The PI3K/Akt/mTOR pathway in innate immune cells: emerging therapeutic applications

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ABSTRACT

The phosphatidylinositol-3 kinases (PI3Ks) and the mammalian target of rapamycin (mTOR) pathway have long been recognised as critically regulating metabolism, growth or survival. Recent data indicate that these molecules are also integral players in coordinating defence mechanisms in the innate immune system. In this respect, PI3K and mTOR positively regulate immune cell activation in neutrophils and mast cells. In plasmacytoid dendritic cells, these pathways have recently emerged as important regulators for type I interferon production via activation of the interferon-regulatory factor 7. Interestingly, in myeloid immune cells, PI3K and mTOR seem to constrain full immune cell activation by upregulation of the key anti-inflammatory cytokine interleukin 10 and inhibition of proinflammatory cytokines. These new insights into innate immune cell regulation may pave the way for manipulating distinct features of the innate immune system for therapeutic treatment of various inflammatory diseases and for implementation of improved vaccination strategies.

The phosphatidylinositol-3 kinases (PI3Ks) as well as the mammalian target of rapamycin (mTOR) pathways are two key cellular signalling pathways that affect broad aspects of cellular functions, including metabolism, growth and survival.[1 2] Although initially viewed as two separate pathways, it has been shown that PI3K and mTOR signalling are connected via the serine/threonine kinase Akt.[3] Akt, also termed PKB (protein kinase B), is one of the most important survival kinases involved in regulating a similarly wide array of cellular processes as PI3K and mTOR, including metabolism, growth, proliferation and apoptosis.[4]

The PI3K/Akt/mTOR pathway has been long known to be important in regulating adaptive immune cell activation. For example, different PI3K heterodimers, but also mTOR, critically control cell survival, proliferation, B- and T-cell receptor (BCR and TCR, respectively) signalling and chemotaxis in B and T lymphocytes.[5 6] However, during recent years, it has increasingly been recognised that the PI3K/Akt/mTOR pathway has broad and yet distinct roles also in innate immune cells, including neutrophils, mast cells, monocytes, macrophages and myeloid as well as plasmacytoid dendritic cells (mDCs and pDCs, respectively).

THE PI3K/AKT/mTOR PATHWAY

The PI3K/mTOR pathway is activated by a broad array of different stimuli via specific receptors, including the BCR, TCR, cytokine receptors (eg, interleukin (IL)2), insulin receptor, insulin-like growth factor I receptor, but also Toll-like receptors (TLRs). Stimulation of these pathways activates tyrosine kinase adaptor molecules on the cell membrane leading to the recruitment of the class I family of PI3K to the receptor complex.[7] Class I PI3K are heterodimeric lipid kinases that phosphorylate the 3-hydroxy group of phosphatidylinositol and related inositol phospholipids and contain a regulatory subunit (p85α or p85β) and a catalytic subunit (p110α, p110β or p110δ).[7] p110γ is also a member of the class I PI3K subfamily, but is mainly activated from G protein-coupled receptors.[8] PI3Kγ and PI3Kδ are preferentially expressed in cells of haematopoietic origin, whereas expression of PI3Kα and PI3Kβ is ubiquitous.[1] After receptor engagement, PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3) as second messenger to recruit and activate downstream targets including Akt (fig 1). The tumour suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a lipid phosphatase and dephosphorylates PIP3 to negatively regulate PI3K signalling.[1]

One main effector of PI3K and Akt is the high molecular weight kinase mTOR, which together with PI3K belongs to the family of phosphatidylinositol kinase-related kinases (PIKK). Interestingly, mTOR is a serine/threonine protein kinase instead of a lipid kinase. mTOR, which is also known as FKBP (FK506-binding protein) 12-rapamycin-associated protein (FRAP), is phosphorylated in vitro and in vivo, although the significance for its activation is not completely understood.[9 10] mTOR controls protein synthesis through the direct phosphorylation and inactivation of the repressor of mRNA translation, eukaryotic initiation factor 4E-binding protein 1 (4E-BP1 or PHAS-I) and through phosphorylation and activation of S6 kinase (S6K1 or p70S6K).[11] The macrolide rapamycin forms a complex with FKBP12 and as a complex inhibits the activity of the mTOR/Raptor complex (fig 1 and see below).[12 13] Cytokines, growth factors, amino acids or insulin activate mTOR and dramatically increase the phosphorylation status of 4E-BP1 and S6K1 in a rapamycin-sensitive manner.[11] Loss of mTOR function leads to an arrest in the G1 phase of the cell cycle along with a severe reduction in protein synthesis.

Recent data demonstrate that mTOR is in a complex with various proteins. Raptor, a conserved 150 kDa protein, which recruits S6K1 and 4E-BP1, forms a rapamycin-sensitive complex with mTOR and the adaptor protein mLST8 named mTORC1.[14 15] Rapamycin inhibits mTORC1

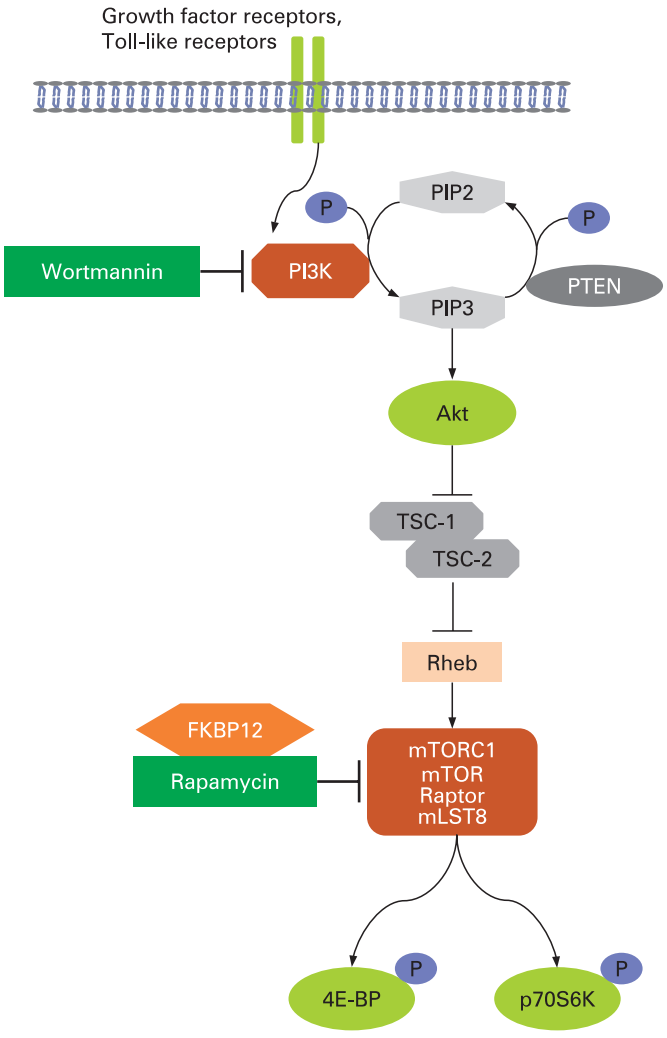

Figure 1 The PI3K/Akt/mTOR pathway. Akt, also termed PKB (protein kinase B); 4E-BP, 4E-binding protein; FKBP12, FK506-binding protein 12; mTOR, mammalian target of rapamycin; mTORC1, a rapamycin-sensitive complex with mTOR and the adaptor protein mLST8; PI3K, phosphatidylinositol-3 kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; p70S6K, a protein serine/threonine kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; Rheb, Ras homologue enriched in brain; TSC, tumour suppressor complex

activity by blocking its interaction with Raptor.[16] More recently, the Hall group identified a second mTOR complex (mTORC2), which constitutes the adaptor protein Rictor and Sin1 instead of Raptor.[17] This mTORC2 complex is insensitive to rapamycin and is thought to regulate actin cytoskeleton and has recently been shown to control Akt Ser 473 phosphorylation.[18] Interestingly, long-term treatment with rapamycin (>18 h) alters the mTORC1/C2 equilibrium resulting in reduced mTORC2 levels, thereby also leading to impaired Akt signalling.[19] Recently, is was shown that the GTPase Rheb (Ras homologue enriched in brain) is essential for mTOR-mediated phosphorylation of S6K1.[20 21] These results indicate that Rheb is a positive regulator of mTORC1 acting downstream of PI3K, Akt and the tumour suppressor complex (TSC-1/2), which itself negatively regulates activation of mTORC1.[22 23] Collectively, receptor engagement leads to a coordinated activation of PI3K/Akt, TSC-1/2 and Rheb, which are integrated at the level of mTORC1. The functional role of mTORC2 within these signalling circuits, however, is far from understood.[24]

THE PI3K/mTOR PATHWAY IN NEUTROPHILS

Neutrophils are terminally differentiated cells that play a vital role in host defence.[25] Neutrophils are attracted by cytokines and quickly move to the focus of an infection. They can kill micro-organisms via phagocytosis, degranulation and oxidative burst.[25] It has recently become evident that PI3K and mTOR play import roles in many neutrophil functions (fig 2).[26 27] For example, PI3Kγ-deficient neutrophils exhibit severe defects in migration in response to heterotrimeric GTP-binding protein (G protein)-coupled receptor (GPCR) agonists and chemotactic agents.[28] Similarly, the PI3K inhibitors wortmannin and LY294002 inhibit IL8-induced cell migration of human neutrophils.[29] Moreover, PI3K is a main regulator of neutrophil phagocytosis, especially during engulfment and for the internalisation of large particles.[26] Granulocyte monocyte-colony-stimulating factor (GM-CSF)-mediated priming of formyl methionyl leucyl phenylalanine-induced respiratory burst is dependent on PI3K.[30] Likewise, chemoattractant-stimulated PI3Kγ−/− neutrophils display an impaired respiratory burst.[31]

Similarly, some evidence suggests a role of the mTOR pathway in neutrophils. For example, activation of neutrophils induces translation of the pre-existing mRNA of retinoic acid receptor (RAR)-α, a vital transcription factor for many neutrophil genes.[32] Interestingly, rapamycin specifically inhibits RAR-α translation to modulate IL8 production. A similar mechanism of rapamycin-sensitive activation-induced translation of pre-existing IL6 receptor mRNA and urokinase plasminogen activator receptor mRNA has been described in these cells, suggesting an important role of signal-dependent translation in activated neutrophils to regulate immune responses.[27] Moreover, GM-CSF, which is a chemoattractant for neutrophils, induces phosphorylation of p70S6K. Phosphorylation of p70S6K as well as migration are suppressed by mTOR inhibition

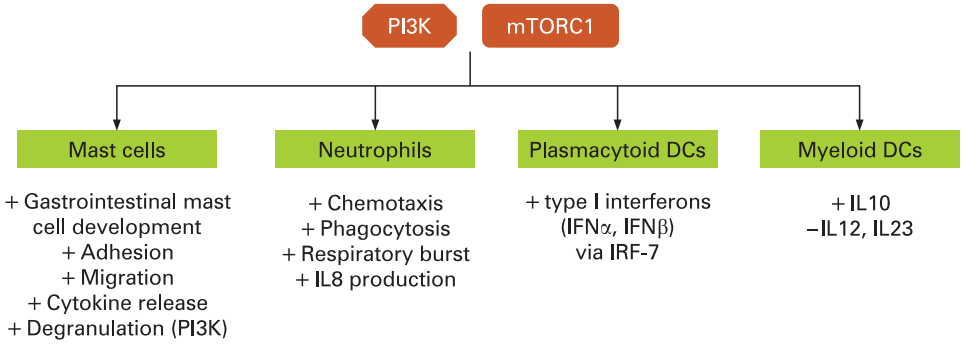

Figure 2 The divergent positive (+) and negative (−) regulatory roles of PI3K and mTOR in distinct immune cells. DCs, dendritic cells; IFN, interferon; IL, interleukin; IRF, IFN regulatory factor; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol-3 kinase.

with rapamycin.[33] Hence, PI3K and mTOR control and affect many crucial immunomodulatory functions of neutrophils.

MAST CELLS

Mast cells are important effector cells mast that not only regulate type IV hypersensitivity reactions but also many tissue functions, such as blood flow and coagulation, smooth muscle contraction and peristalsis of the intestine, mucosal secretion, wound healing, regulation of innate and adaptive immune responses and peripheral tolerance.[34] The development of gastrointestinal, but not dermal mast cells is dependent on signalling mediated by class IA PI3Ks (fig 2).[35] Genetic or pharmacological inactivation of the p110δ isoform of PI3K in mast cells leads to defective stem cell factor (also known as Kit ligand)-mediated in vitro proliferation, adhesion and migration and to impaired allergen-IgE-induced degranulation and cytokine release.[36] Importantly, inactivation of p110δ protects mice against anaphylactic allergic responses. Similarly, the p110γ and p110δ isoforms of PI3K control in vitro degranulation of mast cells induced by cross linking of the high-affinity receptor of IgE (FcεRI).[37] In vivo, however, only p110δ is required for optimal IgE/Ag-dependent hypersensitivity responses in mice.[37]

Little is known about the role of mTOR in mast cell homoeostasis and function. Stimulation via FcεRI or kit results in a PI3K-dependent activation of the mTORC1 pathway.[38] Interestingly, rapamycin inhibits cytokine production, Kit-mediated chemotaxis and cell survival, but it has no effect on FcεRI-mediated degranulation or Kit-mediated cell adhesion.[38] These data suggest that mTORC1 is a point of divergence for the PI3K-regulated downstream events of FcεRI and Kit for the selective regulation of mast cell functions.

PLASMACYTOID DCs

Human and mouse pDCs constitute a specialised cell population that produce large amounts of type I interferons in response to viruses via activation of TLRs.[39] Stimulation of TLR7 by influenza virus or TLR9 via CpG oligonucleotides activates PI3K in primary human pDCs.[40] By using specific inhibitors, Guiducci *et al* could demonstrate that PI3Kδ is critical for type I interferon (IFN) production by pDCs but not for other proinflammatory responses, including tumour necrosis factor (TNF)α and IL6 production, DC differentiation and uptake as well as endosomal trafficking of TLR ligands.[40] Mechanistically, PI3K inhibition prevents the nuclear translocation of IFN regulatory factor (IRF)-7, the main transcription factor for type I IFN production in pDCs (fig 2).

Furthermore, Colina *et al* provided compelling evidence that activation of IRF-7 in pDCs depends on the 4E-BP1 pathway.[41] They showed that in mouse embryonic fibroblasts and pDCs lacking 4E-BP1 and 4E-BP2, the production of type-I IFN is enhanced after TLR stimulation. Consequently, replication of encephalomyocarditis virus, vesicular stomatitis virus, influenza virus and sindbis virus is suppressed. The enhanced type-I IFN response in 4E-BP1−/− 4E-BP2−/− double-knockout mouse embryonic fibroblasts is caused by upregulation of IRF-7 mRNA translation indicating that 4E-BPs might be negative regulators of type-I IFN production via translational repression of IRF-7 mRNA. Although not formally shown, these results together with data of Guiducci *et al* indicate that activation of PI3K enhances mTOR activity, which inhibits 4E-BP1 to stimulate type I IFN production in pDCs via enhanced translation of IRF-7.

MONOCYTES, MACROPHAGES AND mDCs

A growing body of evidence indicates that in monocytes, macrophages and mDC PI3K is crucially implicated in TLR signalling and may serve as a possible "safety mechanism" to control the cellular response to pathogens mainly by limiting proinflammatory cytokine production (eg, IL12) and enhancing the synthesis of the anti-inflammatory IL10 (fig 2).[42–45] Pharmacological or genetic disruption of PI3K results in excessive IL12 production in murine splenic DCs.[43] Interestingly, overproduction of this cytokine in *Leishmania major*-infected PI3K p85−/− mice leads to a healing phenotype. Moreover, pharmacological inhibition of PI3K in a murine cecal ligation and puncture-induced polymicrobial sepsis model increases mortality caused by an amplified production of proinflammatory cytokines, including IL1β, IL6, IL12 and TNFα, supporting the PI3K pathway as a negative inflammatory feedback regulator.[45] Conversely, pharmacological activation of PI3K by glucan phosphate significantly prevents mortality in this model. Furthermore, stimulation of monocytes with *Porphyromonas gingivalis* lipopolysaccharide (LPS) during suppression of PI3K activity leads to increased IL12, but suppressed IL10 synthesis.[46] As a molecular mechanism Fukao *et al* suggested that PI3K selectively controls p38 activation in myeloid DCs,[43] while Martin *et al* demonstrated a selective suppression of ERK-1/2 phosphorylation in human monocytes.[46] Guha and Mackman reported that PI3K inhibition in peripheral blood mononuclear cells and in the monocytic cell line THP-1 upregulates LPS-induced TNFα and tissue factor production via JNK, p38, Erk and the proinflammatory master transcription factor NF-κB.[44]

Recently, Polumuri *et al* demonstrated that FcγR ligation after TLR4 engagement in murine macrophages activates the PI3K/Akt pathway, inhibits IL12 and promotes IL10 production that is reversed by PI3K inhibition.[47] Interestingly, PI3K controls nuclear translocation of IkBα, leading to inhibition of Rel family members binding to the NF-κB site within the IL12 promoter.[47] Collectively, mounting evidence indicates that the PI3K/Akt pathway is a major regulator of innate immunity by controlling the production, accumulation and binding of central transcription factors required for the production of key inflammatory cytokines like IL12 and IL10. However, PI3K might regulate different signalling pathways depending on the cell type and the organism and in some cases even positively regulate some inflammatory mediators.[8] Clearly, further work is needed to integrate these phenomena into a unifying model.

The role of mTOR was investigated in a limited set of studies. Some investigators reported that rapamycin inhibits DC function at various levels.[48] Notably, the DCs employed in those studies were generated in vitro from human peripheral monocytes cultured with GM-CSF and IL4. Of note, rapamycin disrupts the signalling pathways of both GM-CSF and IL4 in diverse myeloid cells like neutrophils, macrophages and DCs.[49–51] However, data from freshly isolated untouched cells support a role for mTOR as negative feedback regulator similar to PI3K in monocytes. In the presence of rapamycin, human peripheral blood mononuclear cells increase IL12 secretion after stimulation with *Staphylococcus aureus* cells, data that were confirmed by Tsiavou *et al* employing intracellular IL12p40-staining in LPS-activated human monocytes.[52 53] Moreover, rapamycin abrogates IL10 production in human monocytes after stimulation with both HIV-1 Nef protein and the HIV transmembrane protein gp41 suggesting that HIV may foster IL10-mediated immunosuppression by innate immune cells via activation of S6K1.[54–56] This reciprocal cytokine regulation is of considerable

biological importance, since IL10 exerts an essential counter-regulatory role during inflammatory responses.[57 58] Moreover, mTOR downregulates IL23 production in human macrophages.[59] In conclusion, several reports point to a pivotal regulatory role of mTOR in innate immune cells for a proinflammatory versus an anti-inflammatory cytokine commitment. Further mechanistic insights into the precise molecular mechanisms underlying this skewing of the cytokine profile are of substantial importance for the regulation of immunity in cancer, allergies, autoimmune diseases or infectious diseases like HIV, tuberculosis and listeriosis.

PHARMACOLOGICAL INHIBITION OF PI3K AND mTOR: EMERGING PRECLINICAL AND CLINICAL DATA

In contrast to PI3K inhibitors, mTOR inhibitors are already prescribed in the clinic. Rapamycin, the prototypic inhibitor of mTOR and its clinically evaluated derivates like RAD001, CCI-779 or AP23573 have potent immunosuppressive and anti-tumour activities by preventing proliferation and cell-cycle progression.[60 61] Moreover, rapamycin is also currently being evaluated for the treatment of tuberous sclerosis and lymphangioleiomyomatosis.[62] The cell-cycle arrest induced by rapamycin might in part explain its potent anti-tumour action, including antimetastatic and antiangiogenic effects.[63 64] Owing to the exquisite sensitivity of T cells, rapamycin treatment was introduced in clinical transplantation and is currently employed as an alternative immunosuppressive treatment to ameliorate chronic allograft damage.[65] However, distinct proinflammatory side effects such as lymphocytic alveolitis, interstitial pneumonitis and also de novo glomerulonephritis have been recognised with the extended use of rapamycin in transplantation including pulmonary and renal inflammatory disorders despite the concurrent use of other immunosuppressive and anti-inflammatory drugs.[66–69] Similarly, anaemia associated with chronic inflammation is seen in rapamycin-treated renal transplant patients along with enhanced proinflammatory cytokines and defective induction of the anti-inflammatory cytokine IL10.[70] While the precise molecular mechanisms underlying these inflammatory conditions await further study, it is tempting to hypothesise that deactivating negative regulatory pathways of the innate immune system upon inhibition of mTOR may be causally linked to these clinical observations.

mTOR inhibitors have shown promising results in advanced clinical trials against certain malignancies like renal cell carcinoma, mantle cell lymphoma and endometrial cancers.[71 72] Upon mTOR inhibition, the altered cytokine milieu in primary innate immune cells characterised by increased production of the anti-tumour cytokine IL12 and ablation of IL10 might foster escape from immunosurveillance. These observations may help to better understand the anti-tumour potency of mTOR inhibitors.

The development of PI3K inhibitors for clinical use has been hampered by their high toxicity, as conventional inhibitors like wortmannin or LY294002 do not discriminate between different PI3K isoforms, and PI3Ks are crucial for all organ systems. Moreover, these inhibitors block many PI3K-related kinases such as mTOR, DNA-PKcs, ATM, ATR or PtdIns-4-kinase (type 3).[73] However, with the design of isoform-specific PI3K inhibitors, it was possible to show in proof-of-concept studies that selective PI3Kγ inhibitors alleviate disease progress in murine models of rheumatoid arthritis and systemic lupus erythematosus. For example, AS-252424, AS-604850 and AS-605240 block neutrophil chemotaxis in vivo and minimise joint destruction in passive models of rheumatoid arthritis.[74] Similarly, PI3Kγ inhibition can block glomerulonephritis and extend lifespan in a mouse model of systemic lupus.[75] Moreover, PI3K inhibition is well tolerated in mice and results in greater efficiency in comparison with the reference drug dexamethasone. Hence, murine models encourage the clinical development of PI3Kγ inhibitors for the treatment of systemic lupus erythematosus.[73] Many other isoform-specific PI3K inhibitors are currently in preclinical and clinical development for the treatment of cancer, inflammation or coronary heart disease (for an excellent review see Marone *et al*[73]).

In conclusion, the importance of the PI3K/Akt/mTOR pathway for many immunological defence mechanisms is increasingly recognised not only for the adaptive immune system, but also for innate immune cells. New discoveries for critical roles of PI3K and mTOR in monocytes, DCs and mast cells can be expected, which may open new therapeutic possibilities for the treatment of many inflammatory diseases.

Competing interests: None.

REFERENCES

1. **Deane JA,** Fruman DA. Phosphoinositide 3-kinase: diverse roles in immune cell activation. *Annu Rev Immunol* 2004;**22**:563–98.
2. **Wullschleger S,** Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;**124**:471–84.
3. **Sekulic A,** Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, *et al*. A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res* 2000;**60**:3504–13.
4. **Brazil DP,** Yang ZZ, Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. *Trends Biochem Sci* 2004;**29**:233–42.
5. **Koyasu S.** The role of PI3K in immune cells. *Nat Immunol* 2003;**4**:313–9.
6. **Okkenhaug K,** Vanhaesebroeck B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* 2003;**3**:317–30.
7. **Fruman DA.** Towards an understanding of isoform specificity in phosphoinositide 3-kinase signalling in lymphocytes. *Biochem Soc Trans* 2004;**32**:315–9.
8. **Hazeki K,** Nigorikawa K, Hazeki O. Role of phosphoinositide 3-kinase in innate immunity. *Biol Pharm Bull* 2007;**30**:1617–23.
9. **Brown EJ,** Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL. Control of p70 s6 kinase by kinase activity of FRAP in vivo. *Nature* 1995;**377**:441–6.
10. **Jacinto E,** Hall MN. Tor signalling in bugs, brain and brawn. *Nat Rev Mol Cell Biol* 2003;**4**:117–26.
11. **Hay N,** Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;**18**:1926–45.
12. **Chiu MI,** Katz H, Berlin V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci USA* 1994;**91**:12574–8.
13. **Sabatini DM,** Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 1994;**78**:35–43.
14. **Hara K,** Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, *et al*. Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* 2002;**110**:177–89.
15. **Kim DH,** Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, *et al*. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 2002;**110**:163–75.
16. **Oshiro N,** Yoshino K, Hidayat S, Tokunaga C, Hara K, Eguchi S, *et al*. Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells* 2004;**9**:359–66.
17. **Jacinto E,** Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, *et al*. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* 2004;**6**:1122–8.
18. **Sarbassov DD,** Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005;**307**:1098–101.
19. **Sarbassov DD,** Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, *et al*. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 2006;**22**:159–68.
20. **Saucedo LJ,** Gao X, Chiarelli DA, Li L, Pan D, Edgar BA. Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat Cell Biol* 2003;**5**:566–71.
21. **Stocker H,** Radimerski T, Schindelholz B, Wittwer F, Belawat P, Daram P, *et al*. Rheb is an essential regulator of S6K in controlling cell growth in Drosophila. *Nat Cell Biol* 2003;**5**:559–65.
22. **Yang Q,** Guan KL. Expanding mTOR signaling. *Cell Res* 2007;**17**:666–81.
23. **Rosner M,** Hanneder M, Siegel N, Valli A, Hengstschlager M. The tuberous sclerosis gene products hamartin and tuberin are multifunctional proteins with a wide spectrum of interacting partners. *Mutat Res* 2008;**658**:234–46.
24. **Huang J,** Dibble CC, Matsuzaki M, Manning BD. The TSC1-TSC2 complex is required for proper activation of mTOR complex 2. *Mol Cell Biol* 2008;**28**:4104–15.

25. **Nathan C.** Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006;**6**:173–82.
26. **Hannigan MO,** Huang CK, Wu DQ. Roles of PI3K in neutrophil function. *Curr Top Microbiol Immunol* 2004;**282**:165–75.
27. **Lindemann SW,** Yost CC, Denis MM, McIntyre TM, Weyrich AS, Zimmerman GA. Neutrophils alter the inflammatory milieu by signal-dependent translation of constitutive messenger RNAs. *Proc Natl Acad Sci USA* 2004;**101**:7076–81.
28. **Sasaki T,** Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, *et al*. Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* 2000;**287**:1040–6.
29. **Knall C,** Worthen GS, Johnson GL. Interleukin 8-stimulated phosphatidylinositol-3-kinase activity regulates the migration of human neutrophils independent of extracellular signal-regulated kinase and p38 mitogen-activated protein kinases. *Proc Natl Acad Sci USA* 1997;**94**:3052–7.
30. **Kodama T,** Hazeki K, Hazeki O, Okada T, Ui M. Enhancement of chemotactic peptide-induced activation of phosphoinositide 3-kinase by granulocyte-macrophage colony-stimulating factor and its relation to the cytokine-mediated priming of neutrophil superoxide-anion production. *Biochem J* 1999;**337**(Pt 2):201–9.
31. **Hirsch E,** Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, *et al*. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 2000;**287**:1049–53.
32. **Yost CC,** Denis MM, Lindemann S, Rubner FJ, Marathe GK, Buerke M, *et al*. Activated polymorphonuclear leukocytes rapidly synthesize retinoic acid receptor-alpha: a mechanism for translational control of transcriptional events. *J Exp Med* 2004;**200**:671–80.
33. **Gomez-Cambronero J,** Horn J, Paul CC, Baumann MA. Granulocyte-macrophage colony-stimulating factor is a chemoattractant cytokine for human neutrophils: involvement of the ribosomal p70 S6 kinase signaling pathway. *J Immunol* 2003;**171**:6846–55.
34. **Bischoff SC.** Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat Rev Immunol* 2007;**7**:93–104.
35. **Fukao T,** Yamada T, Tanabe M, Terauchi Y, Ota T, Takayama T, *et al*. Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. *Nat Immunol* 2002;**3**:295–304.
36. **Ali K,** Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, *et al*. Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. *Nature* 2004;**431**:1007–11.
37. **Ali K,** Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, *et al*. Isoform-specific functions of phosphoinositide 3-kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo. *J Immunol* 2008;**180**:2538–44.
38. **Kim MS,** Kuehn HS, Metcalfe DD, Gilfillan AM. Activation and function of the mTORC1 pathway in mast cells. *J Immunol* 2008;**180**:4586–95.
39. **Colonna M,** Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol* 2004;**5**:1219–26.
40. **Guiducci C,** Ghirelli C, Marloie-Provost MA, Matray T, Coffman RL, Liu YJ, *et al*. PI3K is critical for the nuclear translocation of IRF-7 and type I IFN production by human plasmacytoid predendritic cells in response to TLR activation. *J Exp Med* 2008;**205**:315–22.
41. **Colina R,** Costa-Mattioli M, Dowling RJ, Jaramillo M, Tai LH, Breitbach CJ, *et al*. Translational control of the innate immune response through IRF-7. *Nature* 2008;**452**:323–8.
42. **Aksoy E,** Vanden Berghe W, Detienne S, Amraoui Z, Fitzgerald KA, Haegeman G, *et al*. Inhibition of phosphoinositide 3-kinase enhances TRIF-dependent NF-kappaB activation and IFN-beta synthesis downstream of Toll-like receptor 3 and 4. *Eur J Immunol* 2005;**35**:2200–9.
43. **Fukao T,** Tanabe M, Terauchi Y, Ota T, Matsuda S, Asano T, *et al*. PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat Immunol* 2002;**3**:875–81.
44. **Guha M,** Mackman N. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. *J Biol Chem* 2002;**277**:32124–32.
45. **Williams DL,** Li C, Ha T, Ozment-Skelton T, Kalbfleisch JH, Preiszner J, *et al*. Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. *J Immunol* 2004;**172**:449–56.
46. **Martin M,** Schifferle RE, Cuesta N, Vogel SN, Katz J, Michalek SM. Role of the phosphatidylinositol 3 kinase-Akt pathway in the regulation of IL-10 and IL-12 by Porphyromonas gingivalis lipopolysaccharide. *J Immunol* 2003;**171**:717–25.
47. **Polumuri SK,** Toshchakov VY, Vogel SN. Role of phosphatidylinositol-3 kinase in transcriptional regulation of TLR-induced IL-12 and IL-10 by Fc{gamma} receptor ligation in murine macrophages. *J Immunol* 2007;**179**:236–46.
48. **Abe M,** Thomson AW. Influence of immunosuppressive drugs on dendritic cells. *Transpl Immunol* 2003;**11**:357–65.
49. **Gomez-Cambronero J.** Rapamycin inhibits GM-CSF-induced neutrophil migration. *FEBS Lett* 2003;**550**:94–100.
50. **Hartman ME,** O'Connor JC, Godbout JP, Minor KD, Mazzocco VR, Freund GG. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem* 2004;**279**:28045–50.
51. **Woltman AM,** van der Kooij SW, Coffer PJ, Offringa R, Daha MR, van Kooten C. Rapamycin specifically interferes with GM-CSF signaling in human dendritic cells, leading to apoptosis via increased p27KIP1 expression. *Blood* 2003;**101**:1439–45.
52. **Tsiavou A,** Degiannis D, Hatziagelaki E, Koniavitou K, Raptis S. Flow cytometric detection of intracellular IL-12 release: in vitro effect of widely used immunosuppressants. *Int Immunopharmacol* 2002;**2**:1713–20.
53. **Uthaisangsook S,** Day NK, Hitchcock R, Lerner A, James-Yarish M, Good RA, *et al*. Negative regulation of interleukin-12 production by a rapamycin-sensitive signaling pathway: a brief communication. *Exp Biol Med (Maywood)* 2003;**228**:1023–7.
54. **Barcova M,** Speth C, Kacani L, Uberall F, Stoiber H, Dierich MP. Involvement of adenylate cyclase and p70(S6)-kinase activation in IL-10 up-regulation in human monocytes by gp41 envelope protein of human immunodeficiency virus type 1. *Pflugers Arch* 1999;**437**:538–46.
55. **Speth C,** Joebstl B, Barcova M, Dierich MP. HIV-1 envelope protein gp41 modulates expression of interleukin-10 and chemokine receptors on monocytes, astrocytes and neurones. *AIDS* 2000;**14**:629–36.
56. **Tangsinmankong N,** Day NK, Good RA, Haraguchi S. Different mechanisms are utilized by HIV-1 Nef and staphylococcal enterotoxin A to control and regulate interleukin-10 production. *Immunol Lett* 2002;**84**:97–101.
57. **Conti P,** Kempuraj D, Kandere K, Di Gioacchino M, Barbacane RC, Castellani ML, *et al*. IL-10, an inflammatory/inhibitory cytokine, but not always. *Immunol Lett* 2003;**86**:123–9.
58. **Mocellin S,** Panelli MC, Wang E, Nagorsen D, Marincola FM. The dual role of IL-10. *Trends Immunol* 2003;**24**:36–43.
59. **Yang CS,** Song CH, Lee JS, Jung SB, Oh JH, Park J, *et al*. Intracellular network of phosphatidylinositol 3-kinase, mammalian target of the rapamycin/70 kDa ribosomal S6 kinase 1, and mitogen-activated protein kinases pathways for regulating mycobacteria-induced IL-23 expression in human macrophages. *Cell Microbiol* 2006;**8**:1158–71.
60. **Hartford CM,** Ratain MJ. Rapamycin: something old, something new, sometimes borrowed and now renewed. *Clin Pharmacol Ther* 2007;**82**:381–8.
61. **Fingar DC,** Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 2004;**23**:3151–71.
62. **Paul E,** Thiele E. Efficacy of sirolimus in treating tuberous sclerosis and lymphangioleiomyomatosis. *N Engl J Med* 2008;**358**:190–2.
63. **Guba M,** von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, *et al*. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002;**8**:128–35.
64. **Stallone G,** Schena A, Infante B, Di Paolo S, Loverre A, Maggio G, *et al*. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N Engl J Med* 2005;**352**:1317–23.
65. **Mulay AV,** Hussain N, Fergusson D, Knoll GA. Calcineurin inhibitor withdrawal from sirolimus-based therapy in kidney transplantation: a systematic review of randomized trials. *Am J Transplant* 2005;**5**:1748–56.
66. **Haydar AA,** Denton M, West A, Rees J, Goldsmith DJ. Sirolimus-induced pneumonitis: three cases and a review of the literature. *Am J Transplant* 2004;**4**:137–9.
67. **Pham PT,** Pham PC, Danovitch GM, Ross DJ, Gritsch HA, Kendrick EA, *et al*. Sirolimus-associated pulmonary toxicity. *Transplantation* 2004;**77**:1215–20.
68. **Singer SJ,** Tiernan R, Sullivan EJ. Interstitial pneumonitis associated with sirolimus therapy in renal-transplant recipients. *N Engl J Med* 2000;**343**:1815–6.
69. **Ekberg H,** Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, *et al*. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007;**357**:2562–75.
70. **Thaunat O,** Beaumont C, Chatenoud L, Lechaton S, Mamzer-Bruneel MF, Varet B, *et al*. Anemia after late introduction of sirolimus may correlate with biochemical evidence of a chronic inflammatory state. *Transplantation* 2005;**80**:1212–9.
71. **Faivre S,** Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. *Nat Rev Drug Discov* 2006;**5**:671–88.
72. **Hudes G,** Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, *et al*. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 2007;**356**:2271–81.
73. **Marone R,** Cmiljanovic V, Giese B, Wymann MP. Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim Biophys Acta* 2008;**1784**:159–85.
74. **Camps M,** Ruckle T, Ji H, Ardissone V, Rintelen F, Shaw J, *et al*. Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* 2005;**11**:936–43.
75. **Barber DF,** Bartolome A, Hernandez C, Flores JM, Redondo C, Fernandez-Arias C, *et al*. PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat Med* 2005;**11**:933–5.