, Herholz P, Trujillo-Vargas CM, Phadwal K, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *Am J Hum Genet* (2012) 90:986–1001. doi:10.1016/j.ajhg.2012.04.015
94. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of phenotypes associated with mutations in LRBA. *J Clin Immunol* (2016) 36:33–45. doi:10.1007/s10875-015-0224-7
95. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M, et al. The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. *J Allergy Clin Immunol* (2016) 137:223–30. doi:10.1016/j.jaci.2015.09.025
96. Charbonnier L-M, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. *J Allergy Clin Immunol* (2015) 135:217–27. doi:10.1016/j.jaci.2014.10.019
97. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* (2015) 348:56–61. doi:10.1126/science.aaa8172
98. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science* (2015) 349:436–40. doi:10.1126/science.aaa1663
99. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* (2011) 332:600–3. doi:10.1126/science.1202947
100. Schubert D, Bode C, Kenefeck R, Hou TZ, Wing JB, Kennedy A, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat Med* (2014) 20:1410–6. doi:10.1038/nm.3746
101. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science* (2014) 345:1623–7. doi:10.1126/science.1255904
102. Lo B, Fritz JM, Su HC, Uzel G, Jordan MB, Lenardo MJ. CHAI and LATAIE: new genetic diseases of CTLA-4 checkpoint insufficiency. *Blood* (2016) 128:1037–42. doi:10.1182/blood-2016-04-712612
103. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev* (2009) 229:12–26. doi:10.1111/j.1600-065X.2009.00770.x
104. Schneider H, Prasad KV, Shoelson SE, Rudd CE. CTLA-4 binding to the lipid kinase phosphatidylinositol 3-kinase in T cells. *J Exp Med* (1995) 181:351–5. doi:10.1084/jem.181.1.351
105. Schneider H, Valk E, Leung R, Rudd CE. CTLA-4 activation of phosphatidylinositol 3-kinase (PI 3-K) and protein kinase B (PKB/AKT) sustains T-cell anergy without cell death. *PLoS One* (2008) 3:e3842. doi:10.1371/journal.pone.0003842
106. Walker LSK, Sansom DM. Confusing signals: recent progress in CTLA-4 biology. *Trends Immunol* (2015) 36:63–70. doi:10.1016/j.it.2014.12.001
107. Pengo N, Scolari M, Oliva L, Milan E, Mainoldi F, Raimondi A, et al. Plasma cells require autophagy for sustainable immunoglobulin production. *Nat Immunol* (2013) 14:298–305. doi:10.1038/ni.2524
108. Chen M, Hong MJ, Sun H, Wang L, Shi X, Gilbert BE, et al. Essential role for autophagy in the maintenance of immunological memory against influenza infection. *Nat Med* (2014) 20:503–10. doi:10.1038/nm.3521
109. Muñoz LE, Lauber K, Schiller M, Manfredi AA, Herrmann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. *Nat Rev Rheumatol* (2010) 6:280–9. doi:10.1038/nrrheum.2010.46
110. Roche MI, Ramadas RA, Medoff BD. The role of CARMA1 in T cells. *Crit Rev Immunol* (2013) 33:219–43. doi:10.1615/CritRevImmunol.2013007056
111. Jun JE, Wilson LE, Vinuesa CG, Lesage S, Blery M, Miosge LA, et al. Identifying the MAGUK protein Carma-1 as a central regulator of humoral immune responses and atopy by genome-wide mouse mutagenesis. *Immunity* (2003) 18:751–62. doi:10.1016/S1074-7613(03)00141-9
112. Hamilton KS, Phong B, Corey C, Cheng J, Gorentla B, Zhong X, et al. A Carma1/MALT1-dependent, Bcl10-independent, pathway regulates antigen receptor-mediated mTOR signaling in T cells. *Sci Signal* (2014) 7:ra55. doi:10.1126/scisignal.2005169
113. Turvey SE, Durandy A, Fischer A, Fung S-Y, Geha RS, Gewies A, et al. The CARD11-BCL10-MALT1 (CBM) signalosome complex: stepping into the limelight of human primary immunodeficiency. *J Allergy Clin Immunol* (2014) 134:276–84. doi:10.1016/j.jaci.2014.06.015
114. Jabara HH, Ohsumi T, Chou J, Massaad MJ, Benson H, Megarbane A, et al. A homozygous mucosa-associated lymphoid tissue 1 (MALT1) mutation in a family with combined immunodeficiency. *J Allergy Clin Immunol* (2013) 132:151–8. doi:10.1016/j.jaci.2013.04.047
115. McKinnon ML, Rozmus J, Fung S-Y, Hirschfeld AF, Del Bel KL, Thomas L, et al. Combined immunodeficiency associated with homozygous MALT1 mutations. *J Allergy Clin Immunol* (2014) 133:1458–62, 1462.e1–7. doi:10.1016/j.jaci.2013.10.045

116. Stepensky P, Keller B, Buchta M, Kienzler A-K, Elpeleg O, Somech R, et al. Deficiency of caspase recruitment domain family, member 11 (CARD11), causes profound combined immunodeficiency in human subjects. *J Allergy Clin Immunol* (2013) 131:477–85.e1. doi:10.1016/j.jaci.2012.11.050
117. Greil J, Rausch T, Giese T, Bandapalli OR, Daniel V, Bekeredjian-Ding I, et al. Whole-exome sequencing links caspase recruitment domain 11 (CARD11) inactivation to severe combined immunodeficiency. *J Allergy Clin Immunol* (2013) 131:1376–83.e3. doi:10.1016/j.jaci.2013.02.012
118. Torres JM, Martinez-Barricarte R, García-Gómez S, Mazariegos MS, Itan Y, Boisson B, et al. Inherited BCL10 deficiency impairs hematopoietic and nonhematopoietic immunity. *J Clin Invest* (2014) 124:5239–48. doi:10.1172/JCI77493
119. Ma CA, Stinson JR, Zhang Y, Abbott JK, Weinreich MA, Hauk PJ, et al. Germline hypomorphic CARD11 mutations in severe atopic disease. *Nat Genet* (2017) 49:1192–201. doi:10.1038/ng.3898
120. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol* (2014) 14:435–46. doi:10.1038/nri3701
121. Nakaya M, Xiao Y, Zhou X, Chang J-H, Chang M, Cheng X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* (2014) 40:692–705. doi:10.1016/j.immuni.2014.04.007
122. Snow AL, Xiao W, Stinson JR, Lu W, Chaigne-Delalande B, Zheng L, et al. Congenital B cell lymphocytosis explained by novel germline CARD11 mutations. *J Exp Med* (2012) 209:2247–61. doi:10.1084/jem.20120831
123. Brohl AS, Stinson J, Su HC, Badgett T, Jennings CD, Sukumar G, et al. Germline CARD11 mutation in a patient with severe congenital B cell lymphocytosis. *J Clin Immunol* (2015) 35:32–46. doi:10.1007/s10875-014-0106-4
124. Buchbinder D, Stinson JR, Nugent DJ, Heurtier L, Suarez F, Sukumar G, et al. Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation. *J Allergy Clin Immunol* (2015) 136:819–21.e1. doi:10.1016/j.jaci.2015.03.008
125. Chan W, Schaffer TB, Pomerantz JL. A quantitative signaling screen identifies CARD11 mutations in the CARD and LATCH domains that induce Bcl10 ubiquitination and human lymphoma cell survival. *Mol Cell Biol* (2013) 33:429–43. doi:10.1128/MCB.00850-12
126. Darte JM, McClure PD, Saunders EF, Weber JL, Donohue WL. Congenital lymphoid hyperplasia with persistent hyperlymphocytosis. *N Engl J Med* (1971) 284:431–2. doi:10.1056/NEJM197102252840807
127. Wang Y, Ma CS, Ling Y, Bousfiha A, Camcioglu Y, Jacquot S, et al. Dual T cell- and B cell-intrinsic deficiency in humans with biallelic RLTPR mutations. *J Exp Med* (2016) 213:2413–35. doi:10.1084/jem.20160576
128. Schober T, Magg T, Laschinger M, Rohlfs M, Linhares ND, Puchalka J, et al. A human immunodeficiency syndrome caused by mutations in CARMIL2. *Nat Commun* (2017) 8:14209. doi:10.1038/ncomms14209
129. Liang Y, Niederstrasser H, Edwards M, Jackson CE, Cooper JA. Distinct roles for CARMIL isoforms in cell migration. *Mol Biol Cell* (2009) 20:5290–305. doi:10.1091/mbc.E08-10-1071
130. Roncagalli R, Cucchetti M, Jarmuzynski N, Grégoire C, Bergot E, Audebert S, et al. The scaffolding function of the RLTPR protein explains its essential role for CD28 co-stimulation in mouse and human T cells. *J Exp Med* (2016) 213:2437–57. doi:10.1084/jem.20160579
131. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. *Exp Hematol Oncol* (2012) 1:36. doi:10.1186/2162-3619-1-36
132. Carter RH, Doody GM, Bolen JB, Fearon DT. Membrane IgM-induced tyrosine phosphorylation of CD19 requires a CD19 domain that mediates association with components of the B cell antigen receptor complex. *J Immunol* (1997) 158:3062–9.
133. Carter RH, Fearon DT. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* (1992) 256:105–7. doi:10.1126/science.1373518
134. Kanegane H, Agematsu K, Futatani T, Sira MM, Suga K, Sekiguchi T, et al. Novel mutations in a Japanese patient with CD19 deficiency. *Genes Immun* (2007) 8:663–70. doi:10.1038/sj.gene.6364431
135. van Zelm MC, Smet J, van der Burg M, Ferster A, Le PQ, Schandené L, et al. Antibody deficiency due to a missense mutation in CD19 demonstrates the importance of the conserved tryptophan 41 in immunoglobulin superfamily domain formation. *Hum Mol Genet* (2011) 20:1854–63. doi:10.1093/hmg/ddr068
136. Van Zelm MC, Reisli I, van der Burg M, Castaño D, van Noesel CJM, van Tol MJD, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med* (2006) 354:1901–12. doi:10.1056/NEJMoa051568
137. Vince N, Boutboul D, Mouillot G, Just N, Peralta M, Casanova J-L, et al. Defects in the CD19 complex predispose to glomerulonephritis, as well as IgG1 subclass deficiency. *J Allergy Clin Immunol* (2011) 127:538–41.e1–5. doi:10.1016/j.jaci.2010.10.019
138. Van Zelm MC, Bartol SJW, Driessen GJ, Mascart F, Reisli I, Franco JL, et al. Human CD19 and CD40L deficiencies impair antibody selection and differentially affect somatic hypermutation. *J Allergy Clin Immunol* (2014) 134:135–44.e7. doi:10.1016/j.jaci.2013.11.015
139. Artac H, Reisli I, Kara R, Pico-Knijnenburg I, Adin-Çinar S, Pekcan S, et al. B-cell maturation and antibody responses in individuals carrying a mutated CD19 allele. *Genes Immun* (2010) 11:523–30. doi:10.1038/gene.2010.22
140. Vences-Catalán F, Kuo C-C, Sagi Y, Chen H, Kela-Madar N, van Zelm MC, et al. A mutation in the human tetraspanin CD81 gene is expressed as a truncated protein but does not enable CD19 maturation and cell surface expression. *J Clin Immunol* (2015) 35:254–63. doi:10.1007/s10875-015-0148-2
141. Van Zelm MC, Smet J, Adams B, Mascart F, Schandené L, Janssen F, et al. CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J Clin Invest* (2010) 120:1265–74. doi:10.1172/JCI39748
142. Otero DC, Omori SA, Rickert RC. Cd19-dependent activation of Akt kinase in B-lymphocytes. *J Biol Chem* (2001) 276:1474–8. doi:10.1074/jbc.M003918200
143. Morbach H, Schickel J-N, Cunningham-Rundles C, Conley ME, Reisli I, Franco JL, et al. CD19 controls TLR9 responses in human B cells. *J Allergy Clin Immunol* (2016) 137:889–98.e6. doi:10.1016/j.jaci.2015.08.040
144. Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, et al. Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. *Science* (1999) 283:393–7. doi:10.1126/science.283.5400.393
145. Rickert RC, Rajewsky K, Roes J. Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. *Nature* (1995) 376:352–5. doi:10.1038/376352a0
146. Engel P, Zhou LJ, Ord DC, Sato S, Koller B, Tedder TF. Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. *Immunity* (1995) 3:39–50. doi:10.1016/1074-7613(95)90157-4
147. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* (2002) 2:116–26. doi:10.1038/nri727
148. Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* (1999) 397:263–6. doi:10.1038/16717
149. Coyle AJ, Lehar S, Lloyd C, Tian J, Delaney T, Manning S, et al. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity* (2000) 13:95–105. doi:10.1016/S1074-7613(00)00011-X
150. Parry RV, Rumbley CA, Vandenberghe LH, June CH, Riley JL. CD28 and inducible costimulatory protein Src homology 2 binding domains show distinct regulation of phosphatidylinositol 3-kinase, Bcl-xL, and IL-2 expression in primary human CD4 T lymphocytes. *J Immunol* (2003) 171:166–74. doi:10.4049/jimmunol.171.1.166
151. Warnatz K, Bossaller L, Salzer U, Skrabl-Baumgartner A, Schwinger W, van der Burg M, et al. Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood* (2006) 107:3045–52. doi:10.1182/blood-2005-07-2955
152. Akiba H, Takeda K, Kojima Y, Usui Y, Harada N, Yamazaki T, et al. The role of ICOS in the CXCR5+ follicular B helper T cell maintenance in vivo. *J Immunol* (2005) 175:2340–8. doi:10.4049/jimmunol.175.4.2340
153. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat Immunol* (2003) 4:261–8. doi:10.1038/ni902
154. Schepp J, Chou J, Skrabl-Baumgartner A, Arkwright PD, Engelhardt KR, Hambleton S, et al. 14 Years after discovery: clinical follow-up on 15 patients with inducible co-stimulator deficiency. *Front Immunol* (2017) 8:964. doi:10.3389/fimmu.2017.00964
155. Chou J, Massaad MJ, Cangemi B, Bainter W, Platt C, Badran YR, et al. A novel mutation in ICOS presenting as hypogammaglobulinemia with susceptibility

to opportunistic pathogens. *J Allergy Clin Immunol* (2015) 136:794–7.e1. doi:10.1016/j.jaci.2014.12.1940

156. Salzer U, Maul-Pavicic A, Cunningham-Rundles C, Urschel S, Belohradsky BH, Litzman J, et al. ICOS deficiency in patients with common variable immunodeficiency. *Clin Immunol* (2004) 113:234–40. doi:10.1016/j.clim.2004.07.002
157. Takahashi N, Matsumoto K, Saito H, Nanki T, Miyasaka N, Kobata T, et al. Impaired CD4 and CD8 effector function and decreased memory T cell populations in ICOS-deficient patients. *J Immunol* (2009) 182:5515–27. doi:10.4049/jimmunol.0803256
158. Robertson N, Engelhardt KR, Morgan NV, Barge D, Cant AJ, Hughes SM, et al. Astute Clinician Report: a novel 10 bp frameshift deletion in exon 2 of ICOS causes a combined immunodeficiency associated with an enteritis and hepatitis. *J Clin Immunol* (2015) 35:598–603. doi:10.1007/s10875-015-0193-x
159. Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* (2014) 5:162. doi:10.3389/fimmu.2014.00162
160. Fos C, Salles A, Lang V, Carrette F, Audebert S, Pastor S, et al. ICOS ligation recruits the p50alpha PI3K regulatory subunit to the immunological synapse. *J Immunol* (2008) 181:1969–77. doi:10.4049/jimmunol.181.3.1969

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

PERSPECTIVE
published: 02 August 2018
doi: 10.3389/fimmu.2018.01758

Genetic Defects in Phosphoinositide 3-Kinase δ Influence CD8+ T Cell Survival, Differentiation, and Function

*Jennifer L. Cannons[1,2], Silvia Preite[1,2], Senta M. Kapnick[1], Gulbu Uzel[2] and Pamela L. Schwartzberg[1,2]**

[1] National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States, [2] National Institutes of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD, United States

Activated phosphoinositide 3-kinase delta syndrome (APDS), also known as p110 delta-activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency (PASLI), is an autosomal dominant primary human immunodeficiency (PID) caused by heterozygous gain-of-function mutations in *PIK3CD*, which encodes the p110δ catalytic subunit of PI3K. This recently described PID is characterized by diverse and heterogeneous clinical manifestations that include recurrent respiratory infections, lymphoproliferation, progressive lymphopenia, and defective antibody responses. A major clinical manifestation observed in the NIH cohort of patients with *PIK3CD* mutations is chronic Epstein–Barr virus (EBV) and/or cytomegalovirus viremia. Despite uncontrolled EBV infection, many APDS/PASLI patients had normal or higher frequencies of EBV-specific CD8+ T cells. In this review, we discuss data pertaining to CD8+ T cell function in APDS/PASLI, including increased cell death, expression of exhaustion markers, and altered killing of autologous EBV-infected B cells, and how these and other data on PI3K provide insight into potential cellular defects that prevent clearance of chronic infections.

Keywords: Epstein–Barr virus, activated phosphoinositide 3-kinase delta syndrome, p110δ activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency, cytotoxic T lymphocyte, primary human immunodeficiency

OPEN ACCESS

Edited by:
Carrie L. Lucas, Yale University, United States

Reviewed by:
Shigeaki Nonoyama, National Defense Medical College, Japan
Michel Massaad, American University of Beirut Medical Center, Lebanon

***Correspondence:**
Pamela L. Schwartzberg
pams@mail.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *21 March 2018*
Accepted: *16 July 2018*
Published: *02 August 2018*

Citation:
Cannons JL, Preite S, Kapnick SM, Uzel G and Schwartzberg PL (2018) Genetic Defects in Phosphoinositide 3-Kinase δ Influence CD8+ T Cell Survival, Differentiation, and Function. Front. Immunol. 9:1758. doi: 10.3389/fimmu.2018.01758

INTRODUCTION

Cytotoxic CD8+ T lymphocytes (CTLs) are critical for the elimination of virally infected and tumor targets. Following T cell receptor (TCR) engagement in conjunction with cytokine signals, such as IL-2 and IL-12, naïve CD8+ T cells rapidly proliferate and differentiate from a "naïve" antigen-inexperienced state into an effector state characterized by the expression of cytolytic proteins (1). Upon subsequent engagement with targets, CTLs carry out their effector function through the directed release of cytoplasmic granules containing granzymes and other cytolytic effectors, as well as *via* cytokine secretion (1). CTLs tightly regulate the initiation and termination of granule secretion, a process critical for efficient and precise serial killing (2, 3).

After the resolution of infection, most CTLs are eliminated, although a fraction persist as long-lived memory cells to provide protection against subsequent pathogen encounter (4). However, in chronic infections where antigens persist over time, T cells can acquire an "exhausted" phenotype characterized by expression of inhibitory receptors that limit effector functions (5). While T cell exhaustion serves to dampen immune-mediated damage, it can also permit viral persistence and hinder anti-tumor responses (5). Recent data suggest that a small population of CD8+ T cells, marked

by expression of the transcriptional regulator T cell factor 1 (TCF1), is required to maintain T cell responses during exhaustion in chronic infections (6–8).

The dynamic regulation of $CD8^+$ T cell differentiation, proliferation, survival, and function is essential for generating effective immune responses. Mutations in genes affecting the function of CTLs and natural killer (NK) cells, an innate cell population that is also important for killing tumorigenic and virally infected cells, have been identified in numerous primary human immunodeficiencies (PIDs) associated with impaired viral clearance and tumor development (9). Such immunodeficiencies are also often associated with hemophagocytic syndrome, exemplified by secondary activation of the immune system in response to IFN-γ and other cytokines (9, 10). Thus, proper regulation of CTL function plays vital roles in both host protective immunity and immune cell homeostasis.

One condition where abnormal $CD8^+$ T cell function can lead to substantial pathology is Epstein–Barr virus (EBV) infection. EBV is a common human gamma-herpesvirus that infects the oropharyngeal epithelium and B cells and is primarily controlled by CTLs and NK cell responses (11). Although infection in children is usually associated with mild symptoms, teenagers and adults can develop infectious mononucleosis with fever, enlarged secondary lymphoid organs, and flu-like symptoms, accompanied by a pronounced lymphocytosis, with increased $CD8^+$ T cell numbers. In the normal host, Following initial infection, EBV persists latently in B cells. However, in immunocompromised patients, EBV can cause multiple severe complications that include lymphoproliferative disorders and lymphoid malignancies (12, 13).

Consistent with a critical role for CTLs in EBV control, as evidenced by the successful use of EBV-specific CTLs to treat EBV-induced disease after bone marrow transplantation (14), a growing number of PIDs have been associated with poor EBV clearance (10). Among these is the recently described autosomal-dominant immunodeficiency, activated phosphoinositide 3-kinase delta syndrome (APDS)/PASLI, associated with activating mutations affecting the p110δ catalytic subunit of phosphoinositide 3-kinase (PI3K) (15–19). PI3Ks are lipid kinases that are critical for the regulation of metabolism, differentiation, cell survival, and motility (20). Class Ia PI3Ks consist of two subunits, a regulatory subunit and a p110 catalytic subunit that phosphorylates phophosphoinositide $PI(4,5)P_2$ to generate $PI(3,4,5)P_3$, which recruits molecules to the plasma membrane, facilitating their activation. The p110δ catalytic isoform (encoded by *PIK3CD*) is expressed primarily in hematopoietic cells and is an important component of signaling pathways involved in T and B cell activation and differentiation in response to antigen, costimulatory, cytokine, and chemokine receptors (20).

Activated phosphoinositide 3-kinase delta syndrome/PASLI is associated with frequent respiratory infections, progressive blood lymphopenia, mucosal lymphoid nodules, defective antibody responses, and lymphoma (15, 17–19, 21, 22). Patients have few naïve T cells, with evidence of increased T cell activation, whereas B cell development appears partially blocked, with few memory B cells (19, 23). Although recurrent respiratory infections are the most common feature of this PID (24), an inability to control viremia with EBV and cytomegalovirus (CMV) occurs in nearly half of all reported cases (16). Despite uncontrolled EBV viremia, many APDS/PASLI patients have normal or higher frequencies of EBV-specific $CD8^+$ T cells, as detected by HLA tetramers loaded with lytic or latent EBV peptides (19). These data suggest that gain-of-function (GOF) mutations in p110δ do not result in a global impairment in the generation of antigen-specific T cell responses, raising the question of how activated p110δ affects $CD8^+$ T cell differentiation, homeostasis, and function. Here, we discuss several features of $CD8^+$ T cells in APDS/PASLI that may prevent clearance of EBV, including increased TCR-induced cell death, T cell exhaustion and immune senescence, and how activated PI3K might contribute to these phenotypes (**Figure 1**).

$CD8^+$ T CELL DEATH AND IMMUNE HOMEOSTASIS

Although APDS/PASLI patients can have increased percentages of EBV-specific $CD8^+$ T cells (19), *in vitro* TCR stimulation results in pronounced cell death of both $CD4^+$ and $CD8^+$ T cells (15, 25). Thus, although abundant EBV-specific T cells are detected in the peripheral blood of APDS/PASLI patients, these cells may be more prone to death following re-stimulation. Instead of killing EBV-infected targets, $CD8^+$ T cells may themselves die following TCR engagement and, therefore, not be able to clear the virus, particularly one that chronically remains in the body and continually "tickles" activated T cells.

How might PI3K/p110δ signaling affect TCR-mediated pro-apoptotic pathways? One of the main targets of PI3K activation is protein kinase B (AKT), which directly phosphorylates members of the Forkhead box O (FOXO) family of transcription factors resulting in their nuclear export and degradation (20, 26). Multiple FOXO transcriptional targets influence cell survival, both positively and negatively, depending on the cell type and experimental setting (26, 27). Although FOXO transcription factors drive the expression of genes encoding numerous cylin-dependent kinase inhibitors and the pro-apoptotic proteins BIM, PUMA, and FasL (26, 27), they can also suppress FasL expression in certain cell types (28). Deletion of *Foxo1* in murine T cells also decreases expression of *Il7ra*, which is important for T cell survival (29).

Additionally, the increased frequency of EBV positive cells in the peripheral blood may not accurately reflect tissue-specific frequency. PI3K regulates a number of molecules that affect lymphocyte recruitment and migration. Notably, FOXO1 transcriptional targets, such as *Ccr7* and *Kruppel-like factor 2* (*Klf2*) have profound effects on lymphocyte activation and trafficking in mice (29–31). CCR7 and its ligands play key roles in lymphocyte homing to the lymph nodes and intestinal Peyer's patch (32). KLF2 is required for the effective transcription of *Sell* (encoding L-selection, CD62L) and *S1pr1* (encoding sphingosine-1-phosphate receptor-1, $S1P_1R$), two key regulators of lymphocyte entry and egress from lymph nodes, respectively (33, 34). Notably, both CCR7 and CD62L are expressed at lower

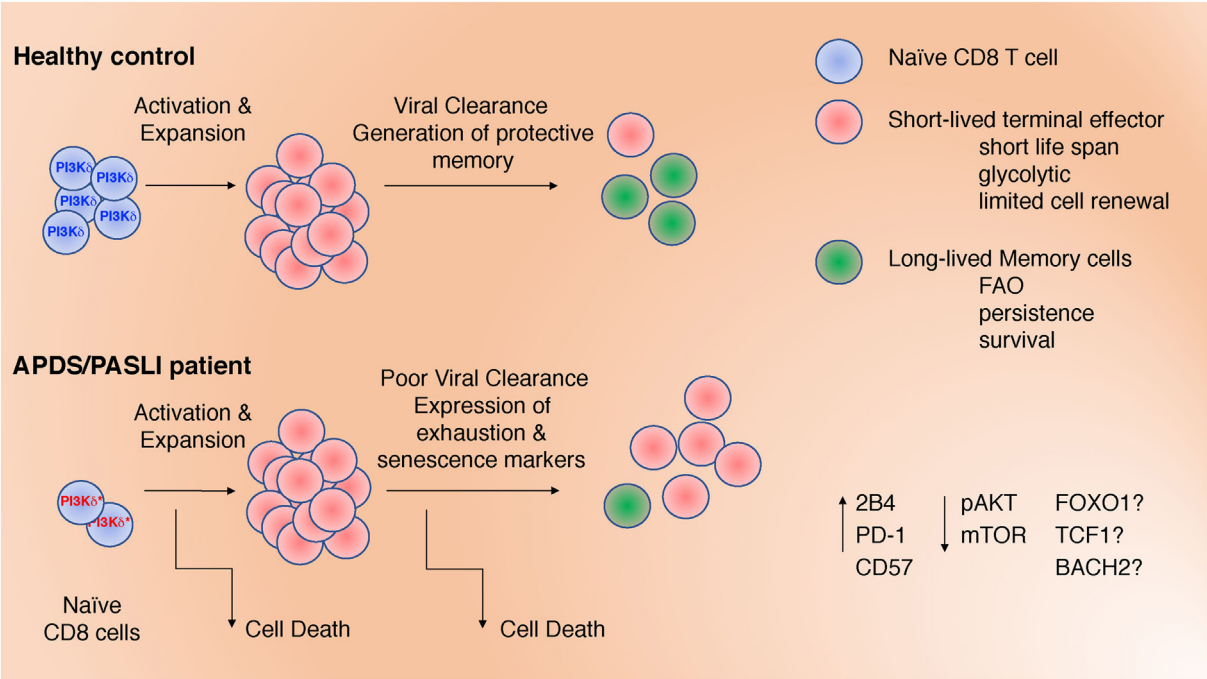


FIGURE 1 | Defects in CD8+ T cells may contribute to impaired clearance of Epstein–Barr virus and CMV in activated phosphoinositide 3-kinase delta syndrome/PASLI. These include: (1) decreased naïve T cells; (2) increased T cell receptor -stimulated cell death; (3) altered differentiation with increased effector cell function at the expense of memory cell formation; and (4) expression of inhibitory receptors associated with exhaustion and/or senescence. These defects are associated with altered signaling (pAKT and mTOR) that, in turn, could affect the activation and/or expression of transcription factors (FOXO1, TCF1, and BACH2).

levels on T cells in peripheral blood from APDS/PASLI patients, which exhibit reduced CCR7+ naïve and central memory T cells, and a greater abundance of CD45RA−CCR7− effector memory and CD45RA+CCR7− terminal effector memory CD8+ T cells relative to controls (19).

A second major PI3K effector that influences lymphocyte migration and homeostasis is the mammalian target of Rapamycin, mTOR. MTOR is a conserved serine/threonine kinase that participates in two complexes, mTORC1 and 2. MTORC1 regulates cell growth, proliferation, survival, protein synthesis, and transcription (35, 36). Although some data convincingly argue that mTORC1 is not solely a PI3K effector in CTLs (37), T cells from APDS/PASLI patients show increased Rapamycin-sensitive phosphorylation of S6, a downstream target of the mTORC1 pathway (19). These data suggest that PI3K activation may be sufficient to activate mTORC1, even if it is not strictly required. Interestingly, mTORC1 and the downstream transcription factor hypoxia inducible factor 1-α (HIF1α) also affect expression of a large number of genes encoding chemokine and homing receptors. HIF1α-deficient murine T cells have higher expression of genes encoding CXCR4, CCR7, $S1P_1$, and CD62L (37). The converse would be expected to occur in the presence of activated PI3Kδ. It is therefore of interest that APDS/PASLI patients are lymphopenic, yet have lymphadenopathy and splenomegaly as well as mucosal lymphoid nodules in their gastrointestinal and upper respiratory tracts, suggestive of altered lymphocyte homing (15, 19). Together, these data argue that continual PI3K signaling alters expression of key trafficking and survival proteins that influence the localization of T lymphocytes to tissues required for effective elimination of infection. Whether this affects responses to chronic infections, such as EBV, remains an interesting question.

ALTERED CD8+ T CELL DIFFERENTIATION

Despite the dramatic increase in TCR-induced cell death in APDS/PASLI patient CD8+ T cells, a fraction of blasts survived TCR stimulation *in vitro* and expand. Strikingly, these CD8+ T cell blasts displayed characteristics of enhanced effector function (19). Indeed, both AKT and mTOR are important for inducing and maintaining expression of cytolytic effector molecules in CTLs, including perforin and various granzymes (37, 38). These observations raise the question of whether continual PI3Kδ signaling promotes CD8+ T cell terminal effector differentiation.

Indeed, many transcription factors inhibited by PI3K activation, including FOXO1, TCF1, and BTB and CNC homology 2 (BACH2), influence CD8+ T cell differentiation. Mutations affecting these proteins promote effector T cell differentiation at the expense of memory formation (31, 39–43). Unequal PI3K

signaling during cell division has been associated with bifurcation of sibling fates, with robust PI3K signaling promoting effector differentiation, associated with decreased expression of TCF1 (44). TCF1 is required for a $CD8^+$ memory stem cell-like population that is necessary for continual responses to chronic infection (6–8), suggesting that reduced TCF1 due to sustained PI3K-signaling may prevent effective control of chronic viral infections.

Other data have implicated HIF1α downstream of mTOR as critical for the expression of cytolytic effectors, including granzymes and perforin (37). Consistent with PI3K promoting mTORC1 activation, $CD8^+$ T cell blasts from patients showed increased effector function, as determined by elevated IFN-γ production, and increased granzyme B expression and TCR-induced degranulation (19). Moreover, patient CTLs effectively killed the Fc receptor-expressing P815 murine target cell line coated with anti-CD3 in a re-directed lytic assay [**Figure 2B** and Ref. (19)]. Thus, APDS/PASLI patients have functional CTLs that even show evidence of enhanced effector cell function relative to controls.

How else might PI3K affect differentiation of $CD8^+$ T cells? It is now appreciated that differentiation into effector cells is accompanied by major changes in cellular metabolism, associated with increased aerobic glycolysis (45). In contrast, memory cell formation is accompanied by increased use of fatty acid oxidation pathways, amino acid degradation, and a return to a more catabolic state (45). A number of metabolic and nutrient sensing pathways are mediated by PI3K and its downstream effectors: AKT and mTOR. AKT can induce the trafficking and surface expression of the glucose transporter, Glut1 (46, 47). Although the mechanism by which AKT alters Glut1 surface expression is still not clear, in other cell types this may occur *via* regulation of thioredoxin-interacting protein and inhibition of Glut1 internalization (48). Indeed, T cell blasts from APDS/PASLI patients demonstrate elevated glucose uptake compared to controls (19). Other data have implicated mTOR and HIF1α in the induction of genes encoding key glycolytic enzymes including hexokinase 2, phosphofructokinase, and pyruvate kinase, as well as Glut1 and Glut3 in CTLs (37). Whether differences in the metabolic profile of patient T cells contribute to, or are secondary to differences in their effector differentiation state and activation remains an open question. Nonetheless, these data point to a multi-faceted polarization to effector cells at the expense of long-term memory and efficient responses to chronic infection in the presence of activated PI3K.

T CELL EXHAUSTION AND/OR SENESCENCE

T Cell Exhaustion

During chronic infections and/or persistent antigen exposure, T cell exhaustion can occur. Exhaustion manifests with several distinct features that include progressive loss of effector function, expression of multiple inhibitory receptors including PD-1, 2B4, Tim3, and LAG3, and an altered transcriptional program (5). In response to TCR stimulation, an elevated percentage of APDS/PASLI patient $CD8^+$ T cells express PD-1 and 2B4 [Ref. (19, 25, 49) and **Figure 2A**], which may prevent effective CTL function.

2B4 is cytolytic receptor that is a member of the family of signaling lymphocyte activation molecule (SLAM) receptors, which associate with the small intracellular adaptor molecule SLAM-associated protein (SAP). Mutations affecting *SH2D1A,* which encodes SAP, cause X-linked lymphoproliferative disease type 1 (XLP1), which is perhaps the classic example of a PID associated with an inability to clear EBV (50–52). In the absence of SAP, or under conditions where SAP:2B4 ratios are low (53), 2B4 switches to function an inhibitory receptor, recruiting SH2-domain-containing tyrosine phosphatases 1 and 2 (SHP-1 and 2) and other negative signaling molecules, whose activities impair TCR signaling and subsequent T cell function (54–59). Because EBV-infected B cells express very high levels of CD48, the ligand for 2B4, killing of EBV-infected targets is specifically impaired in SAP-deficient (XLP1) NK and CTLs (50–52).

Although $CD8^+$ EBV-specific T cells from APDS/PASLI patients killed P815 targets efficiently [**Figure 2B** and Ref. (19)], the P815 mouse mastocytoma cell line does not express ligands that stimulate human PD-1 and 2B4, preventing potential inhibitory effects of these receptors. In contrast, we and others found that patient $CD8^+$ EBV-specific T cell blasts displayed variable defects in killing of autologous EBV-transformed lymphoblastoid B cell (LCL) targets [**Figure 2B** and Ref. (25)]. It is therefore of interest that in addition to high 2B4 levels, we have also observed reduced SAP levels in CTLs grown from APDS/PASLI patients (**Figure 2A**). We, therefore, propose that APDS/PASLI may share features with XLP1, with 2B4 acting as an inhibitory receptor that decreases killing EBV-infected B cells and possibly other hematopoietic cells infected by CMV. Notably, higher CD48 levels have been observed on APDS/PASLI patient B cells and LCLs compared to controls (25), which could also enhance 2B4 inhibitory signals. Interestingly, we have observed that control EBV-specific CTLs kill HLA-matched patient LCLs better than HLA-matched control LCLs, as might be expected if the increased CD48 on patient LCLs engage more 2B4, which acts as an activating receptor to enhance killing in control CTLs that express normal levels of SAP and 2B4. However, APDS/PASLI patients do not appear to develop the most severe phenotypes of XLP1, including hemophagocytic syndrome. This may be the result of less severe defects in cytolysis of EBV-infected B cells, other intrinsic T cell defects such as elevated cell death (15, 16) or alterations in macrophage activation (16) that may prevent secondary immune hyperactivation.

Intriguingly, 2B4 can also recruit SH2-containing inositol 5′ phosphastase (SHIP) (55), which hydrolyzes $PI(3,4,5)P_3$ to $PI(3,4)P_2$ (20). PD-1 can also dampen PI3K signals *via* the recruitment of phosphatases that preferentially downregulate signaling from CD28, a potent activator of PI3K (60). In addition, PD-1 ligation augments expression of PTEN (61), a lipid phosphastase that converts PIP_3 to $PI(4,5)P_2$, counteracting PI3K signaling (20). Thus, strong upregulation of PD-1 and 2B4 could serve as compensatory mechanisms to temper sustained PI3K activity,

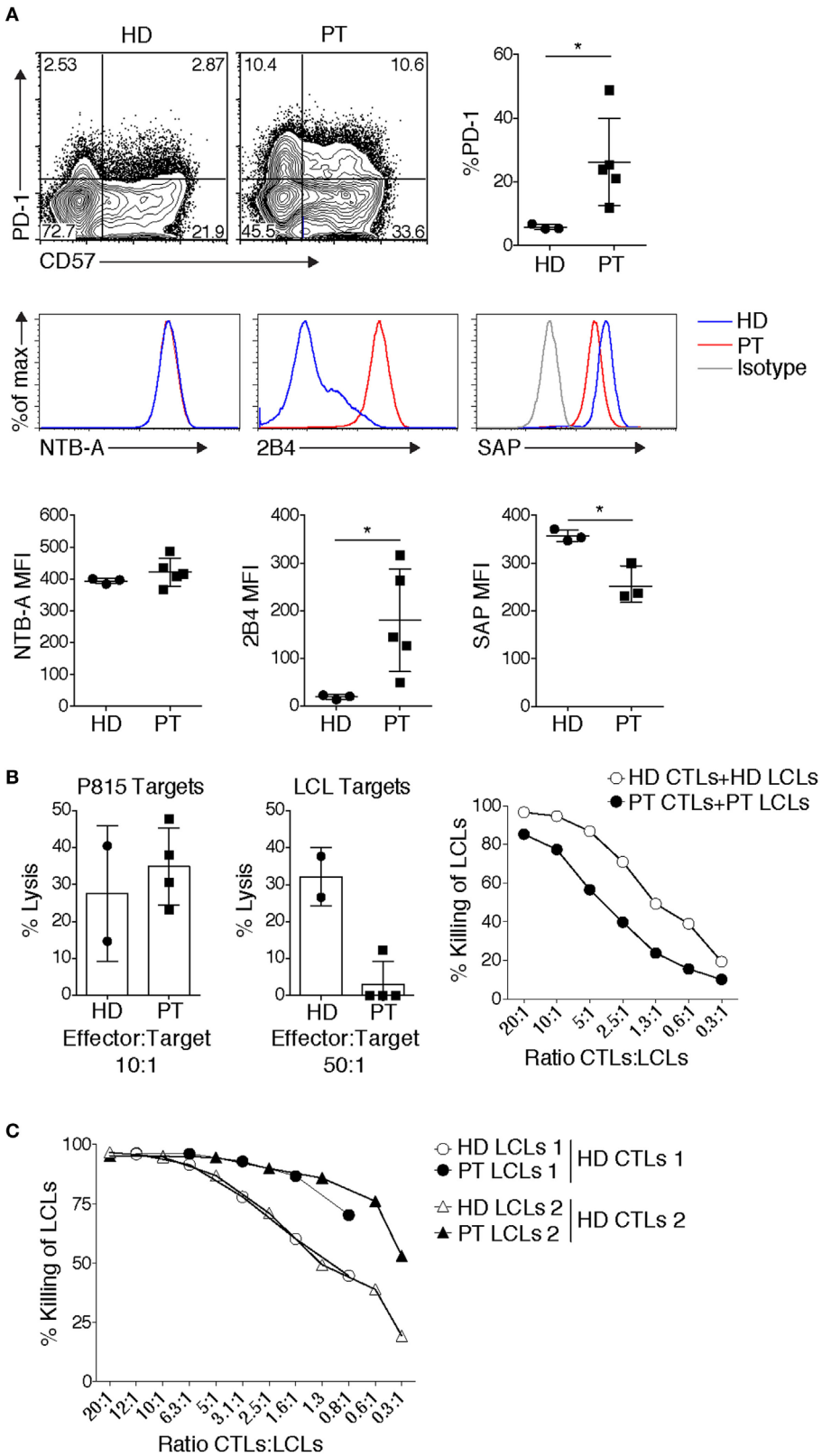


FIGURE 2 | Continued

FIGURE 2 | Patient CD8+ T cells display elevated expression of inhibitory receptors and impaired killing of autologous targets. **(A)** Elevated expression of inhibitory receptors PD-1 and 2B4, and senescence marker CD57 on allo-reactive CD8+ T cells. Expression of the signaling lymphocyte activation molecule family receptor, NTB-A, remained unchanged, while SAP expression can be reduced. Representative example shown [3 healthy donor (HD) controls, 3–5 patients] [PT], small horizon line represents mean, $^*p < 0.05$ (Mann–Whitney test). **(B)** Defects in Epstein–Barr virus (EBV)-specific CD8+ T cells. Cytolysis of P815 targets by anti-CD3-mediated redirected lysis (left panel) and cytolysis of peptide-pulsed autologous LCLs (middle panel), (cytolysis from 2 HD controls and 4 patients cytotoxic T lymphocytes done in duplicate are shown, representative of 4 independent experiments). Right panel: an example of cytolysis of healthy donor (HD) and patient (PT) peptide-pulsed autologous LCLs, with titration of effector:target ratios. **(C)** HD EBV-specific CD8+ T cells cytolysis of HD or PT LCLs. Two examples are shown, which are representative of three independent experiments.

which may paradoxically result in greater defects in CD8+ T cell function.

Senescence

In addition to expression of exhaustion markers, APDS/PASLI CD8+ T cells can also exhibit higher percentages expressing CD57, a marker of senescent T cells [Ref. (19, 25, 49) and **Figure 2A**]. During initial antigen encounter, T cell activation is followed by telomerase activation that preserves telomere length. However, subsequent TCR engagement can inactivate the telomerase promoter and decrease telomerase expression (62). Continual "tickling" of activated TCRs during chronic infections could result in telomere crisis and activation of DNA damage signals, followed by cell cycle arrest leading to replicative senescence or cell death (62). Although the elevated frequency of CD57+ T cells in APDS/PASLI could result from chronic infection, patients who were not overtly viremic also displayed an increased percentage of CD8+ T cells expressing CD57 (19). Alternatively, CD57 expression and detrimental telomere shortening observed in APDS/PASLI patient CD8+ T cells may reflect elevated basal PI3K signaling, evidenced by phosphorylated AKT and S6 (15, 19, 63). However, it has also been proposed that senescent cells survive for extended periods and are often more resistant to apoptotic cell death (62). This is not consistent with the observation that APDS/PASLI T cells rapidly die following *in vitro* activation (15). Nonetheless, these experiments collectively revealed that APDS/PASLI patients display CD8+ T cell dysfunction that includes features of both senescence and exhaustion that may contribute to their inability to clear chronic infections. Whether heterogeneity of these phenotypes is related to age of diagnosis, chronic infection, and/or environmental exposure remains an intriguing question as more patients are followed.

ALTERNATIVE HYPOTHESES

Although we have focused on CD8+ T cell function, alterations in other cells may also contribute to the inability to clear EBV, including recently documented defects in NK cell function (25, 64). Another hypothesis is that altered B cell development and populations may provide reservoirs for continual EBV infection in APDS/PASLI (65). It is important to highlight that Herpes viruses express proteins that converge on PI3K pathways to expidite viral entry, latency, and reactivation (66). Alternatively, although the role of humoral immunity in clearing EBV is not well defined, humoral defects may affect EBV infection in the context of immunodeficiency (67, 68). Altered properties of EBV-infected targets may contribute to poor EBV clearance. Sustained PI3K signals can rescue B cells from cell death in the absence of B cell receptor signaling (69); thus, increased PI3K signals may give EBV-infected B cells a survival advantage. However, we have found that LCLs from APDS/PASLI patients are actually killed better by control CTLs (**Figure 2C**). Finally, EBV occasionally infects T cells (70), and whether this affects CD8+ T cell function in APDS/PASLI remains unknown.

CONCLUDING REMARKS

Here, we review some of the defects that may affect the ability of patients with APDS/PASLI to clear chronic infections such as EBV and CMV, with a focus on CD8+ T cells. A recent report has shown promising results using a PI3Kδ-specific inhibitor, Leniosilib, in a small group of APDS/PASLI patients (71). Inhibition of PI3Kδ rescued both T and B lymphocyte phenotypes, including decreased expression of activation, exhaustion and senescence T cell markers, and decreased lymphadenopathy and splenomegaly. Notably, Sirolimus treatment has also ameliorated lymphadenopathy and hepatosplenomegaly, and NK cell function in some APDS/PASLI patients, implicating mTOR in these phenotypes (19, 64, 72). How these treatments affect clearance of chronic infections is of great interest. However, recent evidence that treatment of mice with PI3Kδ inhibitors results in increased genomic instability in normal and neoplastic B cells (73) suggests that long-term PI3Kδ inhibitor administration could have detrimental consequences. Alternatives that boost T cell function may, therefore, be of continued interest for this disease. Further insight into cellular and molecular defects in APDS/PASLI is, therefore, an important component of understanding how to treat this complex disorder.

METHODS

Samples and Ethics Approval

All human subjects in this study provided signed written informed consent in accordance with Helsinki principles for enrollment in research protocols that were approved by the Institutional Review Board of NIAID (clinical trials registration number NCT00001355, US NIH). Procedures were based on standard of care, under established clinical guidelines. Patients and control samples were described in Ref. (19). Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll–Hypaque gradient centrifugation.

Flow Cytometry and Cytotoxicity

For phenotyping, cells were stained in FACS buffer (74). Staining reagents included: CD8-PECy7 (SK1), CD57-FITC (TB01), PD-1-PE (EBIOJ105), NTBA-PE (NT-7), 2B4-PE (C1.7), and SAP-PE (XLP1D12) (ebioscience). EBV-tetramers were kindly

provided by Stuart Tangye. EBV peptides (HLA-A2-specific CLGGLLTMV and HLA-B8-specific RAKFKQLL) were from AnaSpec. Data were acquired on either a Calibur1 or LSRII flow cytometer (BD) and analyzed using FlowJo software (Tree Star). EBV-specific CTLs were generated from PBMCs that were pulsed with HLA-matched EBV-specific peptides (1 μg/ml) plus rhIL-2 (100 U/ml). Cytotoxicity assays were completed on day 10–12 of culture following assessment of outgrowth of antigen-specific cells *via* tetramer staining. *In vitro* cytolytic activity was determined using a lactate dehydrogenase release or flow-based assay as previously described in Ref. (74).

Statistical Analysis

Data were analyzed *via* Prism 6 (GraphPad Software), using non-parametric unpaired Mann–Whitney *U* tests for comparison of two unpaired groups. p values <0.05 were considered statistically significant.

AUTHOR CONTRIBUTIONS

JC and SP performed experiments. GU provided patient samples and experimental advice. JC, SP, SK, and PS wrote the manuscript and helped prepared the figures. All authors edited and approved the manuscript.

ACKNOWLEDGMENTS

We thank the patients and their families for their many contributions. This work was supported in part by the intramural programs of NHGRI and NIAID, NIH.

REFERENCES

1. Stinchcombe JC, Griffiths GM. Secretory mechanisms in cell-mediated cytotoxicity. *Annu Rev Cell Dev Biol* (2007) 23:495–517. doi:10.1146/annurev.cellbio.23.090506.123521
2. Ritter AT, Kapnick SM, Murugesan S, Schwartzberg PL, Griffiths GM, Lippincott-Schwartz J. Cortical actin recovery at the immunological synapse leads to termination of lytic granule secretion in cytotoxic T lymphocytes. *Proc Natl Acad Sci U S A* (2017) 114(32):E6585–94. doi:10.1073/pnas.1710751114
3. Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM. Centrosome polarization delivers secretory granules to the immunological synapse. *Nature* (2006) 443(7110):462–5. doi:10.1038/nature05071
4. Jameson SC, Masopust D. Understanding subset diversity in T cell memory. *Immunity* (2018) 48(2):214–26. doi:10.1016/j.immuni.2018.02.010
5. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* (2015) 15(8):486–99. doi:10.1038/nri3862
6. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* (2016) 537(7620):417–21. doi:10.1038/nature19330
7. Utzschneider DT, Charmoy M, Chennupati V, Pousse L, Ferreira DP, Calderon-Copete S, et al. T cell factor 1-expressing memory-like CD8(+) T cells sustain the immune response to chronic viral infections. *Immunity* (2016) 45(2):415–27. doi:10.1016/j.immuni.2016.07.021
8. Wu T, Ji Y, Moseman EA, Xu HC, Manglani M, Kirby M, et al. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci Immunol* (2016) 1(6):eaai8593. doi:10.1126/sciimmunol.aai8593
9. Pachlopnik Schmid J, Cote M, Menager MM, Burgess A, Nehme N, Menasche G, et al. Inherited defects in lymphocyte cytotoxic activity. *Immunol Rev* (2010) 235(1):10–23. doi:10.1111/j.0105-2896.2010.00890.x
10. Cohen JI. Primary immunodeficiencies associated with EBV disease. *Curr Top Microbiol Immunol* (2015) 390(Pt 1):241–65. doi:10.1007/978-3-319-22822-8_10
11. Cohen JI. Epstein-Barr virus infection. *N Engl J Med* (2000) 343(7):481–92. doi:10.1056/NEJM200008173430707
12. Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N Engl J Med* (2004) 350(13):1328–37. doi:10.1056/NEJMra032015
13. Worth AJ, Houldcroft CJ, Booth C. Severe Epstein-Barr virus infection in primary immunodeficiency and the normal host. *Br J Haematol* (2016) 175(4):559–76. doi:10.1111/bjh.14339
14. Liu Z, Savoldo B, Huls H, Lopez T, Gee A, Wilson J, et al. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the prevention and treatment of EBV-associated post-transplant lymphomas. *Recent Results Cancer Res* (2002) 159:123–33. doi:10.1007/978-3-642-56352-2_15
15. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342(6160):866–71. doi:10.1126/science.1243292
16. Carpier JM, Lucas CL. Epstein-Barr viurs susceptibility in activated PI3Kd syndrome (APDS) immunodeficiency. *Front Immunol* (2018) 8:2005. doi:10.3389/fimmu.2017.02005
17. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
18. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33(5):361–9. doi:10.1111/j.1744-313X.2006.00627.x
19. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15(1):88–97. doi:10.1038/ni.2771
20. Okkenhaug K. Signaling by the phosphoinositide 3-kinase family in immune cells. *Annu Rev Immunol* (2013) 31:675–704. doi:10.1146/annurev-immunol-032712-095946
21. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34(3):272–6. doi:10.1007/s10875-014-0012-9
22. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol* (2014) 134(1):233–6. doi:10.1016/j.jaci.2014.02.020
23. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2017) 139(3):1032–5.e6. doi:10.1016/j.jaci.2016.08.028
24. Condliffe AM, Chandra A. Respiratory manifestations of the activated phosphoinositide 3-kinase delta syndrome. *Front Immunol* (2018) 9:338. doi:10.3389/fimmu.2018.00338
25. Edwards ESJ, Bier J, Cole TS, Wong M, Hsu P, Berflund LJ, et al. Activating PI3CD mutations impair cytotoxic lymphocyte differentiation, function and EBV immunity. *J Allergy Clin Immunol* (2018). doi:10.1016/j.jaci.2018.04.030
26. Hedrick SM, Hess Michelini R, Doedens AL, Goldrath AW, Stone EL. FOXO transcription factors throughout T cell biology. *Nat Rev Immunol* (2012) 12(9):649–61. doi:10.1038/nri3278
27. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* (1999) 96(6):857–68. doi:10.1016/S0092-8674(00)80595-4
28. Jonsson H, Allen P, Peng SL. Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. *Nat Med* (2005) 11(6):666–71. doi:10.1038/nm1248
29. Kerdiles YM, Beisner DR, Tinoco R, Dejean AS, Castrillon DH, DePinho RA, et al. Foxo1 links homing and survival of naive T cells by regulating L-selectin,

CCR7 and interleukin 7 receptor. *Nat Immunol* (2009) 10(2):176–84. doi:10.1038/ni.1689
30. Carlson CM, Endrizzi BT, Wu J, Ding X, Weinreich MA, Walsh ER, et al. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* (2006) 442(7100):299–302. doi:10.1038/nature04882
31. Kim MV, Ouyang W, Liao W, Zhang MQ, Li MO. The transcription factor Foxo1 controls central-memory CD8+ T cell responses to infection. *Immunity* (2013) 39(2):286–97. doi:10.1016/j.immuni.2013.07.013
32. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol* (2008) 8(5):362–71. doi:10.1038/nri2297
33. Arbones ML, Ord DC, Ley K, Ratech H, Maynard-Curry C, Otten G, et al. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* (1994) 1(4):247–60. doi:10.1016/1074-7613(94)90076-0
34. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* (2004) 427(6972):355–60. doi:10.1038/nature02284
35. Finlay D, Cantrell DA. Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol* (2011) 11(2):109–17. doi:10.1038/nri2888
36. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* (2017) 169(2):361–71. doi:10.1016/j.cell.2017.03.035
37. Finlay DK, Rosenzweig E, Sinclair LV, Feijoo-Carnero C, Hukelmann JL, Rolf J, et al. PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J Exp Med* (2012) 209(13):2441–53. doi:10.1084/jem.20112607
38. Macintyre AN, Finlay D, Preston G, Sinclair LV, Waugh CM, Tamas P, et al. Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. *Immunity* (2011) 34(2):224–36. doi:10.1016/j.immuni.2011.01.012
39. Gattinoni L, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, et al. Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat Med* (2009) 15(7):808–13. doi:10.1038/nm.1982
40. Hess Michelini R, Doedens AL, Goldrath AW, Hedrick SM. Differentiation of CD8 memory T cells depends on Foxo1. *J Exp Med* (2013) 210(6):1189–200. doi:10.1084/jem.20130392
41. Jeannet G, Boudousquie C, Gardiol N, Kang J, Huelsken J, Held W. Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proc Natl Acad Sci U S A* (2010) 107(21):9777–82. doi:10.1073/pnas.0914127107
42. Roychoudhuri R, Clever D, Li P, Wakabayashi Y, Quinn KM, Klebanoff CA, et al. BACH2 regulates CD8(+) T cell differentiation by controlling access of AP-1 factors to enhancers. *Nat Immunol* (2016) 17(7):851–60. doi:10.1038/ni.3441
43. Zhou X, Yu S, Zhao DM, Harty JT, Badovinac VP, Xue HH. Differentiation and persistence of memory CD8(+) T cells depend on T cell factor 1. *Immunity* (2010) 33(2):229–40. doi:10.1016/j.immuni.2010.08.002
44. Lin WH, Adams WC, Nish SA, Chen YH, Yen B, Rothman NJ, et al. Asymmetric PI3K signaling driving developmental and regenerative cell fate bifurcation. *Cell Rep* (2015) 13(10):2203–18. doi:10.1016/j.celrep.2015.10.072
45. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. *Cell* (2017) 169(4):570–86. doi:10.1016/j.cell.2017.04.004
46. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* (2008) 180(7):4476–86. doi:10.4049/jimmunol.180.7.4476
47. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell* (2007) 18(4):1437–46. doi:10.1091/mbc.e06-07-0593
48. Waldhart AN, Dykstra H, Peck AS, Boguslawski EA, Madaj ZB, Wen J, et al. Phosphorylation of TXNIP by AKT mediates acute influx of glucose in response to insulin. *Cell Rep* (2017) 19(10):2005–13. doi:10.1016/j.celrep.2017.05.041
49. Wentink MWJ, Mueller YM, Dalm V, Driessen GJ, van Hagen PM, van Montfrans JM, et al. Exhaustion of the CD8(+) T cell compartment in patients with mutations in phosphoinositide 3-kinase delta. *Front Immunol* (2018) 9:446. doi:10.3389/fimmu.2018.00446
50. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* (2011) 29:665–705. doi:10.1146/annurev-immunol-030409-101302
51. Pachal N, Booth C, Cannons JL, Schwartzberg PL. X-linked lymphoproliferative disease type 1: a clinical and molecular perspective. *Front Immunol* (2018) 9:666. doi:10.3389/fimmu.2018.00666
52. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol* (2014) 34(7):772–9. doi:10.1007/s10875-014-0083-7
53. Chlewicki LK, Velikovsky CA, Balakrishnan V, Mariuzza RA, Kumar V. Molecular basis of the dual functions of 2B4 (CD244). *J Immunol* (2008) 180(12):8159–67. doi:10.4049/jimmunol.180.12.8159
54. Bottino C, Falco M, Parolini S, Marcenaro E, Augugliaro R, Sivori S, et al. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* (2001) 194(3):235–46. doi:10.1084/jem.194.3.235
55. Dong Z, Davidson D, Perez-Quintero LA, Kurosaki T, Swat W, Veillette A. The adaptor SAP controls NK cell activation by regulating the enzymes Vav-1 and SHIP-1 and by enhancing conjugates with target cells. *Immunity* (2012) 36(6):974–85. doi:10.1016/j.immuni.2012.03.023
56. Eissmann P, Beauchamp L, Wooters J, Tilton JC, Long EO, Watzl C. Molecular basis for positive and negative signaling by the natural killer cell receptor 2B4 (CD244). *Blood* (2005) 105(12):4722–9. doi:10.1182/blood-2004-09-3796
57. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med* (2000) 192(3):337–46. doi:10.1084/jem.192.3.337
58. Tangye SG, Lazetic S, Woollatt E, Sutherland GR, Lanier LL, Phillips JH. Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. *J Immunol* (1999) 162(12):6981–5.
59. Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL. Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytolysis. *Immunity* (2012) 36(6):1003–16. doi:10.1016/j.immuni.2012.05.017
60. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* (2017) 355(6332):1428–33. doi:10.1126/science.aaf1292
61. Wartewig T, Kurgyis Z, Keppler S, Pechloff K, Hameister E, Ollinger R, et al. PD-1 is a haploinsufficient suppressor of T cell lymphomagenesis. *Nature* (2017) 552(7683):121–5. doi:10.1038/nature24649
62. Bellon M, Nicot C. Telomere dynamics in immune senescence and exhaustion triggered by chronic viral infection. *Viruses* (2017) 9(10):E289. doi:10.3390/v9100289
63. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211(13):2537–47. doi:10.1084/jem.20141759
64. Ruiz-Garcia R, Vargas-Hernandez A, Chinn IK, Angelo LS, Cao TN, Coban-Akdemir Z, et al. Mutations in PI3K110delta cause impaired natural killer cell function partially rescued by rapamycin treatment. *J Allergy Clin Immunol* (2018). doi:10.1016/j.jaci.2017.11.042
65. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Scholvinck L, Kalina T, et al. Genetic defects in PI3Kdelta affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
66. Cohen JI. Herpesviruses in the activated phosphatidylinositol-3-kinase-d syndrome. *Front Immunol* (2018) 9:237. doi:10.3389/fimmu.2018.00237
67. Bu W, Hayes GM, Liu H, Gemmell L, Schmeling DO, Radecki P, et al. Kinetics of Epstein-Barr Virus (EBV) neutralizing and virus-specific antibodies after primary infection with EBV. *Clin Vaccine Immunol* (2016) 23(4):363–9. doi:10.1128/CVI.00674-15
68. Panikkar A, Smith C, Hislop A, Tellam N, Dasari V, Hogquist KA, et al. Impaired Epstein-Barr virus-specific neutralizing antibody response during acute infectious mononucleosis is coincident with global B-cell dysfunction. *J Virol* (2015) 89(17):9137–41. doi:10.1128/JVI.01293-15

69. Werner M, Hobeika E, Jumaa H. Role of PI3K in the generation and survival of B cells. *Immunol Rev* (2010) 237(1):55–71. doi:10.1111/j.1600-065X.2010.00934.x
70. Coleman CB, Wohlford EM, Smith NA, King CA, Ritchie JA, Baresel PC, et al. Epstein-Barr virus type 2 latently infects T cells, inducing an atypical activation characterized by expression of lymphotactic cytokines. *J Virol* (2015) 89(4):2301–12. doi:10.1128/JVI.03001-14
71. Rao VK, Webster S, Dalm V, Sediva A, van Hagen PM, Holland S, et al. Effective "activated PI3Kdelta syndrome"-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130(21):2307–16. doi:10.1182/blood-2017-08-801191
72. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase delta syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
73. Compagno M, Wang Q, Pighi C, Cheong TC, Meng FL, Poggio T, et al. Phosphatidylinositol 3-kinase delta blockade increases genomic instability in B cells. *Nature* (2017) 542(7642):489–93. doi:10.1038/nature21406
74. Kapnick SM, Stinchcombe JC, Griffiths GM, Schwartzberg PL. Inducible T cell kinase regulates the acquisition of cytolytic capacity and degranulation in CD8(+) CTLs. *J Immunol* (2017) 198(7):2699–711. doi:10.4049/jimmunol.1601202

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 07 September 2018
doi: 10.3389/fimmu.2018.02043

The Treatment of Activated PI3Kδ Syndrome

Tanya I. Coulter[1]* *and Andrew J. Cant*[2]

[1] Regional Immunology Service, Belfast Health and Social Care Trust, Belfast, United Kingdom, [2] Department of Paediatric Immunology and Stem Cell Transplant Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom

Activated phosphoinositide 3-kinase δ syndrome (APDS), also known as PASLI disease (p110d-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency) are combined immunodeficiencies resulting from gain-of-function mutations in the genes (*PIK3CD* and *PIK3R1*) encoding the subunits of phosphoinositide 3-kinase δ (PI3Kδ) and were first described in 2013. These mutations result in the hyperactivation of the PI3K/AKT/mTOR/S6K signally pathways. In this mini-review we have detailed the current treatment options for APDS. These treatments including conventional immunodeficiency therapies such as immunoglobulin replacement, antibiotic prophylaxis, and hematopoietic stem cell transplant. We also discuss the more targeted therapies of mTOR inhibition with sirolimus and selective PI3Kδ inhibitors.

Keywords: activated PI3 kinase delta syndrome, PASLI, HSCT, primary immunodeficiency, APDs

OPEN ACCESS

Edited by:
Carrie L. Lucas,
Yale University, United States

Reviewed by:
Talal A. Chatila,
Harvard University, United States
Silvia Clara Giliani,
Università degli Studi di Brescia, Italy

***Correspondence:**
Tanya I. Coulter
tanya.coulter@gmail.com

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *08 June 2018*
Accepted: *20 August 2018*
Published: *07 September 2018*

Citation:
Coulter TI and Cant AJ (2018) The Treatment of Activated PI3Kδ Syndrome. Front. Immunol. 9:2043. doi: 10.3389/fimmu.2018.02043

Activated phosphoinositide 3-kinase δ syndrome (APDS), also known as PASLI disease (p110d-activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency) is a combined immunodeficiency resulting from dominant, gain-of-function mutations in the genes encoding p110δ (*PIK3CD*) and p85α (*PIK3R1*), the catalytic and regulatory subunits of phosphoinositide 3-kinase δ (PI3Kδ). These mutations result in the hyperactivation of the PI3K/AKT/mTOR/S6K signally pathways in immune cells (1–3).

Patients with APDS may develop immunodeficient and immunodysregulatory features including recurrent respiratory tract infections, bronchiectasis, herpesvirus infections, autoimmunity, non-neoplastic lymphoproliferation, and lymphoma, as well as neurodevelopment delay and growth retardation (4, 5). Clinical cohort studies show that the manifestations of APDS are highly variable, even within families carrying the same mutation, ranging from asymptomatic adult patients to those with primary antibody deficiency, those with a profound immunodeficiency causing early death, to others suffering from lymphoproliferation and malignancy. Thus, the treatment of patients with APDS has varied considerably from simple observation to haematopoietic stem cell transplant (HSCT) in childhood (4, 5). These studies also suggest that disease manifestations mostly have a pediatric onset with recurrent respiratory infections usually the first manifestation occurring in infancy or childhood [0–10 years of age in (4, 5)] followed by bronchiectasis and autoimmunity in later childhood (4–6).

APDS due to gain-of-function mutations in the genes encoding p110δ (*PIK3CD*) and p85α (*PIK3R1*) are termed APDS1 and APDS2 respectively. Previously we have described a cohort of 53 patients with APDS1, 50 of whom had the E1021K mutation and 3 of with the E525 mutation (4). Similarly, Elkaim et al. (5) studied a cohort of 36 patients with APDS2 due to mutations within splice acceptor and donor sites in exon 11 of *PI3KR1*. Comparing the clinical features of these

APDS1 and APDS2 cohorts suggests a similar phenotype with the exception that the APDS1 cohort had a higher prevalence of bronchiectasis (60 vs. 18%) and lower prevalence of lymphoma (13 vs. 25%) compared to APDS2, growth retardation was only reported in the APDS2 cohort and there was an asymptomatic E1021K mutation carrier in the APDS1 cohort compared no asymptomatic individuals in the APDS2 cohort. To date there has been no evidence that the different *PIK3CD* and *PIK3R1* genes mutations lead to different APDS1 and APDS2 phenotypes, however this does require further study. Also, there has been no suggestion to date that APDS1 and APDS2 should be treated differently.

Although APDS was only first described in 2013, many individuals were already under the care of immunology services with diagnoses such as common variable Immunodeficiency (CVID) or combined immunodeficiency (CID), and being treated with conventional therapies including prophylactic antibiotics, immunoglobulin replacement, and in some cases HSCT (1, 3). As patients with APDS can also develop autoimmune and inflammatory complications, others were on various immunosuppressive therapies. Our increasing understanding of the underlying mechanism of APDS however suggests a role for more targeted treatment in this disorder such as direct inhibition of the activated PI3Kδ by selective PI3Kδ inhibitors or inhibition of the downstream mTOR pathway by Sirolimus (3, 7).

In this mini-review will we review how APDS is treated, examining the experience of using conventional immune deficiency therapies in APDS including HSCT, and the more recent experience of selective PI3Kδ inhibitors and Sirolimus. These treatment options are summarized in **Table 1**.

CONVENTIONAL PID TREATMENTS IN APDS

Individuals with APDS often have demonstrable antibody deficiency and lymphopenia and recurrent infections. These complications have led immunologists to treat APDS with standard supportive therapies such as prophylactic antibiotic therapy, immunoglobulin replacement therapy (IRT), and HSCT. (4) and (5) revealed the frequency of conventional treatments in patients with APDS.

ANTIMICROBIAL PROPHYLAXIS

In our APDS1 cohort 62% of patients currently received and 9% had previously received antibiotic prophylaxis. Similarly, Elkaim et al. (5) found 61% of patients with APDS2 were on antibiotic prophylaxis. The antibiotic prophylactic regimens used were similar to those used in patients with antibody defects, largely Trimethoprim/Sulfamethoxazole or Azithromycin. Antibiotic prophylaxis alone sufficed for some patients, however most needed IRT (4, 5). Combined IRT and antibiotic prophylaxis is especially important for patients with respiratory tract infections in the context of established bronchiectasis (4, 5).

Although APDS is also associated with persistent, severe, or recurrent herpesvirus infections (3, 4), in our cohort of patients with APDS1 only 6% were on long term Acyclovir/Valaciclovir due to previous herpes infections despite 49% of patients having had a significant herpes infection previously. 13% of the patients in our APDS1 cohort received antifungal prophylaxis due to previous mucocutaneous candidiasis. No cases of Aspergillus species infection in APDS have been reported.

In individuals with APDS who have received *Mycobacterium bovis* bacillus Calmette Guèrin (BCG) vaccination, persistent local BCG site skin reactions have been reported (4, 5). Chiriaco et al. (8) reported that APDS patient monocytes-derived macrophages failed to eliminate BCG infection *in vitro*, raising the question as to whether APDS patients should be given anti BCG therapy. However, disseminated BCGosis and other mycobacterial infections have not been reported in APDS, and so whilst individual patients have been treated for local BCG infections, APDS patients are not routinely placed on anti-mycobacterial prophylaxis.

IMMUNOGLOBULIN REPLACEMENT THERAPY

Many patients with APDS have antibody deficiency and associated recurrent respiratory tract infections. These antibody defects range from poor vaccine responses and IgG subclass deficiencies to significantly reduced IgG and IgA with normal or elevated IgM. Many patients with APDS have a historical diagnosis of specific antibody deficiency, subclass deficiency, common variable immune deficiency and hyper IgM syndrome and have been treated with IRT.

The majority of APDS patients, 87% in the Coulter et al. (4) APDS1 cohort, and 89% in the Elkaim et al. (5) APDS2 cohort received long-term IRT with a reduction in bacterial infection burden in most cases. IRT was administered as in other antibody deficiencies, i.e., as a long-term therapy, given as intravenous (IVIG) or subcutaneous (SCIG) infusions. IRT is usually commenced at a dose of 0.4 g/kg/month in antibody deficient patients without bronchiectasis and at a higher dose of 0.6 g/kg/month in patients with bronchiectasis (9–11).

IRT is of reported benefit in many patients with APDS reducing respiratory tract infections (4, 12, 13). However, IRT does not appear to prevent the development of the herpes infections nor lymphoproliferative, autoimmune/inflammatory complications nor lymphoma (4, 12, 14, 15). Furthermore, in some APDS patients bronchiectasis has progressed despite optimal IRT (4, 5, 12, 15).

Many patients with APDS develop recurrent respiratory tract infections which often progress rapidly to bronchiectasis in childhood (4, 5). Individuals with APDS and bronchiectasis should be treated as in other patients with bronchiectasis, including airway clearance, physiotherapy, influenza immunization, patient education antibiotic treatment for infective exacerbations, the consideration of interval intensive

TABLE 1 | Treatment options in Activated PI3Kδ Syndrome (APDS).

Treatment	Benefit in APDS	Proposed mechanism of action in APDS
Antimicrobial prophylaxis (e.g., Trimethoprim/Sulfamethoxazole or Azithromycin)	Reduction in respiratory tract infections	Prevention of respiratory bacterial infections
Immunoglobulin replacement therapy	Reduction in respiratory tract infections	Correction of antibody deficiency secondary to APDS
Haematopoietic stem cell transplantation	Reduction in respiratory & herpes infections Reduction in lymphoproliferation & autoimmunity	Replacement leukocytes effected by PI3Kδ hyperactivation
Sirolimus (Rapmycin)	Reduction in lymphoproliferation	Reduction in mTOR hyperactivation
Selective PI3Kδ inhibitors (e.g., Leniolisib)	Reduction in lymphoproliferation	Reduction in PI3Kδ hyperactivation

physiotherapy and antibiotics, nebulized hypertonic saline and bronchodilators, and prophylactic antibiotics where these therapies may benefit. It is recommended that the management and monitoring of patients with bronchiectasis and immune deficiency should be provided through a joint clinical immunology and respiratory (±pediatricians) care model with access to specialist respiratory nursing and physiotherapy services with an expertise in bronchiectasis (16–18).

HAEMATOPOIETIC STEM CELL TRANSPLANTATION

APDS can present as or evolve into a profound combined immunodeficiency which leads into significant morbidity and early death. Allogenic haematopoietic stem cell transplantation (HSCT) has been performed to treat life-threatening infections and as part of treatment for lymphoma (4, 5, 19). Nademi et al. (19) reported a series of eleven patients with APDS and severe immune deficiency who underwent HSCT in seven pediatric centers. The diagnosis of APDS was made prior to HSCT in five patients and retrospectively in six. Ten of the 11 bore the E102IK *PIK3CD* mutation, and one a *PIK3R1* mutation. All had suffered severe sinopulmonary infection, four had bronchiectasis, six severe Herpes family virus infection, four enteropathy/colitis, eight lymphoproliferation, one intestinal obstruction, three significant progressive liver disease due to Cryptosporidium infection, one had glomerulonephritis and one glomerulosclerosis. Five had received steroids ± Sirolimus and seven immunoglobulin, and all were deteriorating despite their treatment. Age at transplant ranged from 5 to 23 years. Peripheral blood stem cells were used in 7 of the 11, five were transplanted from a matched unrelated donor, four from a matched sibling and two from a mismatched donor. Various conditioning regimes were used, mostly Fludarabine with either Treosulphan or Melphalan, and all but two were given serotherapy. Two patients died from CMV/Adenovirus pneumonitis and idiopathic pneumonitis respectively. Mild (Grade I or II) Graft vs. Host Disease occurred in nine patients, but fully resolved in all. Two patients had autoimmune haemolytic anemia, and one encephalitis, these complications also fully resolving. Eight of the nine surviving patients are off IVIG and well, one has got low donor chimerism and is awaiting a second HSCT (19). These preliminary data show that HSCT can be curative with resolution of pre HSCT signs and symptoms, and survival looks to be similar to that seen after HSCT for other PIDs. Indeed, considering the considerable pre HSCT morbidity in this cohort, less success might have been expected, and so it seems a promising option for patients with progressive disease unresponsive to other treatment. To date, long term sequelae do not seem to be a problem with follow up between 8 months and 16 years. However, the risks and benefits of HSCT in patients with severe lung disease are not clear, and longer term follow up in a larger cohort is needed to ascertain the place of HSCT in treating APDS.

IMMUNOSUPPRESSION

Thirty-four percent of our APDS1 cohort had autoimmune or inflammatory disease with 30% of the cohort treated with at least one course of immunosuppressive therapy. Cytopenias were the most common autoimmune disease in APDS, but conditions described include renal disease, inflammatory colitis, exocrine pancreatic insufficiency, seronegative arthritis, cirrhosis, and sclerosing cholangitis. In some cases renal or liver transplantation was necessary (4, 5).

The autoimmune cytopenias have been found to be responsive to steroids, rituximab and splenectomy (4, 12). Rituximab therapy has been noted to be complicated by sustained B-cell lymphopenia (4). Elgizouli et al. (12) described good clinical response from prednisolone and maintenance mesalazine in inflammatory bowel disease in APDS1. Hartman et al. (20) described two patients with APDS and primary sclerosing cholangitis who underwent liver transplantation. In one case the cholangitis relapsed 3 years after the original transplant, and the patient was awaiting re-transplantation.

Non-neoplastic lymphoproliferation including lymphadenopathy, splenomegaly, hepatomegaly with lymphoid aggregates in the respiratory and gastroenteric tract being a very frequent finding in APDS. We found Rituximab to be of some benefit in the management of these non-neoplastic lymphoproliferation in five patients with APDS1 (4).

SIROLIMUS

The mTOR inhibitor, Sirolimus (Rapamycin) has been found to decrease in non-neoplastic lymphoproliferation (3–5, 21, 22). mTOR (mammalian target of rapamycin) is activated downstream of PI3K and has a prominent role in T cell

metabolism and the regulation of immune responses (2, 3). Previously Sirolimus therapy had been reported in a case of APDS to reduce hepatosplenomegaly and lymphadenopathy, increase naïve T cell frequencies, and restore T cell proliferation and IL-2 secretion (3). Recently Maccari et al. (6) published the initial findings of the ESID APDS registry. They looked specifically at the use of Sirolimus/Rapamycin in APDS and noted a significant benefit in the treatment of non-neoplastic lymphoproliferative disease with 8/25 having complete response and 11/25 having partial response to sirolimus. Sirolimus was found to be of less benefit in treating APDS related-cytopenias and gastrointestinal disease with complete response in 3/14 and 3/15 cases and a partial response in 2/14 and 3/15 of cases respectively. Thus, studies to date support the use of Sirolimus in treating APDS-related non-neoplastic lymphoproliferation, though some patients may only develop partial benefit and lymphoproliferation may reoccur after treatment cession. The long-term benefits and risks of Sirolimus therapy remain to be determined.

SELECTIVE PI3Kδ INHIBITORS

Selective PI3Kδ inhibitors have the potential to offer a targeted treatment option for APDS patients with greater efficacy and fewer side effects. PI3K inhibitor therapies have been tested in oncology trials, resulting in the approval of Idelalisib, for treatment of chronic lymphocytic leukemia and non-Hodgkin lymphoma (23, 24). However, Idelalisib has a considerable side-effect profile, including pneumonitis, transaminitis, and colitis (24).

Leniolisib (CDZ173), is a potent oral inhibitor of the p110δ subunit of PI3Kδ which is currently being studied for the treatment of APDS by Novartis (NCT02435173) (7, 25). Rao et al. (7) described a 12-week, open label, multisite clinical trial involving six individuals with APDS, all with lymphadenopathy and splenomegaly on CT/MRI at baseline. After an initial screening period of up to 50 days, including an immunosuppressive/immunomodulatory treatment wash-out period, all patients received Leniolisib in escalating doses (10, 30, 70 mg BD for 4 weeks each receiving 12 weeks total). After the 12 weeks of Leniolisib treatment, significant reduction in lymph node sizes (mean 40%, 13–65%) and spleen volume (mean 39%, range 26–57%) was seen in all patients. Patients reported an increase in wellbeing (Patient global assessment questionnaire 11 ± 11 mm), decrease in fatigue and less disease activity, as assessed by the physician global assessment questionnaire. There was also an improvement in immunological markers with a normalization of transitional B cell and naive B cell populations, and reduction of senescent (CD57+ CD4-) T cells, PD-1+CD4+ T cells, IgM levels and the cytokines and chemokines TNF, IFNγ, CXCL13 & CXCL10. Five of six patients proceeded to enroll in the open-label long term extension study using Leniolisib 70 mg BD. The sixth patient reportedly did not enroll due to logistical reasons related to traveling. Rao et al. (7) reported that during the first 9 months of this extension study no patient has experienced significant adverse effects. This initial report supports that Leniolisib, like Sirolimus, may be a treatment for APDS-related lymphoproliferation. However, the improvements in patient well-being and immunological parameters also suggests Leniolisib and other PI3Kδ inhibitors could have wider benefits for individuals with APDS, athough further studies are required, particularly with regard to the effect on respiratory complications of APDS.

The inhaled PI3Kδ inhibitor, GSK2269557 or Nemiralisib, is also currently being studied in APDS sponsored by GlaxoSmithKline (NCT02593539) (24, 26). Though an oral inhibitor maybe more effective for lymphoproliferation; it is proposed an inhaled inhibitor could benefit patients primarily affected by airway infection and bronchiectasis. The GSK2269557 clinical trial has not as yet reported results, but is described as a "multi-center, non-randomized, open-label, uncontrolled, single group study to investigate the safety and pharmacokinetics during 84 days repeat dosing treatment with 1,000 micrograms of inhaled in addition to standard of care, in subjects with APDS." GSK2269557 is also currently being investigated as an anti-inflammatory treatment in Chronic Obstructive Pulmonary Disease (COPD) (26).

CONCLUSION

The great clinical heterogeneity of APDS means that treatment needs to be carefully tailored to each patient's needs. Some asymptomatic family members need no treatment at all; patients with defective antibody production and recurrent infections may need supportive treatment such as prophylactic antibiotic and/or IRT. The decision as to which treatment needs to be made on clinical grounds. Patients with recurrent chest infection need very careful management in conjunction with a respiratory specialist as the development of life limiting and life threatening bronchiectasis is one of the major causes of early death in APDS. Sirolimus can be a very effective treatment for lymphoproliferative disease, but whether these benefits are sustained long term remains to be seen. Specific PI3Kδ inhibitor therapies appear very attractive and useful in resolving lymphoproliferation but serious side effects have been reported and the results from ongoing clinical trials will hopefully enable us to titrate the best dose and means of delivery as well as delineate long term risks and benefits. Preliminary data suggests that for younger patients in the process of developing life limiting and life threatening complications of APDS, HSCT is curative, but it carries a 10–20% mortality risk and it is unclear whether severe lung disease is reversed. Only with more outcome data from these therapies will the role of each of these treatment modalities become clear.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi: 10.1126/science.1243292
2. Jung S, Gámez-Díaz L, Proietti M, Grimbacher B. "Immune TOR-opathies," a novel disease entity in clinical immunology. *Front Immunol*. 9:966. doi: 10.3389/fimmu.2018.00966
3. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol*. (2014) 15:88–97. doi: 10.1038/ni.2771
4. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol*. (2017) 139:597–606.e4. doi: 10.1016/j.jaci.2016.06.021
5. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. *J Allergy Clin Immunol*. (2016) 138:210–8.e9. doi: 10.1016/j.jaci.2016.03.022
6. Maccari ME, Abolhassani H, Aghamohammadi A, Aiuti A, Aleinikova O, Bangs C, et al. Disease evolution and response to rapamycin in activated phosphoinositide 3-kinase δ syndrome: the European Society for immunodeficiencies-activated phosphoinositide 3-kinase δ syndrome registry. *Front Immunol*. 9:543. doi: 10.3389/fimmu.2018.00543
7. Rao VK, Webster S, Dalm VASH, Šedivá A, van Hagen PM, Holland S, et al. Effective "activated PI3Kδ syndrome"-targeted therapy with the PI3Kδ inhibitor leniolisib. *Blood* (2017) 130:2307–16. doi: 10.1182/blood-2017-08-801191
8. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased *in vitro* anti-mycobacterial activity in the myeloid compartment. *Clin Immunol*. (2017) 178:20–8. doi: 10.1016/j.clim.2015.12.008
9. Edgar JDM, Richter AG, Huissoon AP, Kumararatne DS, Baxendale HE, Bethune CA, et al. Prescribing immunoglobulin replacement therapy for patients with non-classical and secondary antibody deficiency: an analysis of the practice of clinical immunologists in the UK and Republic of Ireland. *J Clin Immunol*. (2018) 38:204–13. doi: 10.1007/s10875-017-0469-4
10. Berger M, Jolles S, Orange JS, Sleasman JW. Bioavailability of IgG administered by the subcutaneous route. *J Clin Immunol*. (2013) 33:984–90. doi: 10.1007/s10875-013-9876-3
11. Lucas M, Hugh-Jones K, Welby A, Misbah S, Spaeth P, Chapel H. Immunomodulatory therapy to achieve maximum efficacy: doses, monitoring, compliance, and self-infusion at home. *J Clin Immunol*. (2010) 30:S84–9. doi: 10.1007/s10875-010-9400-y
12. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hülsdünker J, Eskandarian Z, et al. Activating PI3Kδ mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol*. (2016) 183:221–9. doi: 10.1111/cei.12706
13. Kannan JA, Dávila-Saldaña BJ, Zhang K, Filipovich AH, Kucuk ZY. Activated phosphoinositide 3-kinase δ syndrome in a patient with a former diagnosis of common variable immune deficiency, bronchiectasis, and lymphoproliferative disease. *Ann Allergy Asthma Immunol*. (2015) 115:452–4. doi: 10.1016/j.anai.2015.08.009
14. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase δ syndrome. *J Allergy Clin Immunol*. (2014) 134:233–6. doi: 10.1016/j.jaci.2014.02.020
15. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol*. (2014) 34:272–6. doi: 10.1007/s10875-014-0012-9
16. Coulter TI, Devlin L, Downey D, Elborn JS, Edgar JDM. Immunodeficiency in Bronchiectasis. In: Chalmers J, Polverino E, Aliberti S, editors. *Bronchiectasis*. Cham: Springer (2018), 77–100.
17. Pasteur MC, Bilton D, Hill AT. British Thoracic Society Bronchiectasis non-CF Guideline Group. 2010 – British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* (2010) 65:i1–58. doi: 10.1136/thx.2010.136119
18. Bonilla FA, Barlan IB, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International consensus document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol Pract*. (2016) 4:38–59. doi: 10.1016/j.jaip.2015.07.025
19. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol*. (2017) 139:1046–9. doi: 10.1016/j.jaci.2016.09.040
20. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol*. (2015) 35:11–4. doi: 10.1007/s10875-014-0109-1
21. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110δ cause Activated PI3K-δ Syndrome 1 (APDS1). *Haematologica* (2017) 102:e278–81. doi: 10.3324/haematol.2017.167601
22. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kδ syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett*. (2017) 190:279–81. doi: 10.1016/j.imlet.2017.08.021
23. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell*. (2017) 170:605–35. doi: 10.1016/j.cell.2017.07.029
24. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kδ and primary immunodeficiencies. *Nat Rev Immunol*. (2016) 16:702–14. doi: 10.1038/nri.2016.93
25. Hoegenauer K, Soldermann N, Zécri F, Strang RS, Graveleau N, Wolf RM, et al. Discovery of CDZ173 (Leniolisib), representing a structurally novel class of PI3K delta-selective inhibitors. *ACS Med Chem Lett*. (2017) 8:975–80. doi: 10.1021/acsmedchemlett.7b00293
26. Cahn A, Hamblin JN, Begg M, Wilson R, Dunsire L, Sriskantharajah S, et al. Safety, pharmacokinetics and dose-response characteristics of GSK2269557, an inhaled PI3Kδ inhibitor under development for the treatment of COPD. *Pulm Pharmacol Ther*. (2017) 46:69–77. doi: 10.1016/j.pupt.2017.08.008

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 26 September 2018
doi: 10.3389/fimmu.2018.02172

Class-Switch Recombination (CSR)/Hyper-IgM (HIGM) Syndromes and Phosphoinositide 3-Kinase (PI3K) Defects

Rekha D. Jhamnani[1], *Cristiane J. Nunes-Santos*[2,3], *Jenna Bergerson*[4]* and *Sergio D. Rosenzweig*[2]*

[1] Allergy and Immunology Fellowship Program, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2] Immunology Service, Department of Laboratory Medicine, National Institutes of Health Clinical Center, National Institutes of Health, Bethesda, MD, United States, [3] Instituto da Crianca, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil, [4] Laboratory of Clinical Immunology and Microbiology, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Klaus Warnatz, Albert-Ludwigs-Universität Freiburg, Germany
Mike Recher, Universität Basel, Switzerland

***Correspondence:**
Jenna Bergerson
jenna.bergerson@nih.gov
Sergio D. Rosenzweig
srosenzweig@cc.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *05 February 2018*
Accepted: *03 September 2018*
Published: *26 September 2018*

Citation:
Jhamnani RD, Nunes-Santos CJ, Bergerson J and Rosenzweig SD (2018) Class-Switch Recombination (CSR)/Hyper-IgM (HIGM) Syndromes and Phosphoinositide 3-Kinase (PI3K) Defects. Front. Immunol. 9:2172. doi: 10.3389/fimmu.2018.02172

Antibody production and function represent an essential part of the immune response, particularly in fighting bacterial and viral infections. Multiple immunological phenotypes can result in dysregulation of the immune system humoral compartment, including class-switch recombination (CSR) defects associated with hyper-IgM (HIGM) syndromes. The CSR/HIGM syndromes are defined by the presence of normal or elevated plasma IgM levels in the context of low levels of switched IgG, IgA, and IgE isotypes. Recently described autosomal dominant gain-of-function (GOF) mutations in *PIK3CD* and *PIK3R1* cause combined immunodeficiencies that can also present as CSR/HIGM defects. These defects, their pathophysiology and derived clinical manifestations are described in depth. Previously reported forms of CSR/HIGM syndromes are briefly reviewed and compared to the phosphoinositide 3-kinase (PI3K) pathway defects. Diseases involving the PI3K pathway represent a distinctive subset of CSR/HIGM syndromes, presenting with their own characteristic clinical and laboratory attributes as well as individual therapeutic approaches.

Keywords: class-switch recombination, somatic hypermutation, CD40L/CD40 pathway, NF-kB pathway, mTOR pathway, gain-of-function mutations, PIK3CD, PIK3R1

INTRODUCTION

Effective humoral immunity relies on the ability of B cells to recognize a wide variety of antigens and respond properly. A diverse BCR repertoire is established by V(D)J recombination in early stages of B-cell development, prior to antigen encounter. Functional commitment of antibodies takes place at a later stage, allowing responses to be tailored upon antigen recognition in a process called class-switch recombination (CSR) (1, 2).

Defective CSR results in an abnormal humoral pattern known as hyper-IgM syndrome (HIGM), characterized by normal or elevated serum levels of IgM in the context of low levels of switched IgG, IgA, and IgE isotypes. A heterogeneous group of primary immunodeficiencies (PID) underlies HIGM, collectively known as CSR defects (1, 3–8).

Two recently described PID, caused by autosomal dominant gain-of-function (GOF) mutations in *PIK3CD* and *PIK3R1*, inducing hyperactivation of the enzyme phosphoinositide 3-kinase δ (PI3Kδ) (9–12), can present with elevated IgM levels in 58–79% of cases, according to the two largest cohorts published to date (13, 14). This finding suggests that PI3K GOF defects disturb CSR and therefore might be part of CSR/HIGM syndromes. As most of the known biology related to the CSR process comes from studies of PID (3, 7), a deeper look into new defects can be instructive to the field.

In this review, antibody maturation steps as well as previously known forms of CSR/HIGM syndromes are briefly described, with special emphasis on the more recently reported PI3K pathway activating mutations. The pathophysiology and clinical manifestations associated with CSR/HIGM syndrome in PIK3CD and PIK3R1 defects are described and analyzed in more detail.

CLASS-SWITCH RECOMBINATION AND SOMATIC HYPERMUTATION

B-cell development occurs in the bone marrow. Upon completion, naïve B cells express unique B-cell receptors (BCRs) in the form of membrane-bound IgD and IgM. Naïve B cells circulate throughout the blood and lymphatics to secondary lymphoid organs where activation occurs upon ligation of the BCR with its cognate antigen. Subsequently, and mostly with the help of T cells, two critical steps for antibody maturation occur predominantly inside germinal centers: CSR and somatic hypermutation (SHM) (1–4, 7, 8, 15).

In T-cell-dependent responses, CD40 ligand (CD40L), which is expressed on activated $CD4^+$ T cells, including T follicular helper cells (Tfh), binds to CD40, a receptor constitutively expressed on B lymphocytes (and monocytes). The CD40L/CD40 engagement in the germinal center, in the presence of the appropriate cytokine milieu, promotes B cells to undergo proliferation, CSR and SHM, through activation of transcription factors, such as nuclear factor-κB (NF-κB). Although in a less effective way, CSR can also be induced in a T-cell-independent manner, via concurrent engagement of BCR or TACI and Toll-like receptors (TLRs) (1–4, 7, 8, 15, 16).

The CSR process involves genomic recombination of the IgM-defining constant (C)μ region in the Ig locus for a downstream Cα, Cγ, or Cε region, coding for the constant regions of IgA, IgG, or IgE isotypes, respectively. Constant regions are flanked by switch (S) regions and CSR occurs when two S regions upstream of a C region undergo switch-region recombination, excising the preceding C regions from the Ig locus. CSR involves a sequential, multi-step process. First, there is a requirement for transcriptional access to the S regions, which allows for activation-induced cytidine deaminase (AID) to catalyze the conversion of cytidine nucleotides to uracil. Next, uracil removal by uracil N-glycosylase (UNG), which, along with endonucleases and the mismatch repair (MMR) machinery, facilitates the generation of double-stranded DNA breaks. Finally, the removal of the intervening DNA as excision circles and repair of the DNA double-stranded breaks, primarily by non-homologous end joining (NHEJ), complete the main steps involved in CSR. Thus, the CSR machinery preserves the Ig variable (V) locus region, thereby maintaining antigen specificity (1–4, 7, 8, 15).

The SHM process randomly introduces mutations into the Ig V regions, altering the affinity for antigens while maintaining the same Ig C isotype. Uracil residues are introduced by AID and can be further modified by UNG. These transitions and transversions generated at the Ig V region are perpetuated during DNA replication, the MMR process, and error-prone polymerase enzymatic activities. SHM additionally allows for affinity maturation by providing a higher diversity of antibodies from which clones with the highest affinity to foreign antigens can be selected. While all HIGM syndromes are determined by CSR defects, SHM abnormalities are not always involved in this group of diseases (1–4, 7, 8, 15).

HYPER-IGM SYNDROMES

Deficiencies in CD40L, CD40, AID, UNG, NEMO, IkBα, ATM, and PMS2 represent the previously identified forms of CSR/HIGM syndromes. While the clinical manifestations can be variable, the HIGM phenotype can be divided into those disorders that directly affect the CD40/CD40L pathway (B- and T-cells are impacted), and those like AID, UNG, NEMO, IkBα, ATM, and PMS2 where mainly B cells are affected in the CSR/HIGM defect (3, 5–7). The more relevant and comparative characteristics of these diseases are summarized in **Table 1**.

CD40L Deficiency

The first identified CSR/HIGM syndrome was X-linked CD40L deficiency, which is due to a defect in *CD40L* (17–21). The inability of mutated CD40L protein to bind to CD40 affects CD4 T-cell and B-cell interactions, impairing CSR, SHM, T-cell co-stimulation, and development of memory B cells, resulting in a combined immunodeficiency. Patients with this disorder present in infancy with recurrent sinopulmonary infections, and opportunistic infections, such as *Pneumocystis jirovecii* pneumonia (PJP), or *Cryptosporidium*-induced diarrhea and sclerosing cholangitis, which may predispose patients to tumors of the liver, pancreas or biliary tree [reviewed in (3–8, 22)]. In addition to the characteristic immunoglobulin findings, neutrophils can also be low. Activated $CD4^+$ T-cells and platelets from patients with X-linked HIGM usually do not express CD40L on their surface; however, some mutations result in defective protein that may still be expressed, thus detecting protein expression is not always a reliable diagnostic tool to rule out this disease, and CD40 binding capacity should be assessed. Past studies have shown that between 7 and 23% of patients with CD40 ligand deficiency have dysfunctional, although detectable, CD40L expression by various methods of testing (5, 23). A normal number of mature B cells is typically observed, but class-switched memory B cells are usually very low to absent. Of note, upon *in vitro* activation, patients' B cells are able to undergo CSR (3). Class-switched $CD27^-IgA^+$ memory B cells can still be produced, although showing limited proliferation (24) and abnormal SHM (25). Pathologically, lymph nodes from these patients are devoid of germinal centers as CD40 and

TABLE 1 | Comparison of Hyper-IgM Syndromes.

	Inheritance	Opportunistic infections	Lymphoid hyperplasia	Lymphoma	Autoimmunity	CSR defect	SHM defect
CD40L deficiency	XL	+	−	−	+	+	+
CD40 deficiency	AR	+	−	−	+	+	+
NEMO deficiency	XL	+	−	−	+	+	+/−
IkBα deficiency	AD	+	−	−	+/−	+	−
AID deficiency	AR/AD	−	++	−	+	+	+/−
UNG deficiency	AR	−	+	+/−	+	+	−
ATM syndrome	AR	+/−	−	+	+	+	−
PMS2 deficiency	AR	−	−	+	−	+	−
Undefined upstream	AR	−	+	+	+	+	+
Undefined downstream	AR	−	+	−	+	+	−
PI3KCD (APDS1)	AD	+/−	+	+	+	+	+/−
PI3KR1 (APDS2)	AD	+/−	+	+	+	+	+/−

NEMO, NF-kB essential modulator; AID, activation-induced cytidine deaminase; UNG, uracil N-glycosylase; ATM, ataxia-telangiectasia mutated; PMS2, post-meiotic segregation increased, S. cerevisae 2; PI3KCD, phosphatidylinositol 3-kinase catalytic subunit delta; PI3KR1, phosphatidylinositol 3-kinase regulatory subunit 1; XL, X-linked; AR, autosomal recessive; AD, autosomal dominant; CSR, class-switched recombination; SHM, somatic hypermutation.

CD40L interaction is imperative for secondary lymphoid organ maturation (3–8, 22).

CD40 Deficiency

CD40 deficiency is rare and inherited in an autosomal recessive (AR) manner (26). Recent reports have enumerated only 17 patients from 13 unrelated families with CD40 deficiency (26, 27). The clinical and immunological phenotype of impaired CSR and SHM is similar to that seen in CD40L deficiency, with one important difference; B cells from CD40 deficient patients are unable to undergo class switching *in vitro* upon activation with agonists and cytokines as per their intrinsic defect. Most patients lack expression of CD40 on the surface of B cells and monocytes (3–8, 26). Although rare, there are reports of CD40 deficient patients in whom a dysfunctional CD40 protein could still be detected (27, 28).

AID and UNG Deficiencies

In contrast to impairments in CD40L/CD40 signaling, defects in the immunoglobulin isotype switching enzymes AID and UNG seem to be primarily limited to deficiencies in antibody production and are mostly inherited in AR manner (29, 30). The most commonly identified cause of AR-HIGM is due to defects in the *AICDA* gene, which encodes AID, an enzyme needed for CSR and SHM in B cells, as described above. Expression of AID is upregulated in response to CD40 signaling, initiating immunoglobulin isotype switching by deaminating deoxycytosine in the immunoglobulin heavy chain switch regions generating deoxyuracils in both DNA strands (31). A limited number of patients carrying *AICDA* heterozygous null mutations that act in a dominant negative way have been associated with autosomal dominant (AD) forms of CSR/HIGM syndromes that preserve SHM and present with a milder clinical phenotype (32, 33). Interestingly, biallelic mutations located in the C terminal region of *AICDA* that do not exert a dominant negative effect have been described in patients with normal SHM, despite drastically impaired CSR (34). Defects in UNG, which removes the deoxyuracils from DNA and initiates the DNA repair pathway, also cause an AR-CSR/HIGM syndrome, and exhibit a skewed pattern of SHM with a predominance of G:C transitions (30). Very few cases of UNG deficiency have been reported. Both AID and UNG deficiencies present clinically with recurrent sinopulmonary infections, mainly caused by encapsulated bacteria. Opportunistic infections and neutropenia are rarely seen. Lymph node hyperplasia and autoimmunity are prominent findings in these diseases; giant germinal centers are typical in AID deficiency. Patients with AID deficiency have also been reported to exhibit gastrointestinal infections, central nervous system infections and arthritis. In both AID and UNG deficiencies patients exhibit no class-switched memory B lymphocytes (3–8, 30, 31).

NEMO and IkBα Defects

X-linked NF-kB essential modulator (NEMO) disease in males can result in CSR deficiency because of the important role of NF-kB in the signaling pathway downstream of CD40 (reviewed in (35)). Since NF-kB is expressed widely and is involved in multiple cell lineages signaling pathways, including both the innate and adaptive immune systems, manifestations of this condition tend to be more diverse and severe. Clinically, male patients carrying hemizygous hypomorphic mutations in this gene can experience a combination of manifestations, such as ectodermal dysplasia and increased susceptibility to viral and bacterial diseases (mainly *Streptococcus pneumoniae*). Osteopetrosis, lymphedema, and mycobacterial infections can also be seen. Interestingly, only about 15% of patients with defects in NF-kB have a HIGM phenotype. Immunoglobulin levels tend to be more variable in this disease as patients have been shown to demonstrate low levels of IgG, low or elevated IgA levels, and normal or increased levels of IgM. Laboratory findings also reflect a defect in switched memory B cells (35).

Heterozygous GOF mutations in *NFKBIA*, encoding IkBα and located downstream of NEMO in the NF-kB signaling pathway, have also been associated to CSR/HIGM syndrome in more than 40% of the patients reported [reviewed in (36)]. These patients share multiple characteristics with those carrying NEMO mutations (e.g., ectodermal dysplasia; viral, bacterial and mycobacterial infection susceptibility) although also presenting some unique features as dysfunctional α/β T cells, very low proportions of memory T cells, and lack of γ/δ Tcells in some but not all affected individuals (36).

Ataxia-Telangiectasia

Ataxia-telangiectasia is an autosomal recessive disorder caused by changes in the Ataxia-telangiectasia mutated (*ATM*) gene, which encodes an enzyme that contributes to DNA mismatch repair. Clinical symptoms include cerebellar ataxia, oculomotor apraxia, telangiectasias and sinopulmonary infections. Because the *ATM* gene is involved in DNA double-stranded repair, patients are also sensitive to ionizing radiation and susceptible to malignancy. Laboratory manifestations can also be variable with some patients presenting with a HIGM phenotype, but most other patients having only reduced levels of IgG2 and IgA. The SHM process is not affected in these patients (37).

PMS2 Deficiency

Though classically associated with Lynch syndrome, an autosomal dominant disorder caused by germline mutations in DNA mismatch repair genes associated with non-polyposis colorectal cancer, biallelic mutations in *PMS2* (post-meiotic segregation increased, *S. cerevisae* 2) result in recurrent severe infections, café-au-lait spots, and a HIGM humoral phenotype. While CSR is defective as in other CSR/HIGM syndromes, SHM is only mildly affected (38) and memory B cells are variably low or normal.

Undefined Hyper-IgM Syndromes

Patients with genetically undefined CSR/HIGM syndromes were reported by Imai et al., and defined as Hyper-IgM type 4 (39). These patients shared clinical and immunologic characteristics with some of the more classical and above-discussed forms of CSR/HIGM syndromes. With the popularization of unbiased genetic testing, many of these undefined CSR/HIGM syndromes can now be attributed to particular underlying molecular defects.

Treatment of CSR/HIGM Syndromes

Since antibody deficiency manifests in all forms of CSR/HIGM syndromes, treatment with immunoglobulin replacement is needed to reduce the frequency and severity of infections. In some cases, prophylactic antibiotics are also needed (40). However, such therapies do not prevent lymphoproliferation, if present, nor is it known if they can mitigate autoimmunity in AR-CSR/HIGM. Patients with CSR/HIGM syndromes that are combined immunodeficiencies (e.g., X-HIGM, CD40 deficiency, NEMO, and NFKBIA) also require *PJP* prophylaxis with trimethoprim-sulfamethoxazole or pentamidine. Neutropenia in X-linked HIGM can be improved using G-CSF (3–8). Hematopoietic stem cell transplant (HSCT) as a treatment for X-linked HIGM syndrome was recently reviewed in a multi-center, international, retrospective (1964–2013), large cohort study of 176 patients. CD40L deficient patients undergoing HSCT showed no statistical differences in terms of overall survival when compared to those not being transplanted. Progress made in HSCT-related issues in recent years, early transplants and better quality of life among transplant survivors, remain the encouraging aspects about HSCT in CD40L deficiency (41).

PHOSPHOINOSITIDE 3-KINASE (PI3K) DEFECTS

Activated phosphoinositide 3-kinase δ syndrome (APDS, also known as PASLI- p110δ-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency) is caused by heterozygous GOF mutations in *PIK3CD* (APDS1/PASLI-CD) or *PIK3R1* (APDS2/PASLI-R1) that induce hyperactivation of the enzyme PI3Kδ (9–12, 42).

Cellular metabolism must be carefully controlled, and in lymphocytes this process requires signaling by a family of enzymes known as phosphoinositide 3-kinases (PI3Ks) that phosphorylate the inositol ring of phosphatidylinositol lipids in the plasma membrane (**Figure 1**). There are three classes of PI3Ks that have been identified in mammals; most relevant to disorders of the immune system are those involving class IA PI3K enzymes, which consist of a catalytic subunit (p110α, p110β, or p110δ) and a regulatory subunit (p85α, p55α, p50α, p85β, or p55γ). Only p110δ (encoded by *PIK3CD*) is restricted to leukocytes, and it is activated by antigen receptors, co-stimulatory receptors, cytokine receptors and growth factor receptors. Briefly, class IA PI3Ks catalyze the phosphorylation of phosphatidylinositol-(4,5)-bisphosphate (PIP2) to generate phosphatidylinositol-(3,4,5)-triphosphate (PIP3) at the plasma membrane of lymphocytes upon receptor activation and leading to downstream Akt-mTOR signaling (42–45). Phosphatase and tensin homolog (PTEN) and SH2 domain-containing inositol 5′-phosphatase (SHIP) serve as negative regulators of PI3K via dephosphorylation of PIP3 (42, 46, 47) (**Figure 1**).

The Akt-mTOR pathway is essential for lymphocyte differentiation, function, and maturation. In T cells, PI3Kδ is mainly activated upon TCR (T-cell receptor), ICOS and IL-2R stimulation. Particularly in $CD8^+$ T cells, the Akt-mTOR pathway is critical for regulating whether naïve cells differentiate into effector or memory cells by controlling the transition from oxidative phosphorylation, a lower energy yielding process, to aerobic glycolysis, better suited for the rapid growth and proliferation needed by effector T cells. Akt also suppresses the transcription factor FOXO1 function via phosphorylation and through PI3Kδ-dependent downregulation of IL-7Rα and CD62L (42, 47, 48).

PI3Kδ is also highly relevant for B-cell development, antibody responses, and B-cell lymphoma prevention. As seen in T lymphocytes, within B cells, PI3Kδ signaling through phosphatidylinositol lipids and Akt promotes the activation of mTOR and suppresses FOXO1. This transcription factor

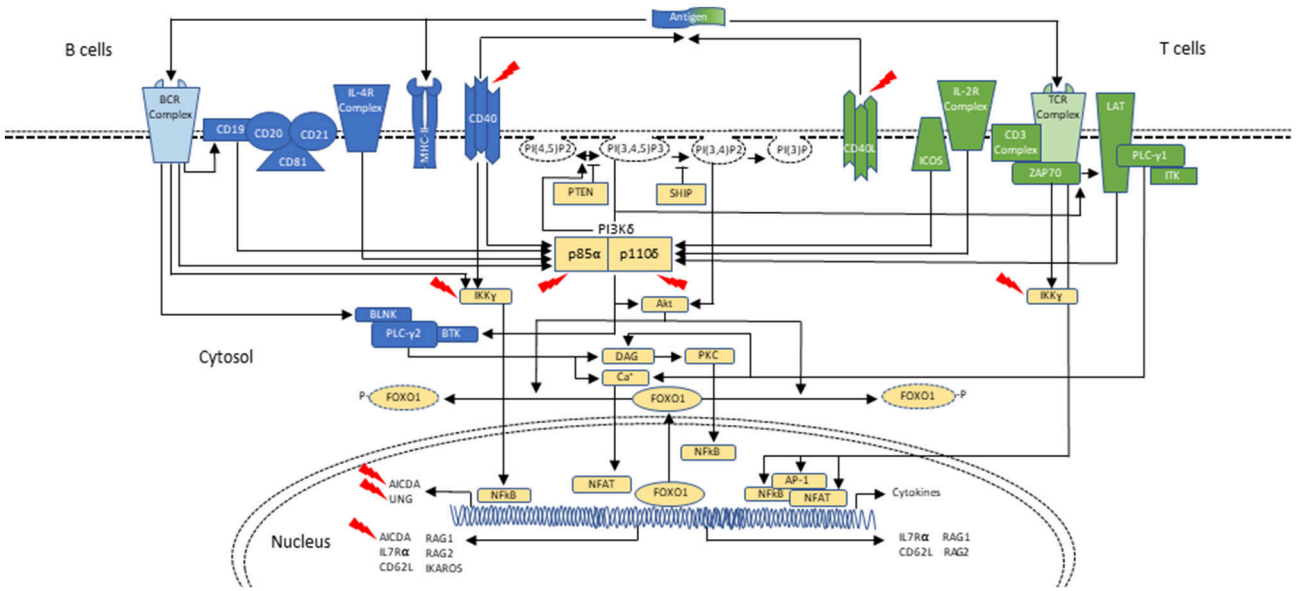


FIGURE 1 | Schematic representation of PI3Kδ activation in B- and T-cells during class-switch recombination. In B cells, PI3Kδ is activated mainly after the BCR (B-cell receptor) cross-linking or upon cytokine stimulation, as with IL-4. CD19 is a co-receptor for the BCR which also p85α can bind. Similar to the T cell, PI3K can signal through Akt to phosphorylate and remove FOXO1 from the nucleus, thus inactivating it. FOXO1 acts as a transcription factor to activate *RAG* genes, *IKAROS*, *CD62L*, and *AICDA* (critical in CSR and SHM). PI3Kδ signals through mTOR to promote B-cell activation (not shown; reviewed in (42)). In T cells, PI3Kδ is mainly activated upon TCR (T-cell receptor), ICOS and IL-2R stimulation. Akt causes FOXO1 to leave the nucleus and helps with PI3Kδ dependent downregulation of IL-7Rα and CD62L. These changes in turn allow the T cell to exit the lymph node and enter circulation. PI3Kδ via mTOR promotes T-cell activation and T-cell effector phenotypes (not shown). In both B and T cells, PTEN and SHIP act as negative regulators of PI3K (reviewed in (42)). Interaction of T cells and B cells in germinal centers through CD40-ligand (CD40L)/CD40 joining is required for class-switch recombination (CSR) and somatic hypermutation (SHM), two events necessary for antibody maturation. Activated $CD4^+$ T cells in the lymph nodes interact with CD40 on B cells, inducing cytokine specific B-cell receptors (IL-R). Signaling through CD40 activates the NF-kB signaling pathway, which ultimately leads to expression of AID and UNG. Mutations affecting components of CD40 mediated B-cell activation can also result in CSR/HIGM syndromes. CD40 has also been associated with PI3K activation. Blue represents signaling elements associated with B cells. Green represents signaling elements associated with T cells. Yellow represents signaling elements that are present in both B- and T- cells. Red arrows point to genes/proteins that when mutated are associated with CSR/HIGM syndromes. Other genes/proteins (e.g., ATM, PMS2, MSH2, and NFKBIA, not included in this figure) can also be associated with CSR/HIGM defects when mutated. BLNK, B-cell linker protein; BTK, Bruton tyrosine kinase; DAG, diacylglycerol; ICOS, inducible T-cell costimulatory; ITK, IL-2 inducible T-cell kinase; LAT, linker for activation of T cells; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PLCγ1, phospholipase Cγ1; PLCγ2, phospholipase Cγ2; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; PI(3)P, phosphoinositide-3-phosphate; PI(3,4)P2, phosphoinositide-3,4-bisphosphate; PI(3,4,5)P3, phosphoinositide-3,4,5-trisphosphate; PI(4,5)P2, phosphoinositide-4,5-bisphosphate; SHIP, SH2 domain-containing inositol-5-phosphatase; ZAP70, zeta-chain-associated protein kinase 70 kD.

activates *RAG*, *IKAROS*, *CD62L* and *AICDA* genes, which as a group are critical for B-cell development, as well as CSR and SHM (42, 49, 50) (**Figure 1**). Furthermore, recent studies identifying patients with loss-of-function (LOF) mutations in PTEN, the oppositional counterpart of PI3K, have revealed hyperactivation of the PI3K-Akt pathway with reduced antibody production and T-cell lymphopenia (42, 51).

In APDS, increased PI3K-Akt-mTOR signaling leads to a state of immune dysregulation and immunodeficiency. While the clinical phenotype is varied, most patients present with recurrent sinopulmonary bacterial infections complicated by bronchiectasis, as well as recurrent and severe viral infections from herpes family viruses (9–12, 42). Such clinical manifestations are indicative of both B- and T-cell deficiencies, and as such, APDS is considered a combined immunodeficiency.

The link between APDS and HIGM syndrome was originally reported in one of the two first descriptions of APDS1 (9, 10). In their work, Angulo et al., described a patient clinically diagnosed with HIGM carrying a heterozygous deleterious mutation in *PIK3CD* detected by next generation sequencing. An active search for this particular defect in a cohort of 15 HIGM patients, led to the identification of three more mutated patients (9).

PIK3CD Mutations

Two groups reported the first patients with heterozygous activating mutations in *PI3KCD* as the cause of APDS/PASLI (9, 10). Angulo et al. reported patients found to have a glutamic acid to lysine change at residue 1,021 (E1021K) in p110δ resulting in a GOF mutation with increased lipid kinase activity. The mutation facilitates enhanced phosphorylation of its lipid substrate PIP2, which increases the amount of PIP3, lowering the activation threshold of PI3Kδ. While these patients had recurrent respiratory infections leading to progressive airway damage, they also had herpes viral infections and an increased proportion of effector T cells. Immunophenotyping in this cohort was notable for decreased T and B cells, with increased transitional B cells and decreased class-switched memory B cells, increased IgM levels,

low IgG2 levels, and diminished vaccine responses. Furthermore, it was noted that $CD4^+$ and $CD8^+$ T cells from these patients were prone to cell death, and an increased proportion of T cells had an activated/memory phenotype (9).

Interestingly, the APDS1 patients reported by Lucas et al. was identified from a cohort of individuals with persistent herpes family viremia, lymphoproliferation, and recurrent sinopulmonary infections (10). In this group, three different mutations in *PIK3CD* were found; two novel (N334K and E525K), and the same E1021K mutation reported by Angulo et al. (9, 10). Immunophenotyping was similar to that seen by Angulo et al.; however, normal to high $CD8^+$ T-cell counts with progressive $CD4^+$ T-cell lymphopenia was observed. Further T-cell phenotyping studies revealed severely reduced naïve and central memory T cells, but increased effector memory T cells and TEMRA (Effector Memory RA^+) cells. *In vitro*, patients' B cells underwent normal proliferation in response to stimuli, but were unable to secrete class-switched immunoglobulin isotypes (9, 10). This is consistent with the normal to elevated IgM, reduced IgA, and variable IgG levels observed.

Crank et al. found mutations in *PIK3CD* when looking at a small cohort of patients diagnosed with HIGM humoral phenotypes. These patients had high numbers of transitional B cells and plasmablasts. In contrast to previously described CSR/HIGM syndromes, none of the patients found in this cohort experienced opportunistic infections, nor did they have enlarged germinal centers. Additionally, these patients had increased cancer susceptibility with a high prevalence of EBV-negative B-cell lymphomas (52).

Subsequently, additional *PIK3CD* mutations have been described and to date 10 heterozygous *PIK3CD* GOF mutations including E1021K, N334K, E525K, C416R, R405C, R929C, E525A, E1025G, E81K, and G124D have been reported. Mutations have now been identified scattered throughout the gene involving not only the kinase domain, but also the helical domain, the C2 domain, the ABD, and the linker region between the ABD and RBD (**Figure 2**) (11, 14, 42, 51–55). Collectively, and as above mentioned, patients with AD *PIK3CD* GOF defects most commonly present with recurrent respiratory tract infections resulting in bronchiectasis in early childhood, lymphoproliferation, and predisposition to develop B-cell lymphomas. The most commonly reported pathogens are *Haemophilus influenzae, Staphylococcus aureus, Moraxella catarrhalis, Pseudomonas aeruginosa*, and *Klebsiella* species. Recurrent episodes of otitis media are also observed, sometimes resulting in hearing loss. A small percentage of infections consisted of conjunctivitis, orbital cellulitis, skin abscesses, and dental abscesses. Lymphadenopathy, mucosal lymphoid hyperplasia, and hepatosplenomegaly are also common features. A broader range of viral infections including varicella zoster, herpes simplex virus, adenovirus, and *molluscum contagiosum* have been reported. Increased susceptibility to herpes virus family infections in these patients is likely due to the combinatory effect of reduced long-lived memory CD8 T cells and increased terminally differentiated effector CD8 T cells (11). While patients with *PIK3CD* GOF mutations had normal/high EBV-specific CD8 T cells (based on tetramer staining), they were predominantly effector memory terminal cells ($CCR7^-/CD45RA^-$) also showing signs of increased activity (by CD38 staining). Persistent Akt hyperactivation was hypothesized as the driver for the increased CD8 T-cell proliferation, that in turn determined higher terminally differentiated CD8 T-effector cells, increased senescent CD8 T cells and decreased long-lived memory CD8 T cells, altogether resulting in impaired control of EBV- and CMV-infected cells (11). Although rare, opportunistic infections in these patients include *Cryptosporidium parvum* and toxoplasmosis. Autoimmune features included cytopenias, glomerulonephritis, and thyroid disease. Aside from EBV-positive lymphoma, other malignancies included EBV- negative diffuse large B-cell lymphoma, Hodgkin lymphoma, nodal marginal zone lymphoma, lymphoplasmacytic lymphoma, and cutaneous anaplastic large cell lymphoma (14, 42, 56). Increased IgM levels and low IgA, IgG, or IgG2 levels were again a defining laboratory finding in symptomatic patients. Flow cytometry revealed similarities to previously reported immunophenotyping: reduced CD4 T-cell counts, increased effector memory CD8 T-cell counts, and increased transitional B cells that can be linked to abnormal B-cell precursor maturation in the bone marrow (14, 42, 54).

PIK3R1 Mutations

Heterozygous loss-of-function mutations in *PIK3R1*, encoding the p85α regulatory subunit, also result in hyperactivation of PI3Kδ signaling due to loss of regulatory control on the catalytic p110δ subunit. Almost all reported patients have splicing mutations leading to skipping of exon 11. More recently, an additional missense mutation located in the SH2 domain of *PIK3R1*, N564K, was described by Wentink et al. and is predicted to affect binding to p110δ (53). Because the net result of these mutants determine an increased PI3Kδ activity, they are considered GOF mutations (11–13, 42).

Similar to patients with mutations in *PIK3CD*, APDS2/PASLI-R1 patients also present with recurrent bacterial sinopulmonary infections, EBV/CMV viremia, chronic lymphoproliferation, and increased risk of lymphoid malignancy. Elevated IgM levels accompanied by low IgG and IgA are frequent, along with reduced naïve T cells and class-switched memory B cells, increased levels of senescent $CD8^+$ T cells and transitional B cells making patients carrying mutations in *PIK3CD* and *PIK3R1* clinical phenocopies (11–13, 42). Consistent with what is seen in *PIK3CD* mutations, patients' lymphocytes revealed hyperactivation of PI3K, Akt, and mTOR. Additionally, increased activation-induced cell death was also seen in APDS2 patients' T cells, with a high proportion of these T cells expressing the senescence marker CD57 (11, 12, 42).

In a cohort study of APDS2, Elkaim et al. reported growth retardation in 14/31 (45%) patients (13). Growth problems are not frequent in APDS1 (14, 57). This particular defect is likely related to non-immunological roles of p85α. In support of this hypothesis, autosomal dominant mutations in the last exons of *PIK3R1* cause SHORT syndrome (short stature, hyperextensibility of joints and/or hernias, ocular depression, Rieger anomaly, and delays of tooth eruption). Other

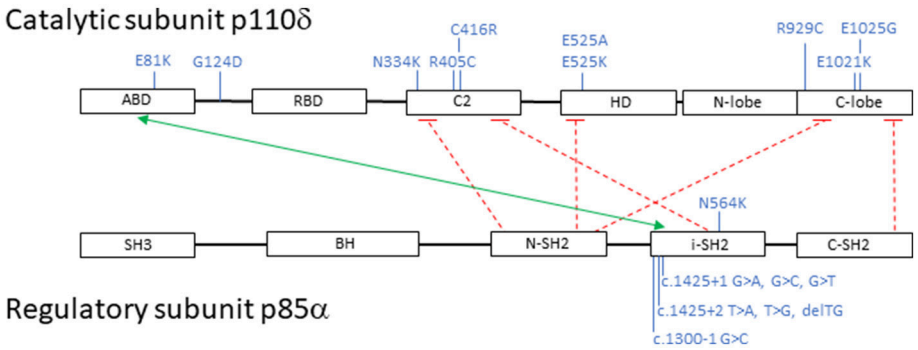


FIGURE 2 | Schematic representation of PI3K catalytic subunit p110δ and regulatory subunit p85α: domains, interactions and mutations. Black boxes represent protein domains: ABD, adaptor-binding domain; RBD, RAS-binding domain; C2, putative membrane-binding domain; HD, helical domain; N-lobe + C-lobe, kinase catalytic domain; SH3, SRC homology 3 domain; BH, breakpoint cluster region homology-domain; N-SH2, N-terminal SRC homology 2 domain; i-SH2, inter-SRC homology 2 domain; C-SH2, C-terminal SRC homology 2 domain. The green arrow points to p110δ and p85α interacting domains; the dashed red lines represent inhibitory contacts between the proteins. In blue, activating mutations affecting p110δ and p85α. Mutations displayed on top of the proteins represent missense changes, mutations displayed below the proteins represent intronic changes.

features consistently seen in patients with SHORT syndrome include mild intrauterine growth restriction, characteristic facies, lipodystrophy and insulin resistance in adolescence progressing to diabetes mellitus in early adulthood. These patients do not typically present with a HIGM humoral phenotype. Immunoblot analysis has shown that levels of p85α were ∼50% lower in SHORT syndrome patients than in controls (58). Normal PI3K activity is critical for adipose tissue differentiation and insulin signaling, thus explaining the lipodystrophy and abnormal glucose tolerance in these patients (59). Interestingly, three patients presenting concomitant features of SHORT syndrome and APDS2 have been described, two of whom showed a HIGM phenotype (60, 61).

While the non-immunological role that p85a plays is certainly highlighted by those affected with SHORT syndrome, evidence for its importance in lymphocyte function is also clearly shown in a patient with homozygous, biallelic, premature stop codon mutations in *PIK3R1* leading to complete absence of p85α. This patient had colitis and history of *Campylobacter* infection, in the context of absent B cells with resulting agammaglobulinemia. Early in life, erythema nodosum and arthritis were also observed (62).

Class-Switch Recombination in PI3K GOF Defects

Early insights on the impact of hyperactivation of PI3Kδ in CSR relate to murine models lacking PTEN expression in B cells. These mice showed a HIGM phenotype with defective CSR that was partially dependent on FOXO1 suppression and reduced AID expression. The CSR process was not completely corrected by ectopic AID expression, but was additionally restored in the presence of a specific PI3Kδ inhibitor (63, 64). These findings were further supported by another mouse model in which mature B cells lacking FOXO1 also expressed lower levels of AID and failed to produce class-switched antibodies, which in this setting could not be rescued by PI3Kδ inhibition (65). Considering the suppressive effect of the PI3K/Akt pathway on FOXO1 and the importance of AID in the CSR process, these results suggest that reduced expression of AID due to inactivation of FOXO1 contribute to the CSR impairment seen in APDS. As shown in other murine models, specific ablation of FOXO1 in germinal center B cells results in abnormal CSR despite normal expression of AID. In these models, germinal center dark zones were ablated and even though proliferation and SHM were preserved, affinity maturation was impaired. Thus, in the absence of FOXO1 in germinal center B cells', AID was normally expressed and yet not sufficient to maintain appropriate CSR. These experiments suggest some additional roles of FOXO1 on AID function, and therefore CSR (66, 67).

Avery et al. (68) developed a novel Pi3kcd^{E1020K} GOF mouse model by CRISPR/Cas9-mediated genome editing. Hyperactivation of PI3Kδ in B cells was documented by increased pAkt and pS6. B cells from mutant mice showed defective class switching and Ig secretion both upon *in vitro* (anti-CD40 + IL-4, LPS, or LPS + TGF-β) and T-dependent *in vivo* stimulation. Of note, Pi3kcd^{E1020K} GOF B cells behaved similarly to WT cells regarding proliferation, germinal center formation, SHM and affinity maturation. Induction of *AICDA* mRNA in B cells following stimulation with anti-CD40/IL-4 was significantly reduced in mutants compared to WT and positively correlated with the percentage of IgG1^{+} B cells generated after activation. *In vitro* treatment with the p110δ inhibitor leniolisib (69) completely restored the class-switch defect in Pi3kcd^{E1020K} GOF B cells, as well as *AICDA* expression levels and secretion of IgG and IgA.

In accordance with the highly prevalent HIGM phenotype observed in patient cohorts, most human studies of APDS found reduced class-switched B cells in the periphery and impaired CSR *in vitro* (10, 13, 14, 53, 68). Increased Akt phosphorylation in B cells, even without stimulation, was another consistent finding (9, 10, 53, 70). While Angulo et al. (9) observed normal SHM in two *PI3KCD* GOF patients, as later reported in the corresponding murine model (68), variable frequencies of SHM were found in

seven APDS patients evaluated by Wentink et al. (53). Altogether, these data suggest that SHM can show some variability in PI3K mutated patients although it seems more consistent in the mouse model.

APDS patients studied by Avery et al. showed reduced *AICDA* expression, in line with their Pi3kcd GOF murine model, as well as with previous experimental models of increased PI3kδ activity (63, 64, 68). This is in contrast to normal expression levels of *AICDA* reported in *PIK3CD* GOF patients by Lucas et al. (10). Targeting of AID to switch regions was assessed in one APDS cohort and shown to be normal (53).

Specific PI3Kδ inhibition is under investigation in APDS patients (71). In a Phase1-phase 2 leniolisib trial, a progressive decrease in patients' serum IgM levels was observed along with a reduced need for IgG supplementation. These findings might reflect a restoration in CSR, however further studies will be needed to confirm and solidify this effect.

Besides this growing evidence pointing to an intrinsic B-cell defect underlying diminished CSR in APDS, given previous data linking PI3Kδ signaling to Tfh cells generation (72) and the requirement of T- and B-cell interaction for effective CSR (2), it is reasonable to speculate whether T-cell related dysfunction additionally impairs CSR in these defects.

To investigate the hypothesis that activating PI3K defects could lead to anomalous germinal center function, Preite et al. (73) generated another mouse model expressing $p110\delta^{E1020K}$. In their animal model increased numbers of Tfh and germinal center B cells were found, along with impaired CSR after immunization. Germinal centers dark zones were poorly formed, with extensive infiltration of Tfh cells. Of particular interest to their model was the observation that, in the setting of hyperactive PI3K/Akt pathway, differentiation to Tfh cells was facilitated by a strong suppression of FOXO1 that occurred independently of ICOS engagement. Adoptive transfer of mutant naïve transgenic T cells into wild type mice resulted in higher Tfh differentiation. These Tfh cells did not lead to increased number of germinal center B cells and were able to provide normal B cell help *in vitro*. Similar findings of disrupted germinal centers infiltrated by Tfh cells were described in APDS1 patients by Coulter et al. (14). Interestingly, while studying an APDS2 patient, Di Fonte et al. found significantly reduced numbers of germinal center Tfh cells in tonsillar tissue (74).

Activating PI3K Defects Compared to Other CSR/HIGM Syndromes

Clinically, all CSR/HIGM syndromes share an increased risk of recurrent bacterial infections, mainly reflecting the absence of isotype specific protective functions (3, 7). Variable impairment of other immune functions that are not a consequence of defective CSR define unique infection patterns observed in specific defects. For instance, the lack of proper interaction between T lymphocytes and monocytes in CD40/CD40L deficiencies confers an increased risk for *Pneumocystis jiroveci* pneumonia, an opportunistic infection not frequent in others CSR/HIGM defects (3). An intact CD40/CD40L interaction is also required to clear *Cryptosporidium parvum* infection of bile duct epithelium leading to sclerosing cholangitis (7). Although sclerosing cholangitis has also been described in a few APDS patients, there was no link to *Cryptosporidium* species infection and therefore is believed to be primary rather than associated to infections. This was hypothesized to be due to the fact that genes associated with primary sclerosing cholangitis often converge at the PI3K/Akt signaling pathway (42, 75). Recent data showed that persistent viral infections (particularly herpesviruses), a hallmark in activating PI3Kδ defects but not common among other CSR/HIGM syndromes, arise from exhaustion of cytotoxic $CD8^+$ T cells and NK cells (76, 77).

Increased autoimmune manifestations are seen in almost all CSR/HIGM syndromes including PI3K defects (3, 7, 13, 14, 78). Functional CD40L seems to be necessary for normal peripheral B cell tolerance, whereas AID is important for both central and peripheral B cell checkpoints. Natural IgM antibodies are also found in AID deficient patients (79). Increased numbers of autoreactive B cells were seen in the GOF PI3Kδ mouse model (73).

Concerning the immunological phenotype, some similarities as well as differences can be seen between PI3K defects vs. the other CSR/HIGM syndromes. While a reduction in class-switched B cells is common to all CSR/HIGM defects, reduced total number of circulating B cells, along with increased transitional B cells are distinctive features of PI3K defects (3, 7, 42, 78). Memory B cells ($CD27^+$), that are shown to be reduced in PI3K defects (10, 42, 53) and absent or very low in CD40/CD40L deficiencies, are normal in AID/UNG deficiencies (3). While total T-cell numbers are usually normal in CD40/CD40L and AID deficiencies, they appear to be reduced in PI3K defects. PI3K defects also show a unique distribution of T-cell subsets, with skewing toward effector and exhausted phenotypes. An inverted CD4/CD8 ratio is also typical, due to both increased $CD8^+$ and reduced $CD4^+$ T cell counts (especially in the naïve compartment) (13, 14, 42). Reduced naïve $CD4^+$ T-cell counts and inverted CD4/CD8 ratios can also be seen in AID deficiency, although less strikingly than in PI3K defects. Circulating Tfh cells, increased in APDS (14) and AID deficient patients (80), are markedly decreased in CD40L deficient patients (3, 81).

Germinal centers' architecture also helps to distinguish between different CSR/HIGM syndromes. While generally absent in CD40/CD40L deficiencies and giant in AID deficiency (3, 79), they appear to be present but disrupted in APDS patients (14, 82).

Treatment of PI3K GOF Defects

Treatment for APDS patients includes prophylactic measures, such as antibiotics, antivirals, and antifungals, as well as immunoglobulin replacement. Most patients undergo immunosuppressive therapy aiming to control lymphoproliferation and/or autoimmunity. Rapamycin was a frequently used immunossupressor in the ESID-APDS-registry cohort, showing positive results in controlling benign lymphoproliferation, but not as effective for gastrointestinal manifestations and cytopenias (83). Rituximab has been successfully used in the treatment of cytopenias and lymphoproliferation, but persistent B-cell lymphopenia can be a common outcome (14).

Therapeutics created for the treatment of leukemia and lymphoma, such as idelalisib, duvelisib, or ibrutinib block PI3Kδ either directly or indirectly, making them exciting potential options for treating patients with APDS (84). Leniolisib, a PI3Kδ inhibitor has been explored as a therapeutic option in patients with PIK3CD GOF mutations. Six patients were treated over a 12-weeks period and demonstrated decreased lymphadenopathy and splenomegaly. Decreased Akt phosphorylation in affected patients' T cells, with noted reductions in transitional B cells, senescent T cells, and IgM levels were also observed (71). However, it has been shown in both mouse and human B cells that by modulating PI3Kδ activity there is a resulting enhancement of AID expression leading to increased SHM and chromosomal translocation to the IgH locus as well as to other AID off-target sites. Thus, PI3Kδ blockade via an AID-dependent mechanism increases genomic instability. Given that such inhibitors could be administered to patients for extended periods of time, several concerns arise including the potential for secondary oncogenic mutations or translocations, and accelerated resistance to targeted therapy by increasing the mutational rate (84). Furthermore, idelalisib, an oral selective inhibitor of PI3Kδ, has been reported to cause enterocolitis and a rash mimicking graft-versus-host disease (85).

When focused on curative treatment, Nademi et al. reported an 81% survival among 11 APDS patients who underwent HSCT in seven pediatric centers. Acute GvHD was a common complication (81%), 2 patients presented low chimerism, and 2/3 of the surviving patients are off immunosuppressive therapy and immunoglobulin replacement (86).

CONCLUSIONS

Diseases involving the PI3K pathway due to *PIK3CD* and *PIK3R1* GOF mutations, have recently been highlighted as forms of combined immunodeficiency compromising both the T- and B-cell compartments. Common clinical features include recurrent bacterial respiratory tract infections, EBV/CMV viremia, T-cell lymphopenia, memory B-cell deficiency, increased transitional Bcells and elevated IgM levels accompanied by low IgG and IgA levels. Due to elevated IgM levels and low IgG, IgA, and IgE levels, PI3K diseases ultimately fit under the umbrella of the CSR/HIGM syndromes.

While all the diseases presenting with HIGM humoral phenotypes described in this review involve by definition, CSR defects, this mechanism is not univocally disrupted in all these defects. Expression and function of CD40L/CD40 and AID are crucial to this process, and when either of them is altered, they markedly affect its outcome. In PI3K-related defects, evidence points at AID expression and function through FOXO1 regulation playing a central role in CSR integrity. In terms of SHM, this process is generally preserved when defects involve *AD-AICDA*, *UNG*, *NEMO*, *NFKBIA, ATM*, and *PMS2*, but shown to be variably affected in patients carrying PI3K-associated diseases, although more consistently normal in animal models (9, 53, 68). Whereas, deficiencies in the CD40L/CD40 and NF-kB pathways are characterized by opportunistic infections, patients with defects in AID and UNG generally do not experience such complications. Patients with *PIK3CD* and *PIK3R1* mutations appear to be intermediate between the above-mentioned defects in terms of opportunistic infections susceptibility. Over time, as with many other immunodeficiencies, autoimmunity has become a more relevant feature demonstrating overall immune dysregulation within HIGM syndromes in general and PI3K defects in particular.

In conclusion, the CSR/HIGM syndromes encompass a wide variety of diseases, each with their own defining features. Diseases involving the PI3K pathway are indeed combined immunodeficiencies, as well as a subset of CSR/HIGM syndromes, presenting with their own characteristic clinical and laboratory attributes as well as individual therapeutic approaches.

AUTHOR CONTRIBUTIONS

RDJ wrote the first draft. RDJ, CJN-S, JB and SDR contributed and reviewed all the data presented. JB and SDR supervised the project.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program, National Institutes of Health Clinical Center. The content of this article does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

REFERENCES

1. Tonegawa S. Somatic generation of antibody diversity. *Nature* (1983) 302:575–81. doi: 10.1038/302575a0
2. Xu Z, Zan H, Pone EJ, Mai T, Casali P. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. *Nat Rev Immunol.* (2012) 12:517–31. doi: 10.1038/nri3216
3. Durandy A. *Class-Switch Recombination Defects*. Elsevier (2014).
4. Notarangelo LD, Duse M, Ugazio AG. Immunodeficiency with hyper-IgM (HIM). *Immunodefic Rev.* (1992) 3:101–21.
5. Geha R, Plebani A, Notarangelo LD. *CD40, CD40 Ligant and the Hyper-IgM Syndrome*. Philadelphia, PA: Oxford University Press (2006).
6. Notarangelo LD, Lanzi G, Peron S, Durandy A. Defects of class-switch recombination. *J Allergy Clin Immun.* (2006) 117:855–64. doi: 10.1016/j.jaci.2006.01.043
7. Qamar N, Fuleihan RL. The hyper IgM syndromes. *Clin Rev Allergy Immunol.* (2014) 46:120–30. doi: 10.1007/s12016-013-8378-7
8. Durandy A, Revy P, Fischer A. Human models of inherited immunoglobulin class switch recombination and somatic hypermutation defects (hyper-IgM syndromes). *Adv Immunol.* (2004) 82:295–330. doi: 10.1016/S0065-2776(04)82007-8
9. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi: 10.1126/science.1243292

10. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PIK catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol.* (2014) 15:88–97. doi: 10.1038/ni.2771
11. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Investig.* (2015) 125:1764–5. doi: 10.1172/JCI81746
12. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med.* (2014) 211:2537–47. doi: 10.1084/jem.20141759
13. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol.* (2016) 138:210–8.e9. doi: 10.1016/j.jaci.2016.03.022
14. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol.* (2017) 139:597–606.e4. doi: 10.1016/j.jaci.2016.06.021
15. Stavnezer J, Schrader CE. IgH chain class switch recombination: mechanism and regulation. *J Immunol.* (2014) 193:5370–8. doi: 10.4049/jimmunol.1401849
16. He B, Santamaria R, Xu W, Cols M, Chen K, Puga I, et al. The transmembrane activator TACI triggers immunoglobulin class switching by activating B cells through the adaptor MyD88. *Nat Immunol.* (2010) 11:836–45. doi: 10.1038/ni.1914
17. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* (1993) 72:291–300 doi: 10.1016/0092-8674(93)90668-G
18. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. *Nature* (1993) 361:541–3. doi: 10.1038/361541a0
19. Fuleihan R, Ramesh N, Loh R, Jabara H, Rosen RS, Chatila T, et al. Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. *Proc Natl Acad Sci USA.* (1993) 90:2170–3. doi: 10.1073/pnas.90.6.2170
20. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* (1993) 259:990–3. doi: 10.1126/science.7679801
21. Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* (1993) 361:539–41. doi: 10.1038/361539a0
22. Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, et al. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine* (2003) 82:373–84. doi: 10.1097/01.md.0000100046.06009.b0
23. Seyama K, Nonoyama S, Gangsaas I, Hollenbaugh D, Pabst HF, Aruffo A, et al. Mutations of the CD40 ligand gene and its effect on CD40 ligand expression in patients with X-linked hyper IgM syndrome. *Blood* (1998) 92:2421–34.
24. Berkowska MA, Driessen GJ, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, Cerutti A, et al. Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. *Blood* (2011) 118:2150–8. doi: 10.1182/blood-2011-04-345579
25. van Zelm MC, Bartol SJ, Driessen GJ, Mascart F, Reisli I, Franco JL, et al. Human CD19 and CD40L deficiencies impair antibody selection and differentially affect somatic hypermutation. *J Allergy Clin Immunol.* (2014) 134:135–44. doi: 10.1016/j.jaci.2013.11.015
26. Ferrari S, Giliani S, Insalaco A, Al-Ghonaium A, Soresina AR, Loubser M, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc Natl Acad Sci USA.* (2001) 98:12614–9. doi: 10.1073/pnas.221456898
27. Murguia-Favela L, Sharfe N, Karanxha A, Bates A, Dadi H, Cimpean L, et al. CD40 deficiency: a unique adult patient with hyper immunoglobulin M syndrome and normal expression of CD40. *LymphoSign J.* (2017) 4:70–6. doi: 10.14785/lymphosign-2017-0004
28. Karaca NE, Forveille M, Aksu G, Durandy A, Kutukculer N. Hyper-immunoglobulin M syndrome type 3 with normal CD40 cell surface expression. *Scand J Immunol.* (2012) 76:21–5. doi: 10.1111/j.1365-3083.2012.02697.x
29. Revy P, Muto T, Levy Y, Geissmann F, Plebani A, Sanal O, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* (2000) 102:565–75. doi: 10.1016/S0092-8674(00)00079-9
30. Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. *Nat Immunol.* (2003) 4:1023–8. doi: 10.1038/ni974
31. Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW. Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature* (2003) 422:726–30. doi: 10.1038/nature01574
32. Kasahara Y, Kaneko H, Fukao T, Terada T, Asano T, Kasahara K, et al. Hyper-IgM syndrome with putative dominant negative mutation in activation-induced cytidine deaminase. *J Allergy Clin Immunol.* (2003) 112:755–60. doi: 10.1016/S0091-6749(03)01860-8
33. Imai K, Zhu Y, Revy P, Morio T, Mizutani S, Fischer A, et al. Analysis of class switch recombination and somatic hypermutation in patients affected with autosomal dominant hyper-IgM syndrome type 2. *Clin Immunol.* (2005) 115:277–85. doi: 10.1016/j.clim.2005.02.003
34. Durandy A, Taubenheim N, Peron S, Fischer A. Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. *Adv Immunol.* (2007) 94:275–306. doi: 10.1016/S0065-2776(06)94009-7
35. Hanson EP, Monaco-Shawver L, Solt LA, Madge LA, Banerjee PP, May MJ, et al. Hypomorphic nuclear factor-kappaB essential modulator mutation database and reconstitution system identifies phenotypic and immunologic diversity. *J Allergy Clin Immunol.* (2008) 122:1169–77.e16. doi: 10.1016/j.jaci.2008.08.018
36. Boisson B, Puel A, Picard C, Casanova JL. Human ikappabalpha gain of function: a severe and syndromic immunodeficiency. *J Clin Immunol.* (2017) 37:397–412. doi: 10.1007/s10875-017-0400-z
37. Noordzij JG, Wulffraat NM, Haraldsson A, Meyts I, van't Veer LJ, Hogervorst FB, et al. Ataxia-telangiectasia patients presenting with hyper-IgM syndrome. *Arch Dis Child.* (2009) 94:448–9. doi: 10.1136/adc.2008.149351
38. Peron S, Metin A, Gardes P, Alyanakian MA, Sheridan E, Kratz CP, et al. Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. *J Exp Med.* (2008) 205:2465–72. doi: 10.1084/jem.20080789
39. Imai K, Catalan N, Plebani A, Marodi L, Sanal O, Kumaki S, et al. Hyper-IgM syndrome type 4 with a B lymphocyte-intrinsic selective deficiency in Ig class-switch recombination. *J Clin Investig.* (2003) 112:136–42. doi: 10.1172/JCI18161
40. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol.* (2010) 149:167–80. doi: 10.1111/j.1365-2141.2010.08077.x
41. de la Morena MT, Leonard D, Torgerson TR, Cabral-Marques O, Slatter M, Aghamohammadi A, et al. Long-term outcomes of 176 patients with X-linked hyper-IgM syndrome treated with or without hematopoietic cell transplantation. *J Allergy Clin Immunol.* (2017) 139:1282–92. doi: 10.1016/j.jaci.2016.07.039
42. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kdelta and primary immunodeficiencies. *Nat Rev Immunol.* (2016) 16:702–14. doi: 10.1038/nri.2016.93
43. Chantry D, Vojtek A, Kashishian A, Holtzman DA, Wood C, Gray PW, et al. p110delta, a novel phosphatidylinositol 3-kinase catalytic subunit that associates with p85 and is expressed predominantly in leukocytes. *J Biol Chem.* (1997) 272:19236–41. doi: 10.1074/jbc.272.31.19236
44. Okkenhaug K, Ali K, Vanhaesebroeck B. Antigen receptor signalling: a distinctive role for the p110delta isoform of PI3K. *Trends Immunol.* (2007) 28:80–7. doi: 10.1016/j.it.2006.12.007
45. Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, et al. P110delta, a novel phosphoinositide 3-kinase in leukocytes. *Proc Natl Acad Sci USA.* (1997) 94:4330–5. doi: 10.1073/pnas.94.9.4330
46. Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, et al. The structure of a human p110alpha/p85alpha complex

elucidates the effects of oncogenic PI3Kalpha mutations. *Science* (2007) 318:1744–8. doi: 10.1126/science.1150799
47. Yu JS, Cui W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development* (2016) 143:3050–60. doi: 10.1242/dev.137075
48. Kim EH, Suresh M. Role of PI3K/Akt signaling in memory CD8 T cell differentiation. *Front Immunol.* (2013) 4:20. doi: 10.3389/fimmu.2013.00020
49. Abdelrasoul H, Werner M, Setz CS, Okkenhaug K, Jumaa H. PI3K induces B-cell development and regulates B cell identity. *Sci Rep.* (2018) 8:1327. doi: 10.1038/s41598-018-19460-5
50. Werner M, Hobeika E, Jumaa H. Role of PI3K in the generation and survival of B cells. *Immunol Rev.* (2010) 237:55–71. doi: 10.1111/j.1600-065X.2010.00934.x
51. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol.* (2016) 138:1672–80.e10. doi: 10.1016/j.jaci.2016.03.055
52. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol.* (2014) 34:272–6. doi: 10.1007/s10875-014-0012-9
53. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Scholvinck L, Kalina T, et al. Genetic defects in PI3Kdelta affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol.* (2017) 176:77–86. doi: 10.1016/j.clim.2017.01.004
54. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol.* (2017) 139:1032–5.e6. doi: 10.1016/j.jaci.2016.08.028
55. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol.* (2017) 181:29–31. doi: 10.1016/j.clim.2017.05.020
56. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol.* (2014) 134:233–6. doi: 10.1016/j.jaci.2014.02.020
57. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kdelta syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol.* (2016) 27:640–4. doi: 10.1111/pai.12585
58. Dyment DA, Smith AC, Alcantara D, Schwartzentruber JA, Basel-Vanagaite L, Curry CJ, et al. Mutations in PIK3R1 cause SHORT syndrome. *Am J Hum Genet.* (2013) 93:158–66. doi: 10.1016/j.ajhg.2013.06.005
59. Chudasama KK, Winnay J, Johansson S, Claudi T, Konig R, Haldorsen I, et al. SHORT syndrome with partial lipodystrophy due to impaired phosphatidylinositol 3 kinase signaling. *Am J Hum Genet.* (2013) 93:150–7. doi: 10.1016/j.ajhg.2013.05.023
60. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol.* (2016) 36:462–71. doi: 10.1007/s10875-016-0281-6
61. Bravo Garcia-Morato M, Garcia-Minaur S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, Lopez-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol.* (2017) 179:77–80. doi: 10.1016/j.clim.2017.03.004
62. Conley ME, Dobbs AK, Quintana AM, Bosompem A, Wang YD, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85alpha subunit of PI3K. *J Exp Med.* (2012) 209:463–70. doi: 10.1084/jem.20112533
63. Suzuki A, Kaisho T, Ohishi M, Tsukio-Yamaguchi M, Tsubata T, Koni PA, et al. Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. *J Exp Med.* (2003) 197:657–67. doi: 10.1084/jem.20021101
64. Omori SA, Cato MH, Anzelon-Mills A, Puri KD, Shapiro-Shelef M, Calame K, et al. Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling. *Immunity (*2006) 25:545–57. doi: 10.1016/j.immuni.2006.08.015
65. Dengler HS, Baracho GV, Omori SA, Bruckner S, Arden KC, Castrillon DH, et al. Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation. *Nat Immunol.* (2008) 9:1388–98. doi: 10.1038/ni.1667
66. Sander S, Chu VT, Yasuda T, Franklin A, Graf R, Calado DP, et al. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. *Immunity* (2015) 43:1075–86. doi: 10.1016/j.immuni.2015.10.021
67. Dominguez-Sola D, Kung J, Holmes AB, Wells VA, Mo T, Basso K, et al. The FOXO1 transcription factor instructs the germinal center dark zone program. *Immunity* (2015) 43:1064–74. doi: 10.1016/j.immuni.2015.10.015
68. Avery DT, Kane A, Nguyen T, Lau A, Nguyen A, Lenthall H, et al. Germline-activating mutations in PIK3CD compromise B cell development and function. *J. Exp Med.* (2018) 215:2073–95. doi: 10.1084/jem.20180010
69. Hoegenauer K, Soldermann N, Zecri F, Strang RS, Graveleau N, Wolf RM, et al. Discovery of CDZ173 (Leniolisib), representing a structurally novel class of PI3K delta-selective inhibitors. *ACS Med Chem Lett.* (2017) 8:975–80. doi: 10.1021/acsmedchemlett.7b00293
70. Asano T, Okada S, Tsumura M, Yeh TW, Mitsui-Sekinaka K, Tsujita Y, et al. Enhanced AKT phosphorylation of circulating B cells in patients with activated PI3Kdelta syndrome. *Front Immunol.* (2018) 9:568. doi: 10.3389/fimmu.2018.00568
71. Rao VK, Webster S, Dalm V, Sediva A, van Hagen PM, Holland S, et al. Effective "activated PI3Kdelta syndrome"-targeted therapy with the PI3Kdelta inhibitor leniolisib. *Blood* (2017) 130:2307–16. doi: 10.1182/blood-2017-08-801191
72. Rolf J, Bell SE, Kovesdi D, Janas ML, Soond DR, Webb LM, et al. Phosphoinositide 3-kinase activity in T cells regulates the magnitude of the germinal center reaction. *J Immunol.* (2010) 185:4042–52. doi: 10.4049/jimmunol.1001730
73. Preite S, Cannons JL, Radtke AJ, Vujkovic-Cvijin I, Gomez-Rodriguez J, Volpi S, et al. Hyperactivated PI3Kδ promotes self and commensal reactivity at the expense of optimal humoral immunity. *Nat Immunol.* (2018) 19:986–1000. doi: 10.1038/s41590-018-0182-3
74. Di Fonte R, Baronio M, Plebani A, Lougaris V, Fousteri G. Reduced germinal center follicular helper T cells but normal follicular regulatory T cells in the tonsils of a patient with a mutation in the PI3KR1 gene. *Clin Immunol.* (2016) 164:43–4. doi: 10.1016/j.clim.2016.01.016
75. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol.* (2015) 35:11–4. doi: 10.1007/s10875-014-0109-1
76. Wentink MWJ, Mueller YM, Dalm V, Driessen GJ, van Hagen PM, van Montfrans JM, et al. Exhaustion of the CD8(+) T cell compartment in patients with mutations in phosphoinositide 3-kinase delta. *Front Immunol.* (2018) 9:446. doi: 10.3389/fimmu.2018.00446
77. Edwards ESJ, Bier J, Cole TS, Wong M, Hsu P, Berglund LJ, et al. Activating PIK3CD mutations impair human cytotoxic lymphocyte differentiation and function and EBV immunity. *J Allergy Clin Immunol.* (2018). doi: 10.1016/j.jaci.2018.04.030. [Epub ahead of print].
78. de la Morena MT. Clinical phenotypes of hyper-IgM syndromes. *J Allergy Clin Immunol Pract.* (2016) 4:1023–36. doi: 10.1016/j.jaip.2016.09.013
79. Durandy A, Cantaert T, Kracker S, Meffre E. Potential roles of activation-induced cytidine deaminase in promotion or prevention of autoimmunity in humans. *Autoimmunity* (2013) 46:148–56. doi: 10.3109/08916934.2012.750299
80. Cantaert T, Schickel JN, Bannock JM, Ng YS, Massad C, Delmotte FR, et al. Decreased somatic hypermutation induces an impaired peripheral B cell tolerance checkpoint. *J Clin Investig.* (2016) 126:4289–302. doi: 10.1172/JCI84645
81. Ma CS, Wong N, Rao G, Avery DT, Torpy J, Hambridge T, et al. Monogenic mutations differentially affect the quantity and quality of T follicular helper cells in patients with human primary immunodeficiencies. *J Allergy Clin Immunol.* (2015) 136:993–1006.e1. doi: 10.1016/j.jaci.2015.05.036
82. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol.* (2015) 159:33–6. doi: 10.1016/j.clim.2015.04.014

83. Maccari ME, Abolhassani H, Aghamohammadi A, Aiuti A, Aleinikova O, Bangs C, et al. Disease evolution and response to rapamycin in activated phosphoinositide 3-kinase delta syndrome: the European society for immunodeficiencies-activated phosphoinositide 3-kinase delta syndrome registry. *Front Immunol.* (2018) 9:543. doi: 10.3389/fimmu.2018.00543
84. Compagno M, Wang Q, Pighi C, Cheong TC, Meng FL, Poggio T, et al. Phosphatidylinositol 3-kinase delta blockade increases genomic instability in B cells. *Nature* (2017) 542:489–93. doi: 10.1038/nature21406
85. Hammami MB A-TA, Meeks M, Fesler M, Hurley MY. Idelalisib-induced colitis and skin eruption mimicking graft-versus-host disease. *Clin J Gastroenterol.* (2017) 10:142–6. doi: 10.1007/s12328-016-0707-y
86. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol.* (2017) 139:1046–9. doi: 10.1016/j.jaci.2016.09.040

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership