Too much of a good thing: immunodeficiency due to hyperactive PI3K signaling

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Primary immune deficiency diseases arise due to heritable defects that often involve signaling molecules required for immune cell function. Typically, these genetic defects cause loss of gene function, resulting in primary immune deficiencies such as severe combined immune deficiency (SCID) and X-linked agammaglobulinemia (XLA); however, gain-of-function mutations may also promote immune deficiency. In this issue of the *JCI*, Deau et al. establish that gain-of-function mutations in *PIK3R1*, which encodes the p85α regulatory subunit of class IA PI3Ks, lead to immunodeficiency. These observations are consistent with previous reports that hyperactivating mutations in *PIK3CD*, which encodes the p110δ catalytic subunit, are capable of promoting immune deficiency. Mutations that reduce PI3K activity also result in defective lymphocyte development and function; therefore, these findings support the notion that too little or too much PI3K activity leads to immunodeficiency.

Hyperactive PI3K signaling and immune deficiency

PI3Ks were discovered by Cantley and colleagues in 1985 (1) and have since been found to play numerous roles in cells (2, 3). Through recruitment to growth factor and cytokine receptor intracellular signaling complexes, class I PI3Ks promote the phosphorylation of phosphatidylinositol-(4,5)-bisphosphate (PIP_2) to phosphatidylinositol-(3,4,5)-triphosphate (PIP_3), which activates several downstream signaling molecules via membrane recruitment to signaling complexes. Class IA PI3Ks are heterodimeric and composed of a catalytic subunit (p110α, p110β, and p110δ) bound to a regulatory subunit (p85α/p55α/p50α, p85β, and p55γ). Although p110α and p110β are ubiquitously expressed, p110δ is restricted to immune cells (4). Indeed, loss of p110δ through gene targeting substantially affects adaptive immunity, impairing B cell development and resulting in a profound loss of T and B cell activation and function (4). Deletion of *Pik3r1*, which encodes PI3K p85α, p55α, and p50α regulatory subunits, leads to defects in B cell development, but does not affect T cell development and function (5, 6), likely due to *Pik3r1*-independent class IA PI3K activity in T cells. PI3K is activated in both T and B cells via antigen receptor signaling, cytokine and chemokine binding, and surface integrin activation (3, 4). These extracellular stimuli depend on PI3K for coordination of downstream signaling enzymes, including the serine/threonine kinases PDK1, AKT, and mTOR. By influencing these pathways, PI3K affects a wide range of cellular functions, modulating cellular metabolism, gene expression, and posttranslational regulation of protein and organelle function. The myriad roles that PI3K plays in the immune system, especially in T and B lymphocytes, are widely documented (3, 7, 8).

In this issue, Deau et al. used whole-exome sequencing and identified two distinct *PIK3R1* heterozygous splice site mutations in four immunodeficient patients. The two different mutations affected the same splice site, leading to aberrant splicing of *PIK3R1* exon 10 and resulting truncation of the p85α protein (9). Similarly aberrant PIK3R1 splicing has been observed in tumor cells and found to increase PI3K enzyme activity through loss of an inhibitory contact between the regulatory and catalytic subunits (10, 11). Consistent with this, Deau et al. found elevated PI3K activity in patient lymphocytes, with enhanced phosphorylation of AKT on serine 473 and threonine 308 (9). The patients did not present with lymphoproliferative symptoms, but instead exhibited enhanced lymphocyte activation-induced cell death (AICD). While overall lymphocyte counts were in the normal range, the naive fraction of $CD4^+$ and $CD8^+$ T cells was dramatically diminished. Some of the patients had elevated levels of transitional B cells, while others presented with a paucity of $IgM^-IgD^-CD27^+$ memory B cells. Furthermore, these patients lacked IgA production, and most of them had much higher titers of IgM compared with IgG. These results suggest that these *PIK3R1* hyperactivating mutations lead to defects in lymphoid homeostasis and B cell Ig class switching.

The findings of Deau et al. (9) are reminiscent of previous observations made in patients bearing activating mutations in *PIK3CD*, which encodes p110δ. In these individuals, the catalytic activity of p110δ is dramatically increased as the result of an E1021K mutation (12). This activating mutation led to the development of a primary immune deficiency, termed "activated p110δ syndrome" (APDS), a condition marked by susceptibility to respiratory tract infection, lymphopenia, and progressive airway damage. Upon closer inspection, Angulo et al. observed elevated serum IgM, an increased fraction of transitional B cells, and heightened AICD and AKT phosphorylation in APDS patient lymphocytes (12). Structural and biochemical evaluation of the E1021K-containing protein revealed an alteration in the C lobe of the kinase domain, which

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interacts with lipid substrates in the cell membrane and is also involved in binding to the SRC homology 2 (SH2) domain of the regulatory subunits. Lucas et al. had similar findings in their characterization of 14 immune-deficient patients from seven families bearing distinct heterozygous germline gain-of-function mutations in *PIK3CD* (13). They found that three different gain-of-function *PIK3CD* mutations led to p110δ hyperactivity, with heightened AKT phosphorylation and mTOR activity. Furthermore, individuals harboring gain-of-function *PIK3CD* mutations had an overrepresentation of senescent effector T cells, with enhanced glucose uptake and terminal effector differentiation. While not specifically addressed by Deau et al. (9), it is likely that the described *PIK3R1* mutation truncates the p85α domain in a manner that alters its interaction with p110δ, resulting in hyperactivation of the catalytic domain (11). Although the symptoms described in the APDS patients are somewhat distinct from those observed in the patients characterized by Deau and colleagues (9), it is possible that a greater concordance in immunodeficiency symptoms will become apparent with the identification of more patients bearing these activating mutations.

Conclusions and future directions

The implications of these findings are important in several respects. First, patients bearing gain-of-function mutations in *PIK3R1*, such as those described by Deau and colleagues (9), may benefit from inhibitors of the PI3K and mTOR pathways that are currently available in the clinic or under scrutiny in clinical trials (14). Indeed, the PI3Kδ inhibitor IC87114 was effective in reducing the hyperactive AKT phosphorylation and heightened AICD in patient samples bearing the *PIK3R1* truncation mutation (9). PI3Kδ inhibitors also affected similar parameters in patients bearing *PIK3CD* mutations (12, 13). Second, given that the identified mutations occur in the germline, it is somewhat surprising that such gain-of-function mutations did not result in an apparent increase in susceptibility to blood cancers. Previous work by Carrera and colleagues has established that a similar truncation in p85α leads to the generation of the oncogene "p65-PI3K," a similarly dysregulated form of p85α that promotes constitutive PI3K catalytic activity (10). Because PI3K and downstream pathways such as mTOR are vital for tumor cell growth and survival, PI3K hyperactivation might be expected to promote oncogenic transformation. Likewise, APDS patients do not appear to have higher susceptibility to cancer, although this may simply be due to the highly specific *PIK3CD* expression pattern (14). *PIK3R1* is more broadly expressed; therefore, it is possible that identification of additional patients bearing such mutations as those described by Deau et al. (9) may reveal a heightened susceptibility to oncogenic transformation in various tissues. Alternatively, the internal truncation caused by these splice site mutations may not increase PI3K catalytic activity as profoundly as was observed with p65-PI3K. It will be important to compare the catalytic activity of p110 catalytic subunits when paired with WT, p65-PI3K, and these exon 10 truncations of p85α.

The work by Deau et al. lays the groundwork for future studies to evaluate the differential effects of PI3K signaling on lymphocyte biology and immune function. A key issue is how hyperactive PI3K signaling affects lymphocyte differentiation. In each study, hyperactive class IA PI3K signaling variably resulted in an enhancement of transitional B cells, with an increase in serum IgM and a consequent decrease in more mature IgG isoforms (9, 12, 13). These findings are intriguing in light of the observation that mice with B cells lacking PTEN, a phosphoinositide phosphatase that counteracts PI3K signaling, have impaired Ig class switch recombination and hyper-IgM syndrome (15). Treatment of these mice with p110δ inhibitors rescued the Ig class switching phenotype. On the other hand, an immunodeficient patient lacking the p85α subunit of PI3K had a profound defect in B cell development and Ig production (16). Together, these observations lend credence to the hypothesis that PI3K activity must be carefully tuned for proper Ig isotype class switching.

Hyperactive PI3K signaling also leads to enhanced cellular metabolism, which was observed in lymphocytes derived from mice bearing gain-of-function p110δ (13). As lymphocytes are highly dependent on metabolic switching after activation (17), it may be that the defective generation of more mature Ig isoforms and enhanced generation of transitional B cell populations associated with hyperactive PI3K is due to activating mutations in the class IA PI3K pathway reprogramming lymphocyte metabolism. Failure to properly control metabolic signaling can result in premature terminal differentiation and cell death (18); therefore, it may be that unrestricted activation of pathways downstream of class IA PI3K lead to the early death of lymphocytes in patients bearing *PIK3CD* and *PIK3R1* mutations. This may explain the observation of heightened AICD in T cells obtained from patients bearing the *PIK3CD* and *PIK3R1* mutations (9, 13). It will thus be important to carefully evaluate lymphocyte metabolic regulation in these patients. PI3K, mTOR, and metabolic regulation are all important for helper T cell differentiation (19, 20). Future studies should also characterize the effect of class IA PI3K gain-of-function mutations on distinct helper cell subsets, as deficiencies in distinct helper subsets could greatly affect immune function in these patients. It is also possible that hyperactive class IA PI3K signaling may lead to overactivation of immune self-tolerance mechanisms; thus, evaluation of regulatory T cells and antiinflammatory cytokine production will be interesting. Perhaps the salient message of these studies is that PI3K signaling does not act in a digital manner; context and signal levels clearly matter to the immune system (3).

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