Phosphoinositide 3-kinase in T cell activation and survival

K. Okkenhaug*, A. Bilancio†, J.L. Emery* and B. Vanhaesebroeck†‡

*Laboratory of Lymphocyte Signalling and Development, Molecular Immunology Programme, Babraham Institute, Cambridge CB2 4AT, U.K.; †Cell Signalling Group, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1W 7BS, U.K., and ‡Department of Biochemistry & Molecular Biology, University College London, Gower Street, London WC1E 6BT, U.K.

Abstract

PI3Ks (phosphoinositide 3-kinases) regulate diverse signalling pathways involved in growth, proliferation, survival, differentiation and metabolism. In T cells, PI3Ks can be activated by a number of different receptors, including the TcR (T cell receptor), co-stimulatory receptors, cytokine receptors and chemokine receptors. However, the specific roles of PI3Ks downstream of these receptors vary. An inactivating mutation in the leucocyte-specific PI3K isoform p110δ results in impaired TcR-dependent proliferation under circumstances where CD28 co-stimulation is blocked or not required. Recruitment and activation of PI3K by CD28 promotes survival by inducing increased expression of Bcl-xL. However, CD28 engages additional signals that regulate proliferation and interleukin-2 production independently of PI3K. Thus a model emerges whereby PI3K is involved in both TcR and CD28 signalling, but each receptor may only exploit a subset of the signalling pathways potentially controlled by PI3K activation.

Introduction

The activation of T cells is initiated upon engagement of the TcR (T cell receptor) by antigenic peptides presented by MHC proteins. In the thymus, T cells with moderate affinity for MHC–self-peptide complexes are selected for further development, whereas T cells with too high or too low affinity for MHC–self-peptide complexes are eliminated [1]. Immature T cells express both CD4 and CD8 receptors, which bind MHC II and MHC I molecules respectively. T cells selected by MHC class I molecules lose the expression of CD4 to become CD8+ T cells, whereas those selected by MHC II molecules lose the expression of CD8 to become CD4+ T cells. The T cells that survive selection in the thymus migrate to the lymph nodes and the spleen, where they interact with specialized APCs (antigen-presenting cells) known as dendritic cells [2]. Upon infection, these APCs ingest and kill the pathogenic organism, digest its proteins into small peptide fragments and present some of these in the context of MHC molecules. For any given pathogen-derived peptide presented, there will be a small subset of T cells that can bind this peptide–MHC complex with an affinity that is sufficiently high that the T cell can distinguish it from self peptides expressed by APCs. The antigen-sensing mechanism of the TcR thus needs to be exquisitely fine tuned in order to distinguish relatively minor differences in affinity and/or avidity. However, presentation of a foreign peptide is not usually sufficient to activate T cells. Dendritic cells also express co-stimulatory ligands, such as CD80 and CD86, which bind CD28 on the T cell surface. The expression of co-stimulatory ligands on the dendritic cells is regulated by Toll-like receptors that recognize conserved microbial molecules not expressed by the host and thus help the immune system to distinguish infectious non-self from non-infectious self [3]. The signals from the co-stimulatory receptors are integrated by the T cell with those from the TcR to initiate gene transcription, cell growth and mitosis. To maintain homeostasis and to prevent T cells with any particular antigen specificity from dominating the repertoire, the majority of T cells are programmed to die after several rounds of division [4]. The few T cells that escape this elimination by apoptosis can persist to provide lifelong immunity against re-infection [5]. In this review, we describe recent investigations into how the TcR and CD28 exploit class IA PI3K (phosphoinositide 3-kinase) activation to influence the response of T cells to antigen stimulation.

TcR signalling and the involvement of PI3K

TcR signalling is initiated by the cytosolic tyrosine kinases Lck and Fyn, which phosphorylate ITAMs (immunoreceptor tyrosine-based activation motifs) in the CD3 invariant chain with which the TcR is associated on the plasma membrane [6,7]. This leads to the SH2 (Src homology 2) domain-mediated recruitment of ZAP-70 (ζ-associated protein, 70 kDa), which phosphorylates the transmembrane adapter protein LAT (linker for activation of T cells) [8]. Grb2, GADS (Grb2-related adaptor downstream of Shc) and PLCγ (phospholipase Cγ) have SH2 domains that bind phosphotyrosine residues in LAT, and phosphorylation of these tyrosines is essential for the TcR to transmit signals to the nucleus [9]. TcR engagement also leads to the activation...
Role of p110δ in T cell activation

The leucocyte-restricted expression of p110δ suggested a unique role in immune receptor signalling. To investigate this possibility, a loss-of-function mutation (D910A) was introduced in the p110δ gene [31]. Indeed, impaired TcR- and CD28-dependent PKB phosphorylation in p110δ(D910A) T cells indicated that p110δ is the main PI3K downstream of these receptors [31]; A. Bilancio, unpublished work). T cell development in the thymus appeared to progress normally, and both CD4+ and CD8+ T cells were found in the spleens and lymph nodes of p110δ(D910A) mice, albeit in reduced numbers. However, the T cells showed reduced CD44 expression, which may be indicative of impaired responsiveness to antigen in vivo. Consistent with this notion, TcR transgenic p110δ(D910A) T cells showed reduced proliferation and interleukin 2 production in response to stimulation by APCs bearing peptide–MHC complexes in vitro [31]. CD28 contributes to the recruitment of lipid rafts, which are rich in associated signalling proteins, to the area of contact between the TcR and the APC [32]. This process is thought to help amplify signals through the TcR. Such raft recruitment stimulated by anti-CD3- and anti-CD28-coated beads was impaired in p110δ(D910A) T cells [31]. However, while p110δ(D910A) T cells showed reduced proliferation in response to stimulation with beads coated only with anti-CD3, proliferation and interleukin 2 production in response to anti-CD3- and anti-CD28-coated beads was unaffected, if not enhanced [31]. It is possible that the high-affinity stimulus provided by antibodies to CD3 and CD28 abrogated any need for signal amplification via raft recruitment, but that such raft recruitment plays a more important role in responses to stimulation with peptide–MHC complexes. Together, these results indicated that the requirement for PI3K activation during T cell activation may be greatest in the context of low-affinity stimuli provided by peptide–MHC complexes in conjunction with sub-optimal co-stimulation. However, in contrast with the LAT-associated signalling proteins, PI3K is probably not an essential component of the TcR signalling complex.

Role of PI3K in CD28 co-stimulation

Upon T cell activation, CD28 becomes tyrosine phosphorylated, providing a docking site for the SH2 domains of PI3K [10,11]. The p85 regulatory subunit of class IA PI3Ks has SH2 domains that probably do not bind directly to LAT, but which bind to a second transmembrane adapter protein called TRIM (TcR-interacting molecule) [12]. It is not yet known to what extent TRIM phosphorylation is required for TcR signals, nor is it known whether TRIM phosphorylation is required for PI3K activation by the TcR. The cytosolic adapter proteins Cbl and Gab2 also contain tyrosine residues to which p85 can bind, but these interactions seem more likely to negatively regulate PI3K signalling [13,14]. The p85 subunit contains additional domains that may contribute to the recruitment and regulation of PI3K activity: Rac can bind the p85 Rho-GAP (GTPase-activating protein) domain [15,16], and Lck and Fyn may interact with proline-rich sequences in p85 via their SH3 domains [17,18]. Upon recruitment of p85/p110 to the membrane, Ras may bind directly to the Ras binding domain of p110 and modulate PI3K activity further [19,20]. However, the precise steps leading to PI3K activation after engagement by the TcR have yet to be mapped out. In particular, it remains to be determined whether several partially redundant mechanisms work to activate PI3K, or whether this is a carefully orchestrated procedure with one or more essential upstream components.

PI3Ks synthesise the phosphorylation of PtdIns(4,5)P₂ on the D3 hydroxy group to yield PtdIns(3,4,5)P₃, a second messenger signalling molecule that remains tethered to the lipid bilayer [21]. Canonical pathways downstream of PI3K are regulated by the PtdIns(3,4,5)P₃-dependent recruitment of PDK1 (phosphoinositide-dependent kinase 1) and PKB (protein kinase B) to the plasma membrane [22]. These enzymes phosphorylate other proteins on serine and threonine residues to regulate metabolism, cell growth, mitosis and apoptosis. Itk is a Tec family tyrosine kinase which is selectively expressed in T cells and which has been implicated in PI3K-dependent regulation of calcium signalling by virtue of its capacity to phosphorylate PLCγ [23]. PI3Ks may also regulate cytoskeletal changes by activating GEFs (guanine nucleotide exchange factors), some of which have PtdIns(3,4,5)P₃-specific pleckstrin homology domains [24]. GEFs catalyse the conversion of small GTTPases, such as Rh, Rac and Cdc42, from the inactive GDP-bound form into the active GTP-bound form. IBP (IRF-4-binding protein; also known as SLAT (SWAP17-like adaptor of T cells)] is a recently described T cell-restricted GEF that regulates Rac and Cdc42 in a PI3K- and Lck-dependent fashion [25]. Hence PI3Ks have the potential to regulate numerous signalling pathways in T cells.

Four class I PI3K isofoms

Of the three known isofoms of class IA PI3K catalytic subunits, p110α and p110β are expressed in most cell types, whereas the expression of p110δ is largely restricted to the leucocyte lineages. Both p110α and p110β are essential for embryonic development, which has thus precluded direct investigation of their role in T cell activation [26,27]. The class IB PI3K isofom p110γ is regulated by G-protein-coupled receptors and is probably not directly involved in antigen receptor signalling, although it may promote the survival of T cells via other ligand/receptor systems, especially in the thymus [28,29]. PI3K signalling is antagonized by the lipid phosphatases Pten (phosphatase and tensin homologue deleted on chromosome 10) and SHIP (SH2-containing inositol phosphatase), which dephosphorylate PtdIns(3,4,5)P₃ on the D3 and D5 positions respectively. Conditional knockout of Pten expression in T cells results in a lethal CD4-mediated lymphoproliferative disease as a consequence of impaired T cell selection in the thymus and enhanced resistance of peripheral T cells to apoptotic stimuli [30]. Thus PI3K is an important regulator of T cell homeostasis.
Although the TcR may regulate PI3K recruitment via phosphorylation of TRIM, it remains unclear precisely how the TcR is coupled to PI3K activation. Available evidence suggests an important role for PI3K in transmitting signals that regulate proliferation and differentiation upon antigen engagement of the TcR. In contrast, CD28 binds directly to the SH2 domains of p85, but promotes proliferation and cytokine production independently of this association. However, CD28 activation of PI3K promotes survival, contributing to the up-regulation of Bcl-X\(_L\) expression. Experiments described in the text suggest that the TcR exploits PI3K activation to regulate proliferation, whereas CD28 requires PI3K to promote survival. This differential utilization of PI3K signalling is indicated by thick compared with dotted lines.

of p85. As such, CD28 had been thought to exploit, rather than circumvent, PI3K signalling [10]. Is there a role for PI3K in CD28 signalling despite the robust responses of p110\(_{\delta}\)(D910A) T cells to CD28 co-stimulation? The immune system of CD28 knockout mice is deficient in several important aspects. The T cells provide inadequate help to the B cells after infection or immunization, such that the B cells fail to form germinal centres and undergo immunoglobulin class switching [33]. As a consequence, while T cell-independent IgM responses are intact, T cell-dependent IgG responses are impaired [34]. To evaluate the role of PI3K in transmitting the CD28 co-stimulatory signal, CD28 knockout mice were reconstituted with a tyrosine-to-phenylalanine (Y170F) mutant of CD28 that cannot bind PI3K [35]. The CD28(Y170F) mutant restored T cell-dependent immune responses, thus indicating that PI3K activation is not essential for the CD28 co-stimulatory signal. Consistent with the results obtained with p110\(_{\delta}\)(D910A) mice, CD28(Y170F) T cells showed unimpaired proliferation and interleukin 2 secretion in response to stimulation with anti-CD3 and anti-CD28. However, T cells expressing the CD28(Y170F) mutant were impaired in other important ways: the PI3K binding site was required for the CD28-dependent induction of Bcl-X\(_L\) expression [35,36]. However, the activation of PI3K is apparently not sufficient to drive Bcl-X\(_L\) expression, because ICOS (inducible co-stimulatory protein), a co-stimulatory receptor that is related to CD28 and which appears to bind PI3K with greater efficiency than does CD28, failed to stimulate increased Bcl-X\(_L\) expression [37]. Therefore, both PI3K-dependent and PI3K-independent signals may be integrated to promote Bcl-X\(_L\) expression in response to CD28 co-stimulation. It is feasible that even though co-stimulation for primary immune responses occurred independently of the CD28/PI3K/Bcl-X\(_L\) pathway, the survival signals provided by Bcl-X\(_L\) could play a role in the long-term survival of memory cells. There is also evidence
suggesting that CD28 regulates glucose uptake and glycolysis via PI3K, with obvious implications for T cell homeostasis [38].

Concluding remarks

In conclusion, p110δ contributes to the response of T cells to exposure to foreign antigen. However, CD28 provides a signal that can surpass the requirement for p110δ to promote T cell proliferation, although the capacity of CD28 to promote survival is PI3K dependent. These results indicate that, despite forming part of the same signalling complex, the TcR and CD28 differentially exploit PI3K activation to drive cell proliferation and survival (Figure 1). Recent progress in analysing PI3K signalling in T cells at the single-cell level should facilitate investigation into the relative contributions of different receptors to PI3K activation in response to antigen [39–42]. Moreover, although CD28 signalling can to some extent overcome the requirement for PI3K signalling of different receptors to PI3K activation in response to anti- gen, it is unlikely that T cells activated independently of PI3K develop normally in other aspects, or indeed are as long lived.

We thank Hanneke Okkenhaug for critical reading of the manuscript. K.O. is the recipient of a BBSRC David Phillips Fellowship. Research in the laboratory of B.V. is supported by the Ludwig Institute for Cancer Research, Diabetes UK, the BBSRC and the European Union Framework V Programme (QLG1-2001-02171). A.B. is supported in part by the Fondazione Italiana per la Ricerca sul Cancro.

References


Received 12 November 2003