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RESEARCH HIGHLIGHT

Do PI3-kinase mutations drive T cells insane?

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The link between the differentiation of effector T lymphocytes and metabolism has become an important area of research. The role played by the phosphoinositide 3-kinase (PI3K)/Akt/mTOR (mammalian target of rapamycin) pathway in this process was recently emphasized by Lucas *et al.*[1] who reported a series of primary immunodeficiency patients with three different germ-line, gain-of-function mutations of the p110δ subunit of PI3K. The patients consistently presented a deficiency in naive T lymphocytes and an accumulation of highly differentiated effector T cells. This phenotype was associated with recurrent respiratory infections and with the reactivation of persistent viruses. The disease was named PASLI (p110δ-activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency). The same disorder was simultaneously reported by Angulo *et al.*[2] and was named the activated PI3K-δ syndrome (APDS). Although the accumulation of effector T cells in the state of immunodeficiency was somewhat unexpected, the description of this syndrome provides important insight into the regulation of T-cell differentiation and T-cell memory.

PI3Ks control many important aspects of immune cell development, differentiation and function. They form a family of intracellular signaling enzymes phosphorylating the 3-position OH of the inositol ring of phosphatidiylinositol. The 3-phosphorylated inositol ring creates a docking site for signaling proteins at the inner leaflet of the plasma membrane. Mammals have eight PI3K catalytic subunits that are divided into three classes based on similarities in structure and function.[3] The class I PI3K subunits are further subdivided into class IA (p110α, p110β and p110δ) and class IB (p110γ). p110α and p110β are broadly expressed in most cell types, whereas the expression of p110γ and p110δ (encoded by the gene *PIK3CD*, containing the mutations identified by Lucas *et al.*) is more limited to cells of the immune system.[3] Class IA PI3Ks, including p110δ, are downstream of signals originating from receptor activation. Extracellular ligands bind to their receptors (such as the T-cell receptor, CD28 or cytokine receptors), stimulate receptor tyrosine kinases and thereby activate PI3K.[4] Activated PI3K then phosphorylates phosphatidiylinositol-4,5-bisphosphate (PIP2) to generate phosphatidiylinositol-3,4,5-trisphosphate (PIP3) (Figure 1). PIP3 interacts with pleckstrin homology domain-containing target proteins, including the serine/threonine kinase Akt and the phosphoinositide-dependent protein kinase (PDK1) (Figure 1). To achieve full activation, Akt has to be phosphorylated by PDK1 and by the mTOR complex 2 (mTORC2).[4] In addition, the PI3K/Akt signaling pathway can be regulated *via* multiple mechanisms.[3,4] When fully activated, Akt becomes a powerful signaling molecule that translocates from the cell membrane to the cytosol and the nucleus where it can alter several important signaling pathways. Among them, mTORC1 is activated by Akt through the phosphorylation of several inhibitory molecules. Activated mTORC1 in turn controls protein synthesis, cell growth and promotes the switch from oxidative phosphorylation to aerobic glycolysis. Although the molecular mechanisms involved remain incompletely understood, mTORC1 promotes the differentiation of effector T cells at the expense of memory cells.[4] Forkhead box O (FOXO) transcription factors are also important targets of Akt. In the nucleus, activated Akt phosphorylates and inactivates FOXOs and thereby reduces the expression of a number of target genes involved in cell proliferation, apoptosis, migration and metabolism (Figure 1). The transcription factor FOXO1 controls multiple functions of T cells including trafficking, tolerance and survival.[4] FOXO1 supports the survival of T cells by inducing the expression of the IL-7Rα chain and influences T-cell trafficking by promoting the expression of CD62L, CCR7 and S1P1 *via* the induction of the transcription factor KLF2 (Figure 1). Through its effects on cell survival and trafficking, FOXO1 could have an important influence on the generation of memory T cells.[4,5] By acting upstream of the Akt/mTORC1/FOXO signaling pathways, PI3K itself could have a profound impact on the balance between effector and memory T cells. The data reported by Lucas *et al.* indicate that this is indeed the case.

The PI3K mutations were identified by Lucas *et al.* in nine patients from seven unrelated families from different ethnic

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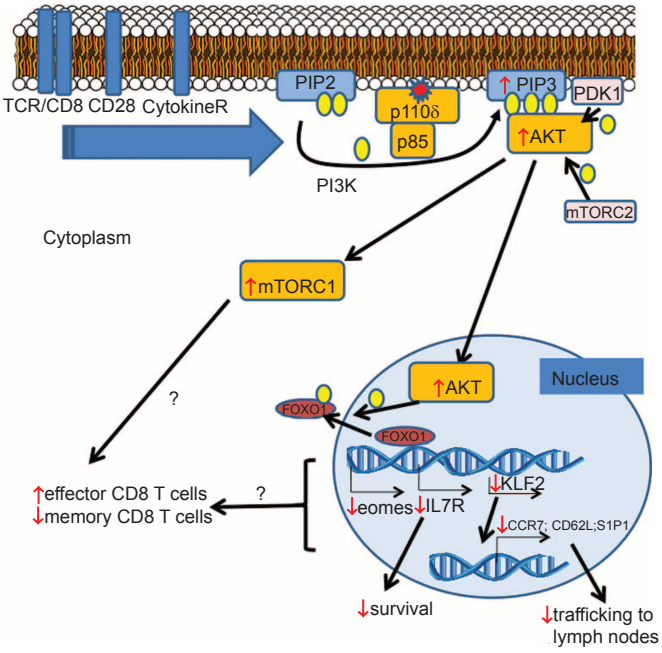

Figure 1 Overview of PI3K/Akt/mTORC1/FOXO1 signaling pathways and consequences of *PIK3CD* mutations in CD8 T cells. Upon binding to their ligands, signaling *via* TCR/CD8, CD28 and/or cytokine receptors results in the activation of PI3K. At the plasma membrane, activated PI3K, composed of the catalytic subunit p110δ in T cells and of the regulatory subunit p85, phosphorylate PIP2 towards PIP3. PIP3 recruits pleckstrin homology-containing proteins such as AKT (also known as protein kinase B) and PDK1. Full activation of Akt requires phosphorylation by PDK1 and mTORC2. In the cytosol, Akt can activate mTORC1 by phosphorylating mTORC inhibitors. Akt goes also to the nucleus and triggers the nuclear exclusion of FOXO1 transcription factors that are important for cell quiescence and apoptosis. FOXO1 drives the transcription of eomes, IL-7R and the transcription factor KLF2. In turn, KLF2 drives the transcription of CCR7, CD62L and S1P1, thus regulating the trafficking of CD8 T cells. T cells from PASLI/APDS patients showed increased levels of PIP3, increased phosphorylation of Akt, increased degradation of FOXO1 (target of Akt) and increased phosphorylation of mTORC1 target genes (red arrows). The red arrows also indicate the possible consequences of these alterations on CD8 T-cell development and function such as effects on effector *vs.* memory formation and effects on survival and trafficking. APDS, activated PI3K-δ syndrome; FOXO1, forkhead box protein O1; KLF2, Krüppel-like Factor 2; mTORC1, mammalian target of rapamycin complex 1; PASLI, p110δ-activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency; PDK1, phosphoinositide-dependent protein kinase; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; S1P1, sphingosine-1-phosphate receptor 1.

backgrounds. All patients had presented childhood onset sinopulmonary infections. Most patients had lymphadenopathies and nodular lymphoid hyperplasia at mucosal surfaces. Epstein–Barr virus viremia was detected in all patients and cytomegalovirus viremia in some, indicating a reduced control of persistent virus replication. Lymphoma was diagnosed in two patients. Similar clinical signs were observed in the series of 17 PASLI/APDS patients reported by Angulo *et al.*[2] In the two studies, whole-exome sequencing was used to identify specific mutations. In exome sequencing, only the exons, i.e., the parts of the gene sequences that code for the mature RNA, are sequenced thereby reducing significantly the amount of DNA that needs to be analyzed. Lucas *et al.* identified three germline-encoded gain-of-function mutations, each located within a different domain of the p110δ subunit of PI3K (encoded by the *PIK3CD* gene).[1] One of the mutations (E1021K) was also identified by Angulo *et al.*[2] Based on a comparison with the described mutations of the PI3K catalytic subunit p110α within cancer cells, Lucas *et al.* proposed that the E1021K mutation would result in enhanced membrane binding.[1] This was confirmed experimentally by Angulo *et al.*[2] T cells from PASLI/APDS patients exhibited increased levels of PIP3, together with elevated Akt phosphorylation and FOXO1 degradation. These were accompanied by increased phosphorylation of mTORC1 target genes and increased glucose uptake (Figure 1, red arrows).[1,2] In contrast, other T-cell receptor (TCR) signaling pathways such as TCR-induced Ca^{2+} flux were not affected by the *PIK3CD* mutations.[1] These alterations in the PI(3)K/Akt/mTORC1/FOXO1 pathways were associated with CD4 T-cell cytopenia and with normal to high CD8 T-cell counts.[1] Importantly, PASLI/APDS patients had reduced frequencies of naive cells and an accumulation of effector and highly differentiated RA^+ effector memory cells within both the CD4 and the CD8 T-cell subsets. The accumulation within the PASLI/APDS patients of CD8 T cells with a highly differentiated phenotype was associated with decreased proliferative responses and IL-2 production following TCR activation and with high production of IFN-γ and cytolytic molecules.[1] Although some of these functional properties were assessed using *in vitro* expanded T-cell lines and not directly *ex vivo*, they are consistent with the marked differentiation towards effector cells. Somewhat different results were reported by Angulo *et al.*[2] who observed a reduced production of IFN-γ by CD8 T cells of PASLI/APDS patients. This difference may be related to the fact that Lucas *et al.* measured cytokine production by flow cytometry following short-term stimulation, whereas Angulo *et al.* measured cytokines in supernatants following several days of stimulation. In the latter conditions, the amount of cytokines produced is influenced by cell proliferation that was shown to be defective in both reports. In addition, Angulo *et al.*[2] reported that the constitutive activation of the PI3K pathway also resulted in an increased susceptibility of T cells to activation-induced cell death and could thereby limit the expression of effector functions. Inhibition of mTOR by the administration of rapamycin to one

PASLI/APDS patient decreased CD8 T-cell counts and increased the proportions of naive and central memory T cells in the CD4 T-cell compartment. Although the reason for the different impact of *PIK3CD* mutations and rapamycin treatment on CD4 and CD8 T-cell subsets remains unclear, these observations are in keeping with the results obtained in the mouse and non-human primate, where rapamycin was shown to promote virus-specific memory CD8 T-cell responses.[6] Together, these results suggest that mTOR inhibition may be an effective strategy to improve memory T-cell responses to vaccines and infectious pathogens in humans.

The association between gain-of-function mutations in a pathway involved in T-cell activation and differentiation and a state of immunodeficiency is remarkable. Primary immunodeficiencies usually relate to mutations preventing T-cell activation or differentiation. The reactivation of persistent viruses in patients with a deficiency in naive T cells and an accumulation of effector T cells is reminiscent of the immunodeficiency associated with the human immunodeficiency virus infection and in which immune activation and immunosenescence probably play a central role.[7] The mechanism by which the accumulation of effector T cells in PASLI/APDS patients may reduce the control of persistent virus replication remains unclear, but it may involve the short lifespan of these cells. On the other hand, a central clinical consequence of the PI3K mutations was the development of respiratory infections of bacterial origin. It is likely that the accumulation of immature B cells and the deficiency in class-switched antibody secreting cells reported by Lucas *et al.* and Angulo *et al.*, rather than the differentiation of effector T cells, played a central role in the development of these complications. PASLI/APDS patients also presented with nodular lymphoid hyperplasia at mucosal surfaces. It is intriguing to consider that this complication may be related to the switch from lymph node homing receptors to tissue-homing receptors induced by the constitutive activity of the PI3K/Akt/FOXO1 pathway[5] (Figure 1). Of note, although autoimmune cytopenia were observed by Lucas *et al.*, the accumulation of effector T cells was not associated with marked autoimmune disorders in PASLI/APDS patients.

As the *PI3KCD* mutations are germline-encoded (in contrast to the frequent mutations in *PIK3CA*, the gene coding for p110α, in human cancers), they could also influence T cells during their development in the thymus. Indeed, PI3K, including p110δ, has been implicated in thymocyte development.[3–5] It would be of interest to determine whether the *PIK3CD* gain-of-function mutations have an impact on thymic output and whether this could contribute to the reduced proportions of naive T cells observed in the circulation of PASLI/APDS patients. Finally, extending the study of PASLI/APDS patients to regulatory T cells and unconventional T cells, including γδ T cells, could provide important insights into the role of PI3K in their development, differentiation and functions.

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