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Phosphoinositide 3-kinase δ is a regulatory T-cell target in cancer immunotherapy

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Summary

Tumour infiltration by regulatory T (Treg) cells contributes to suppression of the anti-tumour immune response, which limits the efficacy of immune-mediated cancer therapies. The phosphoinositide 3-kinase (PI3K) pathway has key roles in mediating the function of many immune cell subsets, including Treg cells. Treg function is context-dependent and depends on input from different cell surface receptors, many of which can activate the PI3K pathway. In this review, we explore how PI3Kδ contributes to signalling through several major immune cell receptors, including the T-cell receptor and co-stimulatory receptors such as CD28 and ICOS, but is antagonized by the immune checkpoint receptors CTLA-4 and PD-1. Understanding how PI3Kδ inhibition affects Treg signalling events will help to inform how best to use PI3Kδ inhibitors in clinical cancer treatment.

Keywords: signal transduction; regulatory T cell; T cell; tumour immunology.

Introduction

$CD4^+$ $Foxp3^+$ regulatory T (Treg) cells can act as a barrier to the effective implementation of cancer therapy.[1,2] Initially identified as a $CD25^{hi}$ T-cell subset with immunosuppressive function, Treg cells assume critical roles in the prevention of autoimmunity.[3] Among the numerous immune cell subsets now known to form the immunosuppressive barrier within the tumour microenvironment, including macrophages and myeloid-derived suppressor cells, Treg cells remain the most intensely studied.[4–8] The therapeutic targeting of tumour Treg cells to enhance anti-tumour immunity is therefore an active area of research.[7,8] The lack of a truly unique surface marker that identifies Treg cells, and the fundamental requirement of Treg cells to prevent lethal autoimmunity, makes the strategy of Treg cell depletion challenging. However, recent advancements using antibodies against CD25 or CCR4, a chemokine receptor that is strongly up-regulated on tumour Treg cells, are showing promise.[9,10]

Alongside the developments in antibody therapies, modulation of cell signalling pathways through small-molecule inhibitors has also gained ground within the immunotherapy field. The functional profiles of immune cells are necessarily shaped in response to environmental cues, which are conveyed to the cellular machinery through a myriad of distinct but overlapping signalling cascades. Many of these pathways are driven by post-translational phosphorylation of downstream targets by kinases, several of which have been targeted with some success in oncology.[11] In particular, the phosphoinositide-3-kinase (PI3K) pathway has a uniquely extensive role in transducing signals from key cell surface receptors in the immune system, influencing many important aspects of the immune response.[12] Inhibition of the PI3K pathway represents a promising approach to the therapeutic manipulation of Treg cell function. Here, we

explore the role of the PI3K pathway in Treg cell biology, particularly in the context of tumour immunosuppression.

A regulatory T-cell subset

Evidence of immune-suppressive T cells, then proposed as 'T suppressor' cells, first emerged half-century ago,[13,14] but detailed study of a defined T-cell subset was only made possible when, 25 years later, Sakaguchi *et al.*[3] described a $CD4^+$ $CD25^+$ population, with a critical role in maintaining immune homeostasis and preventing autoimmunity. The identification of the forkhead box P3 (Foxp3) transcription factor as a master regulator of the Treg cell lineage[15,16] revealed a unique transcriptional programme driving a suppressive profile. Loss-of-function mutations in the *FOXP3* gene result in lethal multi-organ autoimmunity, known as immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome in humans,[17] and as scurfy in mice.[18] A plethora of studies since their initial discovery have shown that Treg cells play prominent roles in preventing pathogenic autoimmunity,[19] and in controlling rejection and graft-versus-host-disease in transplant recipients,[20,21] but can contribute to the persistence of infections.[22]

In addition to Treg cells originating in the thymus, naive conventional CD4 T cells can also be 'induced' by environmental cues in the periphery, most prominently transforming growth factor-β, to express Foxp3 and take on a suppressive phenotype.[23,24] Both thymic Treg cells and peripherally-derived Treg cells employ a broad range of suppressive mechanisms; their application and relative importance appear to depend heavily on the specific physiological context.[25]

Treg cells in the immunosuppressive tumour environment

The proportion of Treg cells within the tumour immune infiltrate often far outstrips homeostatic proportions in circulation – although 5–10% of $CD4^+$ T cells in the spleen or lymph nodes are $Foxp3^+$, in many types of tumour this can be up to 30–50%.[26] In certain types of tumour, most notably colorectal cancers, it has been argued that a large Treg cell infiltrate is beneficial and indicates a favourable prognosis, due to its role in dampening the inflammation driving oncogenic progression.[27,28] In other cancers, however, increased Treg cell infiltration correlates with a poor prognosis,[29,30] and therapies that reduce Treg cell numbers in the tumour have produced positive results.[31,32]

Accumulation of Treg cells is a crucial part of immune evasion by the tumour where cytokines and chemokines, produced by both tumour cells and stromal cells, contribute to Treg cell recruitment, *in situ* proliferation, and conversion from conventional $CD4^+$ T cells.[33] Treg-mediated immunosuppression has been held accountable for the reduced anti-tumour functionality of $CD8^+$ and $CD4^+$ conventional T cells in the tumour.[34,35] Tumour Treg cells have also been implicated in the recruitment of myeloid-derived suppressor cells, playing an accessory role in the formation of the tumour immunosuppressive environment.[36]

The first attempts to treat cancer by depleting Treg cells targeted the interleukin-2 (IL-2) receptor, which is highly expressed by Treg cells. Denileukin diftitox, a fusion molecule combining IL-2 and diphtheria toxin originally designed for the treatment of cutaneous T-cell leukaemia, was demonstrated to deplete Treg cells and enhance anti-tumour immunity in mouse B16 melanoma,[37] but failed to provide clinical benefit in human ovarian or breast cancers.[38,39] A depleting antibody against the IL-2 receptor α subunit, also known as CD25, can mediate tumour rejection in mice when administered before or shortly after tumour implantation, but is not effective against established tumours, possibly because highly activated conventional T cells required for tumour elimination also up-regulate CD25.[40] This approach may still prove effective, however; anti-CD25 with an engineered antibody Fc region to mediate enhanced antibody-dependent cell-mediated cytotoxicity by tumour-resident macrophages can combine with anti-programmed death-1 (anti-PD-1) to effectively eliminate tumours in mice.[9]

Beyond CD25, Treg-selective cell surface markers amenable to antibody targeting are highly sought after, but have remained largely elusive – most markers are also shared with activated T effector cells. Meanwhile, a greater understanding of the phenotypic profile of activated, highly suppressive effector Treg cells – describing the great majority of Treg cells within the tumour – is forming the basis for alternative approaches to Treg cell modulation. Although genetic aberrations that disrupt the development or maintenance of the homeostatic 'resting' Treg cell pool can result in catastrophic autoimmunity, effector Treg cells depend on a partially distinct set of transcriptional, metabolic and signalling conditions to maintain high functionality in specific contexts, such as the tumour microenvironment.[41,42] Tumour-infiltrating Treg cells adapt to an environment characterized by a myriad of cytokines and chemokines, low oxygen availability, and high glucose demand, among other factors.[43,44] Nuclear factor-κB activation through the tumour necrosis factor receptor super family has been shown to have special significance in the effector Treg cell population,[45] as has the promotion of glycolysis through the adenosine-monophosphate-activated protein kinase.[46] Pharmacological manipulation of these cellular pathways therefore holds promise for the therapeutic targeting of tumour Treg cells.

PI3K in the immune system

One cellular signalling pathway that has become increasingly prominent as a pharmaceutical target is the PI3K pathway. One of the master signalling pathways with critical roles in all mammalian cells, PI3K signalling has been shown to be involved in processes including cell survival, proliferation, differentiation and mobility.[47]

Class I PI3Ks are activated by the recruitment of the catalytic p110 subunit to the plasma membrane, through the binding of a regulatory subunit (p85 in the case of the class 1A PI3Ks: p110α, β or δ; p55 or p101 for the class 1B PI3K: p110γ) to a phosphorylated tyrosine on the intracellular domain of a cell surface receptor.[12,47] On the inner surface of the cell membrane, the p110 subunit phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3), a second messenger molecule propagating the signal to downstream effectors.[48] The phosphorylation cascade of the PI3K pathway is regulated at several points by phosphatases, the best-known of which is the phosphatase and tensin homologue conversion of PIP3 back into PIP2.[49] PIP3 recruits phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B/AKT, the latter of which is phosphorylated and activated by the former.[50] Both PDK1 and AKT phosphorylate and activate multiple downstream targets, among which the activation of mammalian target of rapamycin is known to be a central modulator of many transcriptional and metabolic processes.[51]

In contrast to non-immune cells, which dominantly express the p110α or p110β catalytic isoform, immune cells largely depend on the p110δ or p110γ isoforms for PI3K signal transduction.[12] Many principle receptors in lymphocyte biology, e.g. the T-cell receptor (TCR), the B-cell receptor, the IL-2 receptor and various co-stimulatory receptors, activate p110δ (hereafter PI3Kδ) upon ligand binding, making it an integral component in mounting a coherent immune response to extracellular cues.[12] PI3Kγ is more predominant in myeloid cells and can also play a key role in tumour immune suppression.[52–54]

Dysregulation of PI3Kδ signalling leads to altered lymphocyte development and function.[12] Mice with a knock-in kinase-inactivating D910A point mutation in p110δ (PI3Kδ^{D910A}) have a profound defect in B-cell development and function.[55] Remarkably, constitutive activation of PI3Kδ also leads to B-cell dysfunction, resulting in symptoms of immunodeficiency such as recurrent respiratory infections.[56] PI3Kδ^{D910A} mice show a reduced CD8$^+$ T-cell response to pathogenic *Listeria monocytogenes* infection, but improved bacterial clearance due to an enhancement of the innate immune response.[57] Under specific circumstances, however, the attenuated phenotype of PI3Kδ-deficient CD8$^+$ T cells paradoxically results in an overall improvement of the desired immune response – *in vitro* treatment of T cells with inhibitors of the PI3K/Akt pathway has been shown to improve *in vivo* persistence and anti-tumour efficacy when transfused into tumour-bearing mice, by favouring a central-memory phenotype over terminal differentiation as effectors.[58–60]

A requirement for PI3Kδ in Treg-mediated tumour immunosuppression

Treg cell development has been widely reported to be enhanced under PI3K/AKT pathway inhibition.[61–63] Suppression of the PI3K signal has been shown to be necessary for normal Treg cell differentiation,[63] and PI3Kδ^{D910A} mice have increased numbers of Treg cells in the thymus.[61] In circulation, however, Treg cells are reduced in PI3Kδ^{D910A} compared with wild-type,[61] and deletion of Foxo1, a transcription factor inhibited by AKT, abrogates normal Treg cell function.[64] It may be surmised that optimal Treg cell development and homeostatic maintenance require dynamic regulation of PI3K activity (Fig. 1).

PI3Kδ activity is necessary for Treg cell suppressive function. PI3Kδ^{D910A} mice can develop intestinal inflammation, due to a breach in immune tolerance against colon microflora.[55,61] Several patients with genetic loss of PI3Kδ have also been described, who suffer from colon and liver inflammation.[65,66] With the use of PI3Kδ-specific inhibitor idelalisib in the treatment of chronic lymphocytic leukaemia, colitis and transaminitis have been reported as common adverse effects in patients, indicating immune dysregulation.[67] A recent study identified clear defects in human Treg cell activation and suppressive function under PI3Kδ inhibition, both *in vitro* and in idelalisib-treated patients.[68]

Additionally, we and others have shown that PI3Kδ-inactivated Treg cells are impaired in mediating tumour immunosuppression. Loss of PI3Kδ activity, especially by specific deletion in Treg cells, can restrict the growth of transplanted tumours in mice.[69–71] Whereas PI3Kδ inhibition has been reported to preferentially incapacitate Treg cells over effector T cells in the anti-tumour immune response,[59] a different study has shown that loss of PI3Kδ activity abrogates tumour elimination by CD8$^+$ T cells,[72] so the overall effect of PI3Kδ inactivation on tumour growth may depend on a balance of the impact on effector and regulatory T-cell subsets. We have demonstrated that enhancement of anti-tumour immunity by PI3Kδ inactivation occurs in spite of concurrent impairment of CD8$^+$ T-cell cytotoxicity,[69] and correlates with the dependence of the tumour on Treg-mediated immunosuppression.[70] Supporting a requirement for PI3Kδ activity in tumour Treg cell function, constitutive nuclear localization of Foxo1 – as

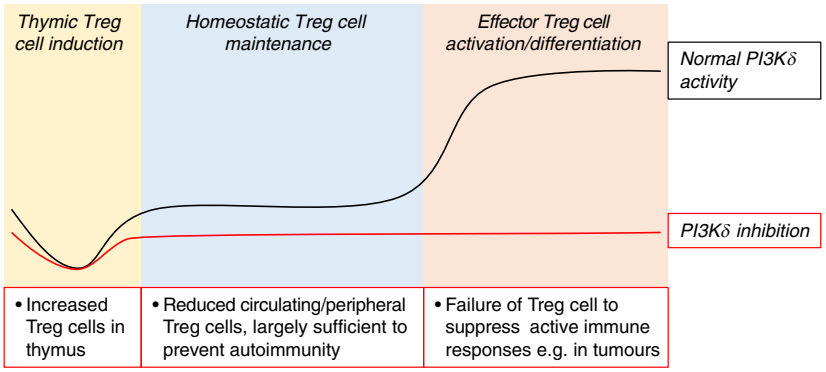

Figure 1. Regulatory T (Treg) cells require different levels of phosphoinositide-3-kinase (PI3Kδ) activity during different phases of development and function. An increase in Treg cells in the thymi of mice with inactive PI3Kδ may indicate a required repression of PI3Kδ signalling in early Treg cell development. In the periphery, a basal level of PI3Kδ activity is needed to maintain a homeostatic Treg cell population, such that PI3Kδ inhibition results in reduced circulating Treg cells in the resting state. For Treg cells to differentiate into fully activated effector Treg cells, the PI3Kδ pathway is required to transduce an array of signals (see Fig. 2); loss of PI3Kδ activity can therefore lead to an acute impairment of Treg-mediated suppression at sites of active inflammation.

would be the case in PI3Kδ inactivation – blocks Treg cell tumour infiltration and boosts anti-tumour immunity.[73]

We therefore suggest that Treg cells have varying requirements for PI3Kδ signalling throughout their lifespan, benefiting from reduced activity during development, but depending on the PI3Kδ pathway in delivering immune suppression as mature regulators.[74] This bifurcation represents an intriguing therapeutic potential to selectively target highly activated effector Treg cells – for example, in the tumour, or less desirably, in the gut – while leaving the homeostatic pool of resting Treg cells intact.

PI3Kδ downstream of core T-cell receptors

Treg cells are thought to develop within a narrow window of TCR–MHC binding affinity above the threshold of negative selection, thus possessing a TCR repertoire skewed towards self-recognition.[75] The signal immediately downstream of the TCR is constricted in Treg cells compared with conventional T cells,[76] protecting, perhaps, against stimulation-induced apoptosis – but rendering them especially vulnerable to inhibition of the TCR signal (Tanaka *et al.*, under review).

Stimulation of the TCR acutely activates PI3Kδ.[55,77] Once activated, it is thought that Treg cells are capable of suppressing in a TCR-independent manner,[78] but tumour-infiltrating Treg cells are enriched for tumour antigen-specific clones, indicating an influence of antigen-binding within the tumour.[79] The TCR can also play a larger coordinating role beyond direct signalling from its own intracellular domains, as a focal point for the formation of immunological synapses, within which many of the receptors we will go on to discuss receive and transduce their respective signals.[80]

Alongside the TCR, T cells need a second signal[81] through co-stimulatory receptors to achieve full activation (Fig. 2). A canonical example of such a receptor is CD28, which binds CD80 and CD86 on antigen-presenting cells. Within Treg cells, CD28 binding is required for the up-regulation of key functional proteins cytotoxic T lymphocyte antigen-4 (CTLA-4), PD-1 and CCR6, the lack of which reduces effector Treg cell suppressive capacity and abrogates the ability to home to tissues.[82] CD28 ligation has been reported to signal through PI3Kδ,[83] although in naive T cells CD28 plays a more important role in amplifying the PI3K signal downstream of the TCR.[77] However, there is evidence that memory T cells can activate PI3K via CD28 in a TCR-independent manner to promote migration into tissues.[84] CD28-dependent activation of PI3K was recently shown to regulate glycolysis by increasing the expression of glucokinase. Interestingly, this was not required for Treg-mediated suppression, but rather for Treg cell migration to non-lymphoid tissues, such as the skin.[85] It will be intriguing to know of CD28-dependent glucokinase expression also regulates Treg cell migration to tumours. CD28 is also a major activator of the nuclear factor-κB pathway, which may be more central to its co-stimulatory function.[86,87]

By contrast to CD28, the inducible co-stimulatory (ICOS) signals almost exclusively through PI3Kδ to enhance T-cell activation and function, and is instrumental in T helper cell differentiation.[88,89] $CD4^+$ T cells, especially, show an almost exclusive dependence on PI3K signalling downstream of ICOS, whereas PI3K-independent signals could mediate some degree of ICOS co-stimulation in $CD8^+$ T cells.[90,91] ICOS expression in Treg cells correlates with a highly activated Blimp-1^+ IL-10^+ effector Treg cell population,[92,93] and is thought to promote a Foxp3-driven transcriptional programme over an effector T-cell phenotype upon cell activation.[94]

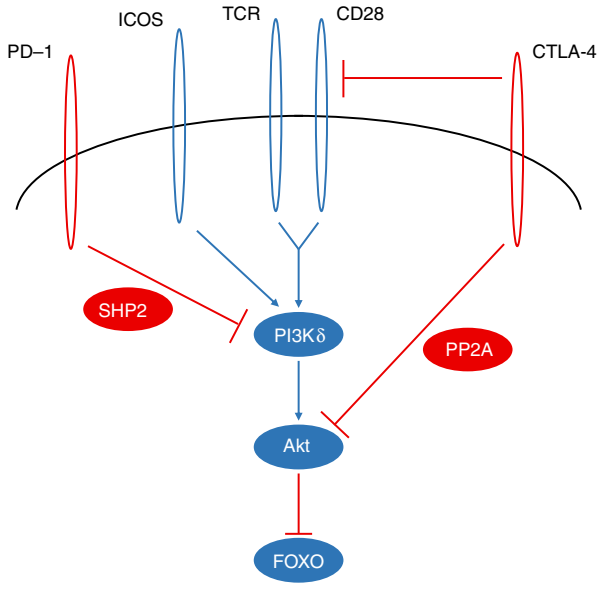

Figure 2. Antigen-binding, co-stimulatory and co-inhibitory receptors shape regulatory T (Treg) cell function through phosphoinositide-3-kinase (PI3Kδ) signalling. T-cell receptor (TCR) stimulation strongly activates PI3Kδ, and binding of CD28 amplifies this signal. ICOS, another co-stimulatory receptor highly up-regulated in effetor Treg cells, can independently activate PI3Kδ. Through phosphorylation of PIP2 to produce PIP3, PI3Kδ then activates AKT, which inhibits FOXO, a transcription factor critical to Treg cell function, in part through its regulation of the *Foxp3* gene. CTLA-4, constitutively expressed in Treg cells, is known to attenuate the co-stimulatory signal by competition with CD28 for its ligands, but can also antagonize AKT activity through activation of the PP2A phosphatase. PD-1, which is up-regulated in Treg cells as well as conventional T cells in the tumour, can restrict the T-cell activation signal through the SHP2 phosphatase, dephosphorylating tyrosine residues required for PI3Kδ recruitment and activation.

A third signal critical to the development and function of Treg cells is IL-2.[95–97] Interleukin-2 can signal through a receptor composed of the β (CD122) and γ (CD132) subunits, which binds the ligand with moderate affinity; the addition of the α subunit, also known as CD25, forms the high-affinity receptor for IL-2.[98] With constitutively higher expression of CD25 on Treg cells compared with other T-cell subsets, the competitive sequestration of IL-2 from the environment has been proposed as a mechanism of suppression against conventional T cells.[99] The IL-2 receptor primarily activates the Janus kinase–signal transducer and activator of transcription (JAK/STAT) pathways but can also lead to TCR-dependent PI3K signalling.[100]

To summarize, there is known PI3Kδ involvement in signalling downstream of the TCR, CD28, ICOS, and possibly the IL-2 receptor, each of which regulates Treg cell homeostasis and function (Fig. 2). The observation that PI3Kδ-deficient Treg cells appear phenotypically normal and are only modestly reduced in number suggests that they are functionally incapacitated in a manner that largely manifests itself in loss of immune tolerance to commensal microorganisms and in the context of tumour immunology. The precise Treg-mediated suppressive mechanism that is impaired by PI3Kδ inhibition remains to be firmly established, as do the receptors that activate PI3Kδ to this end.

PI3Kδ downstream of checkpoint receptors

Checkpoint receptor molecules, such as CTLA-4 and PD-1, have gained much publicity as targets of breakthrough cancer immunotherapies, as evidenced by this year's Nobel Prize in Medicine or Physiology awarded to James Allison and Tasuko Honjo. Checkpoint receptors are so named for their role in restricting the cytotoxic or inflammatory functions of effector T cells, and are often up-regulated in highly activated effector T cells to limit collateral damage to self tissue in the wake of pathogen-induced immune responses, as well as to prevent autoimmune damage.[101] The list of checkpoint receptors continues to grow; this section will focus primarily on the founding members CTLA-4 (also known as CD152) and PD-1 (CD279) (Fig. 2).

CTLA-4 shares its ligands with the co-stimulatory receptor CD28, binding both CD80 and CD86 with higher affinity than CD28. Its presence on both activated conventional T cells and Treg cells is thought to abrogate the co-stimulatory signal through competitive binding,[102] and has indeed been proposed to remove these ligands from the surface of antigen-presenting cells through trogocytosis and transendocytosis.[103] In contrast with conventional T cells, Treg cells constitutively express CTLA-4 at a high level;[104] whereas the anti-CTLA-4 antibody ipilimumab was primarily developed to relieve the 'checkpoint'-induced suppression on cytotoxic $CD8^+$ T cells in the tumour,[105] it has since been reported that Treg cells remain the cell population expressing the highest levels of CTLA-4 within the tumour,[106] and that the efficacy of ipilimumab treatment can at least partially be attributed to antibody-dependent cell-mediated cytotoxicity in the Treg cell population.[107]

Although there are questions about the capacity of the intracellular domain of the CTLA-4 receptor to transduce signals[108] (and some suggestion that the influence of CTLA-4 binding is limited to its own cycling between cytosolic vesicles and the cell surface[109]), it has also been proposed that CTLA-4 binding can antagonize the activation signal in a cell-intrinsic manner by attenuating the PI3K pathway, by activating the protein phosphatase 2A (PP2A).[110]

PD-1 is up-regulated in both activated conventional T cells and effector Treg cells – such as those found in the tumour – and binds ligands PD-L1 and PD-L2 expressed

by a variety of immune and non-immune cell types.[111] PD-1 binding stimulates SHP2, a phosphatase that antagonizes a range of cell-activating signals – including PI3K – so exerting an inhibitory effect on T-cell function.[110,112,113] Like conventional T cells, Treg cells lacking PD-1 show enhanced proliferation; however, PD-1-deficient Treg cells have reduced expression of Bcl2, and may have reduced viability or stability.[114] Intriguingly, PI3Kδ-deficient tumour-infiltrating Treg cells – but not $CD8^+$ T cells – express markedly lower levels of PD-1 than PI3Kδ-sufficient controls;[70] whether a consequent loss of Treg cell stability can explain the anti-tumour effects of PI3Kδ inactivation remains to be determined.

Mice with inactive PI3Kδ become unresponsive to anti-CTLA-4 or anti-PD-L1 treatment, even in tumour models that are usually sensitive to checkpoint blockade.[70] As both receptors are implicated in reducing PI3Kδ signalling, we hypothesize that loss of PI3Kδ may at least partially nullify the benefits of concurrent checkpoint blockade therapy, which act in part by increasing PI3Kδ signalling in $CD8^+$ T cells. These results do not preclude combination of pharmacological PI3Kδ inhibition and checkpoint blockade therapy, however, as others have reported success with this strategy.[115,116] The discrepancy between studies using a genetic model of PI3Kδ inactivation and those using pharmacological inhibition points to important differences in the underlying mechanism of these approaches, warranting additional investigation to inform clinical use. Intriguingly, an intermittent dosing schedule for PI3Kδ inhibition is reported to yield even better enhancement of anti-tumour immunity, relieving Treg cell suppression without compromising effector $CD8^+$ T-cell function,[117] raising the attractive possibility that PI3Kδ inhibition can be further refined to improve synergy with other therapies.

PI3Kδ in the recruitment and localization of Treg cells in tumours

Cells rely on chemokine signals for navigational cues, and the tumour exploits a wide range of chemokines to recruit various immune cell subsets.[118] The chemokine receptors CCR4 (binding CCL17 and CCL22) and CCR8 (binding CCL1) have both been identified as tumour Treg-specific markers,[10,119,120] and are the targets of ongoing efforts to selectively deplete tumour-infiltrating Treg cells.

The PI3K pathway is involved in several aspects of cell mobility. A major consequence of the constitutive nuclear localization of FOXO1 is the failure of Treg cells to down-regulate lymphoid homing receptors such as CD62L, hampering tumour infiltration.[73] PI3Kδ-deficient T cells also fail to down-regulate CD62L and CCR7, well-established markers of naive T cells important for lymphoid homing, even upon strong stimulation *in vitro*.[121] PI3Kδ inactivation also impairs the activation of LFA-1 in $CD4^+$ T cells, leading to defects in cell–cell interaction, which could affect the adhesive contacts necessary for tissue infiltration, or for contact-dependent suppression[122] – particularly as LFA-1 is involved in CTLA-4 transendocytosis of CD80 and CD86 from antigen-presenting cells.[103]

However, a comparison of tumour-infiltrating Treg cells between wild-type and $PI3K\delta^{D910A}$ mice does not show a consistent quantitative reduction in PI3Kδ-deficient mice.[69] We have also not observed gross-level differences in the intratumoural localization of Treg cells with inactivated PI3Kδ, even though a number of chemokine receptors may have lower expression in the absence of PI3Kδ (unpublished observations). Further investigation will be required to determine how prominent this factor is in mediating the effects of PI3Kδ inhibition in tumour Treg cells.

PI3Kδ in cytokine-driven adaptation in tumour Treg cells

As Foxp3 functions as a master lineage regulator in Treg cells, so too can the T helper type 1 (Th1), Th2 and Th17 subsets of $CD4^+$ T helper cells be identified by their expression of T-bet, GATA-3 and RORγt, respectively.[123,124] However, $Foxp3^+$ Treg cells can also up-regulate the lineage-regulating transcription factors of T helper subsets in response to appropriate cytokine stimulation,[125] and such expression has been recognized as a mark of adaptation in Treg cells, allowing them to co-localize with their targets and function optimally within the same environmental conditions.[124,126] Follicular regulatory T cells, for example, share with T follicular helper cells the expression of lineage transcription factor Bcl6, allowing residency in the germinal centre through expression of the chemokine receptor CXCR5.[127,128]

Tumour-infiltrating Treg cells have been widely reported to up-regulate T helper lineage transcription factors, especially T-bet, and in some cases GATA-3.[129–131] Both T-bet-expressing and GATA-3-expressing Treg cells were shown to have enhanced suppression of anti-tumour $CD8^+$ T cells, without up-regulating inflammatory cytokines characteristic of their $Foxp3^-$ counterparts. Strikingly, Treg cells have been reported to rely on granzyme expression in mediating tumour immunosuppression[132,133] but not in the control of graft-versus-host disease,[134] and it is plausible that an adaptive Th1-like transcriptional programme driven by the expression of T-bet is required for the manifestation of these context-specific suppressive features.

We have observed that PI3Kδ-deficient tumour Treg cells have reduced expression of T-bet at the transcriptional level, and further that addition of a PI3Kδ inhibitor to *in vitro* Treg cell cultures with interferon-γ and IL-

12 abrogates the acquisition of T-bet expression (unpublished observations). Further study will reveal whether, and how, loss of PI3Kδ activity disrupts the tumour-adapted functional profile of Treg cells, to the enhancement of anti-tumour immunity.

Summary

Treg cells in the tumour context lose suppressive capacity in the absence of PI3Kδ activity, indicating a central role for PI3Kδ signalling in facilitating tumour Treg cell function. As one of the most pleiotropic signalling cascades in immune cells, the precise sequence of events, leading from stimulus to suppression, which is perturbed in PI3Kδ-deficient Treg cells is not straightforward to pinpoint. Indeed, considering the wide-ranging but subtle phenotypic differences observed in PI3Kδ-inactivated tumour-infiltrating Treg cells, it is distinctly possible that the impairment of suppressive function is not due to the abrogation of any single pathway, but a cumulative result of incomplete restrictions in numerous signalling cascades. We have explored some of the better-studied pathways among these, presenting a picture that we hope will serve as a starting point for understanding how PI3Kδ signals are integrated to shape Treg cells within the tumour environment.

Disclosure

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