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Phosphoinositide 3-kinase and the mammalian target of rapamycin pathways control T cell migration

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The established role for phosphatidylinositol (3,4,5) triphosphate ($PI(3,4,5)P_3$) signaling pathways is to regulate cell metabolism. More recently it has emerged that $PI(3,4,5)P_3$ signaling via mammalian target of rapamycin and Foxo transcription factors also controls lymphocyte trafficking by determining the repertoire of adhesion and chemokine receptors expressed by T lymphocytes. In quiescent T cells, nonphosphorylated active Foxos maintain expression of KLF2, a transcription factor that regulates expression of the chemokine receptors CCR7 and sphingosine 1 phosphate receptor, and the adhesion receptor CD62L that together control T-cell transmigration into secondary lymphoid tissues. $PI(3,4,5)P_3$ mediates activation of protein kinase B, which phosphorylates and inactivates Foxos, thereby terminating expression of KLF2 and its target genes. The correct localization of lymphocytes is essential for effective immune responses, and the ability of phosphoinositide 3-kinase and mammalian target of rapamycin to regulate expression of chemokine receptors and adhesion molecules puts these signaling molecules at the core of the molecular mechanisms that control lymphocyte trafficking.

Keywords: phosphatidylinositol (3,4,5) triphosphate, KLF2, CCR7, S1P1, CD62L, lymphocytes

Background

One critical signal transduction pathway in T cells is initiated by the lipid second messenger phosphatidylinositol (3,4,5) triphosphate ($PI(3,4,5)P_3$).[1] Intracellular levels of $PI(3,4,5)P_3$ are low in quiescent naive T cells but increase rapidly following triggering of the T-cell antigen receptor (TCR) with antigenic peptides presented on major histocompatibility complex (MHC) molecules on the surface of specialized antigen-presenting cells (APCs).[2,3] T cells can maintain contact with antigen-primed APCs for many hours and during this time will sustain high levels of $PI(3,4,5)P_3$.[2] This biochemical response has been visualized using enhanced green fluorescent protein (EGFP)-tagged high-affinity $PI(3,4,5)P_3$ lipid-binding domains as fluorescent reporters is thus known that as T cells respond to antigen, $PI(3,4,5)P_3$ accumulates relatively uniformly around the T-cell plasma membrane creating a signaling platform inside and outside the contact area with the APCs.[2]

The sustained accumulation of $PI(3,4,5)P_3$ in T cells undergoing immune activation requires continual engagement of the TCR and continual activation of phosphatidylinositol 3-kinase (PI3K).[2,4] Signals from co-stimulatory molecules, such as CD28, are important during this sustained response,[5] although it should be emphasized that co-stimulatory signals alone are not sufficient to trigger $PI(3,4,5)P_3$ production in T cells. Hence T cells that encounter activated dendritic cells do not generate $PI(3,4,5)P_3$ unless the APC expresses the cognate peptide/MHC complex.[2,5] There are, however, other physiological stimuli, such as chemokines and cytokines, that can directly induce $PI(3,4,5)P_3$ production in T cells. For example, antigen-primed T cells exposed to IL-2 can sustain high cellular levels of $PI(3,4,5)P_3$ over several days.[6,7] Other cytokines, such as IL-15 and IL-7, also induce $PI(3,4,5)P_3$ accumulation, but it should be emphasized that the relative potency of these different cytokines can vary. For example, in antigen-primed T cells IL-15 can only induce relatively low levels of $PI(3,4,5)P_3$ compared to IL-2.[7]

Cellular levels of $PI(3,4,5)P_3$ in T cells are controlled by the balanced activity of class I PI3Ks that phosphorylate the 3′-OH position of the inositol ring of phosphatidylinositol (4,5) biphosphate ($PI(4,5)P_2$) and lipid phosphatases, particularly PTEN (phosphatase and tensin homologue deleted on chromosome 10), a lipid phosphatase with specificity for the 3′ position of $PI(3,4,5)P_3$.[8–11] Class I PI3Ks comprise a 110-kDa catalytic subunit and an adapter regulatory subunit. Four catalytic isoforms exist (α, β, γ, δ) and three adapter subunits (p85α, p85β, and p55γ).[5,12] In mature T cells the majority of $PI(3,4,5)P_3$ is produced by the actions of the p110δ PI3K catalytic subunit.[5] In simplistic models it is assumed that stimuli, such as TCR ligation, that cause accumulation of $PI(3,4,5)P_3$ do so because they activate PI3K.[13] However, PTEN acts as a critical negative regulator of cellular $PI(3,4,5)P_3$ levels, and it is important to note that deletion of PTEN causes an immediate accumulation of $PI(3,4,5)P_3$, indicating that even in quiescent T cells PI3Ks are constitutively active.[8,10,11] It thus remains to be determined whether cellular levels of $PI(3,4,5)P_3$ increase in activated T cells solely as a consequence of increased PI3K activity/translocation to the plasma membrane or whether T cells respond to immune activation by deleting PTEN. In this context, the importance of PTEN for T-cell pathology has been recognized in a number of studies. Notably, PTEN is downregulated by Notch1 signaling in T-cell acute lymphoblastic leukemias, with a resultant constitutive accumulation of $PI(3,4,5)P_3$.[14] Moreover, tissue-specific deletion of PTEN in T-cell progenitors in the thymus, using Cre-loxP strategies, results in constitutive $PI(3,4,5)P_3$ signaling and causes rapid T leukemogenesis or lymphomagenesis.[11,15]

How does $PI(3,4,5)P_3$ signal?

A simple descriptor of the signaling function of $PI(3,4,5)P_3$ is that it binds to the pleckstrin homology (PH) domains of proteins and controls their activity and subcellular localization. One well-characterized $PI(3,4,5)P_3$-binding protein is the serine/threonine kinase protein kinase B (PKB) or Akt (Fig. 1). A rate-limiting step for PKB activation is phosphorylation of threonine 308 within the PKB catalytic domain by 3-phosphoinositide-dependent protein kinase 1 (PDK1).[16] PDK1 has a PH domain that binds $PI(3,4,5)P_3$ with high affinity; but PDK1 activity is not $PI(3,4,5)P_3$ dependent, rather the binding of $PI(3,4,5)P_3$ to the PDK1 PH domain promotes translocation of the enzyme to the plasma membrane where it can co-localize with PKB.[17–19] The $PI(3,4,5)P_3$ dependence of PKB activation also reflects that $PI(3,4,5)P_3$ binding to the PKB PH domain causes a conformational change that allows PDK1 to phosphorylate threonine 308 within the PKB catalytic domain and activate the enzyme.[20,21] Once activated PKB phosphorylates a number of critical signaling molecules, including the transcription factors Foxo1, 3a, and 4.[22] These Foxo family transcription factors are nuclear and active in quiescent cells but, when phosphorylated by PKB, they exit the nucleus and form a complex with 14-3-3 proteins in the cytosol, thereby terminating their transcriptional activity.[23,24] PKB also controls mammalian target of rapamycin complex 1 (mTORC1) by phosphorylating and inactivating TSC2, the Rheb GTPase-activating protein. PKB activation thus results in accumulation of Rheb-GTP, which activates mTORC1.[22] PKB also regulates mTORC1 by phosphorylating PRAS40, thereby blocking PRAS40-mediated inhibition of mTORC1.[22]

PDK1/protein kinase B control of T-cell metabolism

One evolutionarily conserved function of PDK1/PKB is to control cell metabolism. In particular, PKB-mediated activation of mTORC1 controls translation of a subset of mRNA and controls ribosomal biogenesis. Here it is relevant that the survival and immune function of peripheral T lymphocytes require that these cells match energy metabolism to energy demands. In particular, activated T cells upregulate glucose, amino acid, and iron uptake and switch to glycolysis in a response that increases cellular energy production and nutrient uptake to support the biosynthetic demands of rapid cell division.[6,25–27] The signaling pathways that control this facet of T-cell metabolism have been best characterized in T-cell progenitors in the thymus. In these cells, PDK1/PKB signaling pathways induce and maintain expression of the nutrient receptors CD71 and CD98, which control transferrin and amino acid uptake.[27,28] PKB also coordinates glucose uptake and survival of thymocytes.[29] Hence in the absence of PDK1 or PKB, thymocytes cannot increase metabolism

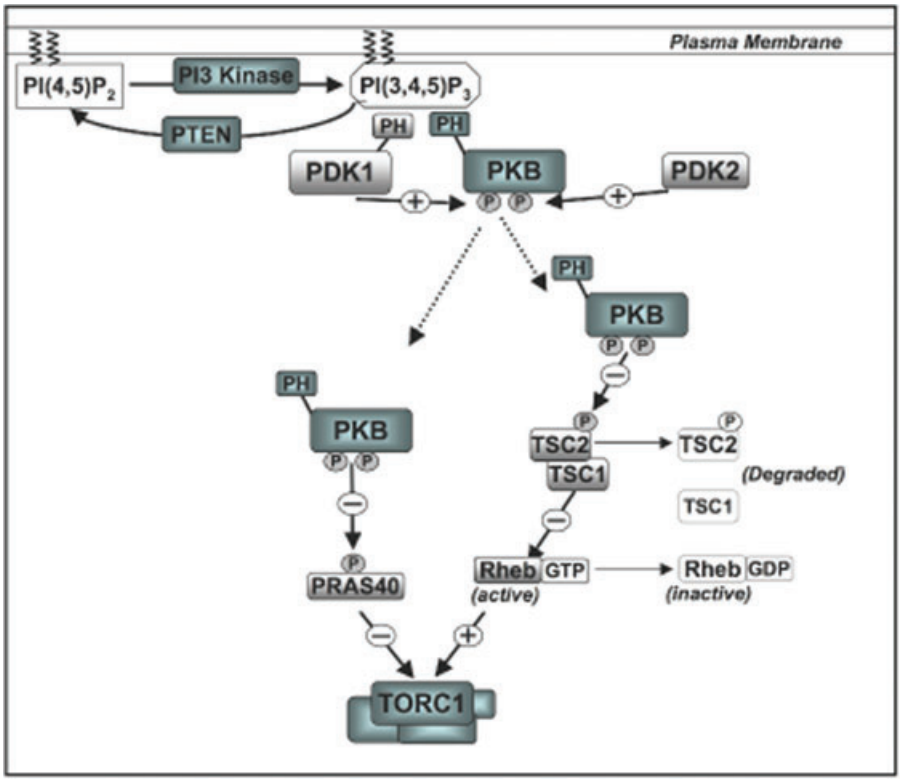

Figure 1. Phosphatidylinositol 3-kinase (PI3K)-dependent activation of protein kinase B (PKB) and mammalian target of rapamycin (mTOR). Elevated $PI(3,4,5)P_3$ levels following PI3K activation or PTEN (phosphatase and tensin homologue deleted on chromosome 10) deletion recruits PKB and its upstream activator, 3-phosphoinositide-dependent protein kinase 1 (PDK1), to the membrane. Full activation of PKB requires the interaction of its pleckstrin homology (PH) domain with $PI(3,4,5)P_3$ followed by its phosphorylation on two key residues by PDK1 and another kinase, termed PDK2, which is cell-type and context specific. PKB activates mTOR through dual mechanisms: (1) phosphorylation and inactivation of PRAS40, a negative regulator of mTOR, and (2) inactivation of the TSC1/TSC2 complex, a GTPase-activating protein that promotes the inactivation of the small GTPase Rheb, a positive regulator of mTOR complex 1 (TORC1).

to match the energy demands of proliferation and they atrophy and fail to develop.[27,30]

One question that has not yet been addressed is whether PI3K/PDK1/PKB signaling has a similar essential role to direct the metabolic program of proliferating peripheral T cells. Certainly antigen receptors, CD28, and the common γ chain cytokines IL-7, IL-15, and IL-2, extrinsic stimuli that control peripheral T-cell function, all activate PKB. Additionally, constitutively active mutants of PKB can substitute for the cytokine IL-7 and stimulate glucose uptake and promote cell growth and survival in mature CD4 T cells.[31] It has also been shown that PI3K inhibitors, which prevent PKB activation, prevent increases in glucose uptake following T-cell activation.[32] However, it is now recognized that some of the PI3K inhibitors used to explore T-cell metabolism have off-target effects. For example, Ly294002 can inhibit mTOR and Pim family serine kinases.[33] There are also other problems with the concept that PKB is the central regulator of T-cell metabolism. For example, constitutively active PKB is effective at inducing survival of CD4 T cells but not CD8 T cells, indicating that the signals that control the metabolism of these two different lymphocyte subsets must differ.[34] Moreover, in CD4 T cells, deletion of PDK1, which is essential for PKB activation, causes a selective defect in

TCR and CD28 activation of nuclear factor (NF)-κB, but the ability of IL-7 to support survival of CD4 T cells is independent of PDK1.[35] There must, therefore, be differences between T-cell progenitors in the thymus and mature T-cell populations in terms of the role of PKB in controlling cell metabolism and survival. This could reflect redundancy between PKB and other AGC kinases and/or contribution of Pim serine kinases. Indeed, it has been shown that Pim kinases have a redundant role with mTOR to control survival of T cells.[36]

PDK1/protein kinase B control of T cell trafficking

The role for PI3K and mTOR in the regulation of T-cell metabolism is a role these kinases recapitulate in many cell lineages. However, there is emerging evidence for a second crucial function for these signaling molecules in T cells, namely to determine the repertoire of adhesion and chemokine receptors expressed by T lymphocytes and control T-cell trafficking.[7,37–39] In fibroblasts, PI3Ks regulate actin dynamics,[40] and, accordingly, there has been a past focus on the role of PI3Ks in regulating leukocyte chemokinesis and chemotaxis and there is indeed a reasonable volume of work showing that PI3Ks can control T-cell motility.[38] However, there is now very strong data that PI3K and mTOR control T-cell recirculation by regulating a Foxo-controlled signaling pathway that dictates the membrane expression of receptors that function to home T cells to secondary lymphoid tissue.[7,41–43]

The background to this discovery is that naive T lymphocytes continually recirculate around the body via the blood and lymphatic system and migrate into secondary lymphoid tissues, such as lymph nodes, by transendothelial migration in specialized high endothelial venules (HEVs). This lymph node entry process is coordinated by chemokine receptors, such as CCR7, adhesion molecules, such as CD62L (L-selectin), and integrins.[44–46] T cells thus move into secondary lymphoid tissue by responding to a gradient of CCR7 ligands, and the first step of transmigration is CD62L-mediated capture and rolling of naive lymphocytes on the endothelium of HEVs. CD62L and CCR7, which are constitutively expressed at high levels on naive T lymphocytes, are thus essential for the entry of these cells into peripheral lymph nodes. Importantly, immune activation of T cells induces striking changes in their migratory patterns. Effector T lymphocytes migrate to a greater extent to nonlymphoid tissues and sites of inflammation and have a reduced capacity to home to peripheral lymph nodes compared to naive and memory T cells.[47]

The changes in the trafficking behavior of activated T cells are important for immune responses and are mediated by changes in the expression of chemokine receptors and adhesion molecules. Effector T cells downregulate CCR7 and CD62L but upregulate expression of receptors that facilitate their homing to sites of inflammation, such as very late antigen 4, P- and E-selectin ligands, and inflammatory chemokine receptors, such as CXCR3 and CCR5.[46,48] The loss of CD62L and CCR7 by activated T cells is an important mechanism that prevents effector T cells re-entering secondary lymphoid organs and allows their redirection to peripheral tissues. Ectopic expression of CCR7 in effector T cells causes them to be retained in secondary lymphoid tissues and impairs T-cell effector function in peripheral tissues.[49] Similarly, perturbations of the normal cycles of CD62L expression have a significant impact on T-cell homing and migration.[50–53] Despite the importance of the dynamic changes in CD62L and CCR7 expression that accompany immune activation, it is only in the last year that the signal transduction pathways that control these processes have been defined and shown to be regulated by PI3K.

PI3K control of CD62L proteolytic cleavage

The expression of CD62L at the plasma membrane is controlled by a balance of two activities: the rate of CD62L gene transcription and the rate of CD62L proteolytic cleavage. The proteolytic cleavage and shedding of CD62L from the cell surface of T lymphocytes is an acute response to triggering of T-cell antigen receptors. This takes place proximal to the cell membrane and is mediated by TNF-converting enzyme (TACE)/disintegrin and metalloprotease (ADAM) 17.[50,53] One mechanism to control ectodomain shedding of proteins is mediated by extracellular signal-regulated kinases (ERKs)1/2, which phosphorylate the metalloprotease TACE/ADAM17 and control its trafficking to the cell surface.[54–56] In T cells, ERKs

activation by the TCR is dependent on the p110δ PI3K catalytic subunit that produces the $PI(3,4,5)P_3$ generated in response to TCR triggering.[57] In T cells where homologous recombination has been used to substitute wild-type p110δ for a catalytically inactive mutant ($p110\delta^{D910A}$), TCR triggering no longer activated ERKs and no longer induced shedding of CD62L from the T-cell membrane.[7] The blocking of CD62L proteolytic cleavage on T cells prevents T cells mediating anti-viral T-cell responses.[58] Accordingly, the key role for the p110δ PI3K catalytic subunit for this response would contribute to the necessary role for this signaling molecule for T-cell immune responses.

PI3K/mTOR control of CD62L gene transcription

Differentiated effector T cells are typically CD62L low, reflecting that these cells have terminated CD62L gene transcription.[59,60] Indeed the loss of CD62L is often used as a marker to distinguish naive, memory, and effector T cells during immune responses. For example, effector $CD8^+$ cytotoxic T lymphocytes (CTL) are CD62L low and preferentially home to sites of inflammation in peripheral tissues, whereas central memory CTL express high levels of CD62L similar to naive T cells and home to secondary lymphoid tissue.[47] Cellular immunologists had not questioned the molecular basis for the loss of CD62L by effector T cells until recently when it became clear that the expression of CD62L reports the PI3K and mTOR signaling status of a T cell and is not simply an epigenetic consequence of immune activation.[7] $PI(3,4,5)P_3$ and mTOR signaling are thus critical negative regulators of CD62L gene expression. Hence, effector T cells that have high levels of $PI(3,4,5)P_3$ and mTOR signaling express low levels of CD62L mRNA and protein. However, treatment of effector T cells with PI3K inhibitors or rapamycin, which inhibits mTORC1, restores expression of CD62L mRNA and plasma membrane expression of CD62L. Conversely, in naive T cells, the production of $PI(3,4,5)P_3$ is sufficient to terminate CD62L gene transcription and protein expression. $PI(3,4,5)P_3$ is normally dephosphorylated by the 3′ phosphatase PTEN to produce $PI(4,5)P_2$. The loss of PTEN in T cells results in accumulation of $PI(3,4,5)P_3$, and in naive antigen-inexperienced T cells this results in loss of CD62L.[7]

How do PI3K and mTOR control expression of CD62L? The answer to this question lies in the roles of these signaling molecules in controlling the expression of the transcription factor KLF2 that directly regulates CD62L gene transcription.[61–63] KLF2-null naive T cells do not express CD62L, fail to home to secondary lymphoid organs, and migrate to peripheral tissues.[62,63] In T cells that express low levels of $PI(3,4,5)P_3$, such as naive T cells, KLF2 levels are very high. In contrast KLF2 expression is downregulated in effector T cells that express high levels of $PI(3,4,5)P_3$. This is more than just correlation because effector T cells treated with PI3K inhibitors or mTORC1 inhibitors regain expression of KLF2. Furthermore, the constitutive production of $PI(3,4,5)P_3$ in PTEN-null naive T cells switches off KLF2 expression. In this context it is important to note that KLF2 not only controls CD62L gene transcription but also simultaneously controls expression of other key lymph node homing receptors, including CCR7 and the sphingosine 1 phosphate receptor (S1P1), which control T-cell entry and egress from lymph nodes, respectively.[61,62] The ability of high levels of PI3K signals to downregulate KLF2 thus acts as a switch to terminate expression of a network of important chemokine and adhesion receptors during immune activation.[7]

How do PI3K and mTOR control KLF2 expression?

The answer to this question is incomplete, although there is strong evidence that the Foxo family transcription factor Foxo1 is an integral component of the process.[41–43] Foxo1 is transcriptionally active in the nucleus of quiescent naive T cells, whereas following activation of PI3K there is a PKB-mediated phosphorylation of Foxo1 that drives it from the nucleus to the cytosol where it forms a complex with 14-3-3-bindng proteins.[13] The presence of active Foxo1 in the nucleus of quiescent T cells but not activated T cells is a perfect correlation with the pattern of KLF2 and CD62L expression. That this is more than a correlation was first suggested by elegant experiments that examined the impact of restoring Foxo1 transcriptional activity in PTEN-null T cells by expressing a mutant of Foxo1 that could not be phosphorylated by PKB.[41] These experiments identified KLF2, CD62L, and S1P1 as Foxo1-regulated genes. More recently is has been

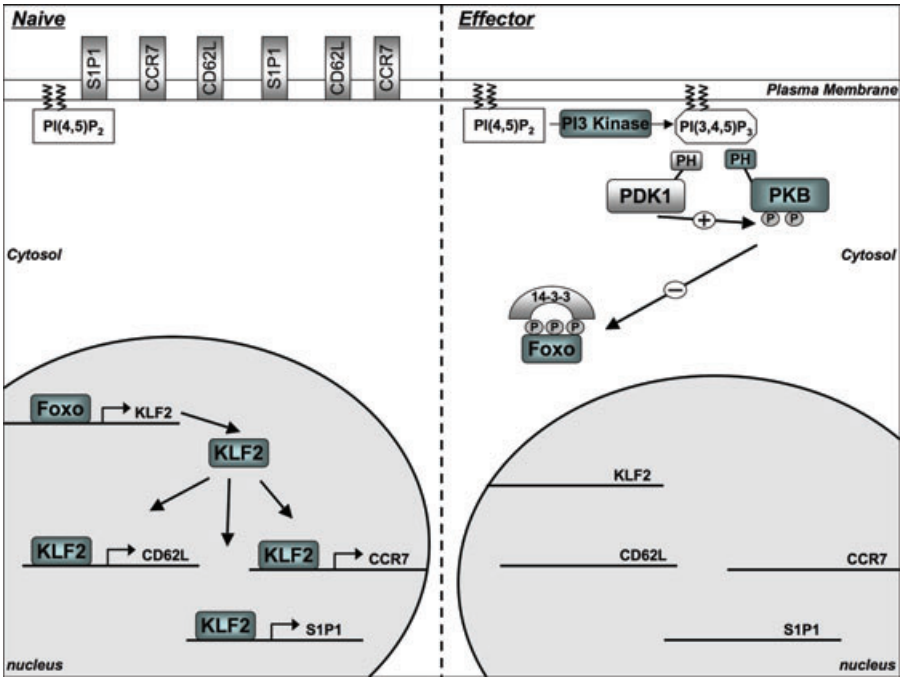

Figure 2. **PKB/Foxo-dependent regulation of KLF2 target genes. Naive T cells contain low levels of PI(3,4,5)P_3 and active PKB. In these cells Foxo transcription factors actively induce the expression of targets genes, such as KLF2. KLF2 in turn induces the expression of an array of genes that determine T-cell trafficking properties, CD62L, CCR7, and S1P1. In contrast, in effector T cells, activated PI3K maintains elevated levels of PI(3,4,5)P_3 and resultant activated PKB; PKB phosphorylates Foxo transcription factors on multiple residues, facilitating 14-3-3 binding, thus maintaining Foxos in the cytosol and repressing KLF2 expression and that of its target genes.**

shown that Foxo1-null T cells fail to express KLF2 and fail to express CD62L.[42] This explains how PI3K/PKB signaling pathways control expression of KLF2 and its target genes. In quiescent T cells, active Foxos drive expression of KLF2, whereas in response to PI(3,4,5)P_3 accumulation, PKB phosphorylation of Foxos occurs, which causes Foxos to be exported from the nucleus, hence terminating expression of KLF2 and its gene targets (Fig. 2).

What is the link between Foxo1 and mTOR in T cells that explains why expression of KLF2, CD62L, and CCR7 are controlled by mTOR?[7] There is as yet no answer to this question except to state that rapamycin treatment of effector CTL, which causes them to re-express KLF2, CD62L, and CCR7, has no discernable effect on PKB activity or Foxo phosphorylation. The molecular detail of mTOR control of KLF2 expression in effector T cells remains to be determined. However, it should be emphasized that mTOR regulation of CD62L and CCR7 expression may be important for the immunosuppressive actions of the mTORC1 inhibitor rapamycin, which is widely used clinically as an immunosuppressant. It was originally thought that rapamycin suppressed immune responses because of the role for mTOR as a nutrient sensor that controls protein synthesis and proliferation of T cells. However, it is now clear that rapamycin treatment restores expression of CD62L and CCR7 on effector CTL and re-directs their trafficking from peripheral tissue to lymph nodes and spleen.[7] The ability of rapamycin to redirect activated cytotoxic T cells to secondary lymphoid tissue could result in the containment of these cells within secondary lymphoid organs and prevent immune destruction of target cells in peripheral tissues. The regulation of lymphocyte trafficking, therefore, might contribute to the clinical efficacy of rapamycin in various immunosuppressive protocols.

Conflict of interest

The authors declare no conflicts of interest.

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