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Rho family GTPases and their regulators in lymphocytes

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Abstract

Rho family GTPases, and the proteins that regulate them, have important roles in many cellular processes, including cell division, survival, migration and adhesion. Although most of this understanding has come from studies using cell lines, more recent gene targeting studies in mice are providing insights into the *in vivo* function of these proteins. Here we review recent progress revealing crucial roles for these proteins in lymphocyte development, activation, differentiation and migration. The emerging picture shows that Rho family GTPases transduce signals from receptors for antigens, chemokines and cytokines, as well as adhesion molecules and pattern recognition receptors, and that they act as focal points for crosstalk between different signalling pathways.

Introduction

Rho family GTPases have been recognized as crucial signal-transducing proteins for almost 20 years. Whereas initial analysis of the prototypical family members RHOA (RAS homologue gene family member A), RAC1 and CDC42 (cell division cycle 42) identified important roles for these proteins in the regulation of the cytoskeleton, Rho GTPases also have crucial functions in cell division, survival, migration and adhesion. Most of our insight into these cellular functions has come from cell-based studies using dominant negative or constitutively active proteins. More recently, however, gene targeting in mice has allowed selective inactivation of different Rho GTPases and their regulators, and subsequent analysis of *in vivo* function¹. In this review we discuss how this approach has led to a large increase in our understanding of the physiological roles of these proteins in lymphocyte development and activation.

General features of Rho GTPases and their regulators

Mammalian Rho GTPases constitute a family of 23 proteins that are related in primary sequence. Phylogenetic analysis shows that these proteins cluster into several subfamilies based on sequence similarity (Figure 1). The prototypical members of the family, RAC1, CDC42 and RHOA, can bind both GTP and GDP. When bound to GTP they are active and able to transduce signals by binding to effector proteins. By contrast, the GDP-bound forms do not bind effector proteins and are generally assumed to be inactive. However the RND,

RHOBTB, RHOH, RHOU and RHOV GTPases are atypical and do not conform to this stereotypical mechanism of regulation as they are mainly GTP bound. They might instead be regulated by expression, phosphorylation or stability.

The switch between the GDP- and GTP-bound forms of typical Rho GTPases is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Figure 2). Most Rho GTPases are modified at the C-terminus by the attachment of a **prenyl group**². This contributes to membrane localization of the GTPases and therefore to their signalling function, as some effectors of the Rho GTPases are membrane-associated proteins and others are recruited to membranes by binding to the GTPases. Guanine nucleotide dissociation inhibitors (GDIs) bind to the C-terminal lipid groups on GTPases, thereby inhibiting membrane binding and maintaining them in a state where they cannot be activated and are unable to interact with effector proteins.

When in the GTP-bound active form, Rho family GTPases transduce signals by binding to effector proteins (Box 1). These in turn are involved in many cellular processes including regulation of the actin cytoskeleton, microtubule dynamics, cell division, migration and adhesion (see recent reviews^{1,3} for more detail regarding effector proteins).

There are 79 identified GEFs for Rho-family GTPases in the mammalian genome⁴. The largest subgroup of these, containing 66 members, is the Dbl family, all of which contain a Dbl homology (DH) domain, which has the catalytic GEF activity. The Dock family of GEFs is distinguished from the Dbl family by not having a DH domain. Instead, catalytic activity resides in a Dock homology region 2 (DHR2). Finally, the related SWAP70 and IBP proteins are GEFs for Rac GTPases but they contain neither DH nor DHR2 domains.

There are currently 65 Rho family GAPs known in mice. All of these are typified by a conserved RhoGAP domain, which contains the catalytic activity of the enzymes⁵. Both the GEFs and the RhoGAPs usually contain other domains, including protein-protein interaction domains and catalytically active domains. The reasons for the large number of GEFs and GAPs relative to Rho family GTPases are not known. Whereas some GEFs are specific for only one or a few GTPases (such as ITSN1 for CDC42), others have a broader specificity (for example, VAV1 acts on RAC1, RHOA, RHOG and CDC42). The same is true for GAPs. It is probable that this diversity of regulators serves to transduce signals from many different receptors to the GTPases, suggesting that these GTPases act as focal points for cross-talk between different signalling pathways.

Finally, there are three GDIs for Rho family GTPases: GDI α , GDI β and GDI γ . Readers are referred to recent reviews^{4–6} for a more comprehensive discussion of the GEFs, GAPs and GDIs that regulate Rho family GTPases.

Analysis of mRNA levels for Rho family GTPases and their regulators shows that approximately half of the members of these large families are expressed by lymphoid cells (Table 1 and Supplementary Table 1). In particular, 14 out of 23 GTPases, 36 out of 79 RhoGEFs, 38 out of 65 RhoGAPs and 2 out of 3 RhoGDIs are expressed in one or more lymphoid lineages, suggesting that this large group of signal transducers has important roles in lymphocyte biology.

GTPases and their regulators in B cells

B cell development starts in the bone marrow, and proceeds through an ordered series of maturation steps resulting in the release of IgM-expressing immature B cells, which migrate to the spleen, where they undergo further maturation to give rise to **follicular B cells** and **marginal zone (MZ) B cells** (Figure 3). Follicular B cells recirculate between **secondary lymphoid organs** through the blood and lymphatic systems. Upon engagement with antigen through the B cell receptor (BCR), they are activated, move to germinal centres, and, with T cell help, differentiate into plasma cells secreting high affinity antibodies and into long-lived memory B cells.

B cell development

The roles of RAC1 and RAC2 in B cell development have been investigated using mice deficient in either or both GTPases. Whereas loss of RAC1 had no apparent effect, a deficiency in RAC2 results in loss of MZ and **B1 cells**, without affecting follicular B cells^{7,8}. By contrast, loss of both RAC1 and RAC2 led to a complete block in the development of all B cell lineages, with arrest at the **transitional B cell** stage in the spleen⁸. Analysis showed that RAC1 and RAC2 were required for these earliest transitional B cells to migrate from the red pulp to the white pulp of the spleen, most probably because of a crucial role for RAC GTPases in transducing chemokine receptor signals (R. Henderson and V. Tybulewicz, unpublished observations). This migration is essential for the survival of transitional B cells and their subsequent maturation into follicular and MZ B cells. The redundancy between RAC1 and RAC2 revealed by these studies is an emerging theme of Rho GTPases and their regulators.

B cell development is also perturbed in the absence of the Vav GEFs. A deficiency of VAV1 results in a decreased number of B1 cells, but has little or no effect on the development of either follicular or MZ B cells, despite impaired BCR signalling (see later)⁹. By contrast, deficiency of VAV2 alone had no effect on B cell development, whereas mice lacking both VAV1 and VAV2 had a strong developmental block which resulted in decreased numbers of follicular and MZ B cells, which was further enhanced in the absence of all three VAV proteins (VAV1, VAV2 and VAV3), once again revealing redundancy of function^{10–13}. The developmental block in these mice occurs between T1 and T2 transitional splenic stages, indicating that VAV proteins have a crucial role in this late step of B cell maturation. This is at least partly due to a function for VAV proteins in transducing nuclear factor- κ B (NF- κ B)-dependent survival signals from the BCR¹³. Interestingly, the developmental block seen in VAV-deficient B cells is distinct from that seen in the absence of RAC1 and RAC2. Whereas deficiency of RAC proteins results in a block in the earliest T0 transitional subset, before migration into the splenic white pulp, lack of all three VAV proteins arrests development at the later T1 transitional stage and has no effect on migration of these cells into the white pulp, or on chemokine responsiveness *in vitro*, implying that a non-VAV GEF, such as a DOCK family member, is required for chemokine-induced RAC activation in transitional B cells (R. Henderson and V. Tybulewicz, unpublished observations).

Deletion in pro-B cells of RACGAP1, a RhoGAP with broad specificity, results in defective interleukin-7 (IL-7)-induced cell proliferation and survival¹⁴. Interestingly, RACGAP1 has

been shown recently to act as a nuclear chaperone that is required for the nuclear import of phosphorylated signal transducers and activators of transcription (STATs), which have essential roles in transducing signals from cytokine receptors¹⁵.

B cell activation and function

Inhibition of RHOA in both primary B cells and a B cell line showed that this GTPase is required for BCR-induced synthesis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂), the substrate for phospholipase Cγ2 (PLCγ2), which hydrolyses it to generate inositol-1,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG)¹⁶ (Figure 4). Thus inhibition of RHOA leads to less InsP₃ production, less calcium flux and decreased B cell proliferation. RHOA might mediate this function by either directly activating or controlling the localization of phosphatidylinositol 4-kinase and phosphatidylinositol-4-phosphate 5-kinase, lipid kinases that are required for the synthesis of PtdIns(4,5)P₂ in response to BCR signalling.

In B cells that lack RAC2 alone, or RAC2 and one allele of RAC1 (*Rac1^{+/-}Rac2^{-/-}*), BCR-induced proliferation of B cells was decreased, as was BCR-induced calcium flux, AKT activation and induction of expression of BCL-X_L and cyclin D28. RAC2-deficient B cells have defects in the formation of an **immunological synapse**, owing to defective activation of lymphocyte function-associated antigen 1 (LFA1)-mediated adhesion¹⁷. This might be due to a role for RAC2 in transducing BCR signals to activate RAP1 and to induce actin polymerization, both of which have been proposed to regulate LFA1 avidity¹⁸. Finally, a deficiency of RAC2 results in defective T cell-independent B cell responses⁷.

RHOG-deficient mice do not have a severe phenotype, showing a mild increase in the humoral immune response to T-cell-dependent antigens and a subtle increase in the proliferation of B and T cells following antigen receptor cross-linking¹⁹. As RHOG is most closely related to the Rac group of GTPases (Figure 1), there may be functional redundancy between RHOG and the Rac proteins.

BCR-induced cell proliferation is partially compromised in the absence of VAV1, a defect that is enhanced in the absence of the other VAV isoforms^{9–12,20}. This is due at least in part to a role for VAV proteins in transducing BCR signals, leading to intracellular calcium flux and activation of phosphatidylinositide 3-kinase (PI3K), AKT and NF-κB^{10–13,21,22} (Figure 4). Interestingly, BCR-induced activation of extracellular signal-regulated kinase (ERK) is normal, even in the absence of all three VAV proteins¹². This is in contrast to a key role for VAV proteins in transducing signals from the T cell receptor (TCR) to ERK activation (see later), illustrating differences in signalling pathways between B and T cells. Microscopy studies have shown that B cells deficient in both VAV1 and VAV2 have defective BCR-induced cell adhesion and spreading on intercellular adhesion molecule 1 (ICAM1)-coated surfaces, decreased actin polymerization, and defective formation of signalling microclusters and the immunological synapse^{17,23}.

The similarity of the BCR signalling defects between B cells deficient for VAV1 and VAV2 and B cells deficient for RAC1 and RAC2 indicates that these proteins lie in the same

pathway, with VAV proteins transducing BCR signals to RAC activation, which in turn leads to calcium flux, activation of PI3K and increases in adhesion and actin polymerization.

In VAV1-deficient mice, both T-cell-dependent and T-cell-independent antibody responses were reduced^{9,20}. This was further exacerbated in the absence of VAV2 and VAV3^{10–12}. The decreased T-cell-dependent response of VAV1-deficient mice was most probably due to defective help from VAV1-deficient T cells⁹.

Follicular B cells deficient in VAV1 and VAV2 were reported to have reduced lipopolysaccharide (LPS)-induced proliferation and activation of AKT and NF- κ B, suggesting that VAV proteins transduce signals from the receptor for LPS, Toll-like receptor 4 (TLR4)^{11,24}. By contrast, MZ B cells that lack all three VAV proteins had normal LPS-induced proliferation²⁵.

α PIX and β PIX are GEFs for RAC1 and CDC42. Mice deficient in α PIX have reduced numbers of mature B cells in lymph nodes and defects in BCR-induced proliferation²⁶. Deficiency of α PIX does not affect BCR-induced calcium flux, but results in decreased phosphorylation, and therefore activation, of PAK1 and PAK2, which are kinases that transduce signals to the regulation of the actin cytoskeleton (Figure 4 and Box 1).

B cell migration

RAC2-deficient follicular B cells have partially decreased migration in response to the chemokines CXC-chemokine ligand 12 (CXCL12) and CXCL13 *in vitro*, and reduced homing to lymph nodes *in vivo*⁷. A more severe phenotype is seen in transitional B cells deficient in both RAC1 and RAC2, which have a severe defect in migration from the blood through the red pulp of the spleen into the white pulp, and are very defective in chemotaxis in response to CXCL12, CXCL13 and CCL21, chemokines which direct the migration of B cells into follicles of secondary lymphoid tissue (R. Henderson and V. Tybulewicz, unpublished observations).

LSC is a haematopoietic-cell-restricted GEF for RHOA, which interacts with **G-protein-coupled receptor** (GPCR)-associated $G_{\alpha 12}$ and $G_{\alpha 13}$ proteins (Figure 5). In addition to its DH domain with GEF activity, LSC has an RGS domain, which binds to G_{α} proteins and increases their intrinsic GTPase activity, thereby turning off GPCR signalling. So LSC seems to carry out two functions. First, it transduces GPCR signals from $G_{\alpha 12/13}$ to the activation of RHOA and second, it attenuates other $G_{\alpha 12/13}$ -coupled GPCR signals. In LSC-deficient mice, the number of B cells is reduced as are T-cell-dependent antibody responses to protein antigens, due to a cell-intrinsic requirement for LSC in B cells^{27,28}. Further analysis has shown that LSC-deficient MZ B cells migrate more effectively to **sphingosine 1-phosphate** (S1P) *in vitro*, which might be due to the loss of LSC-mediated inhibition of signalling from $G_{\alpha 12/13}$ -coupled S1P receptors. By contrast, LSC-deficient MZ B cells have a defect in their ability to detach from ICAM1, and *in vivo*, they do not migrate to follicles following LPS stimulation. These latter defects might reflect a requirement for LSC-mediated RHOA activation in the disengagement of integrin binding at the trailing edge of the migrating B cell²⁸. Further understanding of LSC function would benefit from analysis of mutants that selectively perturb either the GEF or the RGS activity of LSC.

DOCK2 is a RAC-specific GEF with crucial roles in chemokine receptor signalling (Figure 5). DOCK2-deficient B cells have greatly reduced chemotaxis *in vitro* in response to CXCL12 and CXCL13, and defective homing to lymph nodes and splenic white pulp *in vivo*²⁹. The cells also show reduced chemokine-induced activation of RAC1 and RAP1, actin polymerization and integrin-mediated adhesion^{29,30}. **Intravital microscopy** shows that DOCK2-deficient B cells have a reduced ability to adhere strongly to **high endothelial venules** (HEVs) of lymph nodes and show reduced interstitial mobility within lymph node follicles^{30,31}. Finally, DOCK2 is also required for S1P-mediated egress of B cells from lymph nodes³¹.

B cells deficient in the RAC-specific GEF SWAP70 are defective in constitutive trafficking to lymph nodes, as well as in homing to inflamed lymph nodes³². Unlike DOCK2, SWAP70 is not required for chemokine-induced chemotaxis or adhesion to integrin ligands. Instead, SWAP70-deficient B cells have perturbed morphology, with unstable **lamellipodia** and fewer **uropods**. Intravital microscopy shows that SWAP70-deficient B cells roll and adhere normally to the HEVs of lymph nodes, but they are defective in transmigration³². The physiological effect of these defects is to impair the ability of B cells to participate in the germinal centre and memory responses to T-cell-dependent antigens³³. Surprisingly, however, loss of SWAP70 results in increased numbers of high-affinity plasma cells in the bone marrow and splenic red pulp.

In summary, these studies show that in B cells, Rho GTPases lie at points of crosstalk between signalling pathways from multiple receptors. As a result, they have crucial roles in B cell development, migration and activation.

GTPases and their Regulators In T Cells

T cell development begins in the thymus where CD4⁻CD8⁻ double negative (DN) thymocytes differentiate into CD4⁺CD8⁺ double positive (DP) cells and then undergo **positive selection** into either CD4⁺CD8⁻ or CD4⁻CD8⁺ single positive (SP) thymocytes (Figure 6). These cells exit the thymus, becoming naïve CD4⁺ or CD8⁺ T cells, which continuously recirculate between secondary lymphoid organs. Activation of T cells through the TCR leads to their differentiation into effector and memory T cells.

T cell development

Mice deficient in RHOC have no alterations in the development, activation or migration of B or T cells³⁴. RHOB-deficient mice have been generated, but no analysis of the lymphoid compartment has been carried out³⁵. Most information about the role of this subfamily of Rho GTPases has come from transgenic mice expressing in the T cell lineage either a constitutively active form of RHOA (V14RHOA) or *Clostridium botulinum* C3 transferase. C3 is a toxin that ADP-ribosylates RHOA, RHOB and RHOC in the effector-binding domains, thereby inactivating their function. Transgenic expression of V14RHOA results in more efficient positive selection of CD8⁺ SP thymocytes and T cells³⁶. C3-transgenic mice have a marked inhibition of thymopoiesis, with a large decrease in the size of the DN type 2 (DN2) and DN3 subsets because of increased apoptosis, leading in turn to reduced numbers of DP and SP thymocytes and T cells^{37,38}. Thymocytes expressing C3 have specific

reductions in the expression of transcription factors from the EGR1 and AP1 families³⁹. This may account for the developmental arrest in these mice, as these factors are important for the proliferation and differentiation of early thymocyte subsets.

Expression of a constitutively active form of RAC1 (L61RAC1) in the thymus can partially overcome the developmental block in recombination-activating gene 1 (RAG1)-deficient mice. Whereas in the absence of RAG1 T-cell development is arrested at the DN stage due to lack of a pre-TCR, expression of L61RAC1 caused the appearance of some DP thymocytes, although in smaller numbers than normally found in a wild-type thymus⁴⁰. Furthermore, expression of L61RAC1 diverts DP thymocytes from positive to **negative selection**⁴¹. These results imply that RAC1 might normally have a role in these developmental transitions. By contrast, transgenic mice expressing a constitutively active form of RAC2 in the thymus had increased apoptosis of both DP and SP thymocytes⁴². This could reflect a different function for RAC2 compared with RAC1, or might be due to different levels of expression of the transgenes. More recently, however, mice lacking RAC1 or RAC2 or both in the T cell lineage have been analysed. Whereas elimination of either GTPase alone has no effect on T cell development, in mice lacking both GTPases there is a marked block in development at the pre-TCR checkpoint, and also a defect in the positive selection of DP thymocytes, demonstrating functional redundancy between these highly related GTPases^{43,44}. Unexpectedly, loss of both RAC1 and RAC2 leads to the aberrant appearance of a population of thymocytes that lack expression of TCR β , with hallmarks of hyperactive signalling from Notch receptors, which have important roles in controlling differentiation of DN thymocytes⁴⁵. These results suggest that RAC1 and RAC2 may negatively regulate Notch function during early thymic development.

T cell development in mice deficient in VAV1 is partially blocked at the pre-TCR checkpoint, resulting in decreased numbers of DP thymocytes^{46–49}. Furthermore, there is also a marked defect in both positive and negative selection of DP thymocytes^{46,48,50}. These defects are most probably due to a crucial role for VAV1 in transducing pre-TCR and TCR signals. In agreement with this, VAV1-deficient DP thymocytes are defective in TCR-induced calcium flux and TCR-induced activation of ERK MAP kinases, protein kinase D and PI3K^{46,51,52}. The defects in calcium flux and ERK activation most probably result from defective activation of PLC γ 1. Interestingly, the block at the pre-TCR checkpoint in VAV1-deficient mice can be rescued by transgenic expression of constitutively active RAC1, suggesting that VAV1 transduces pre-TCR signals to the activation of RAC1⁴⁰. These blocks in thymic development are more pronounced in the absence of all three VAV proteins, resulting in an almost complete block in development at the pre-TCR and TCR checkpoints and arguing strongly for overlapping redundant functions between these three related GEFs¹². In contrast to its role in the development of T cells expressing $\alpha\beta$ TCRs, VAV1 is not required for the development of $\gamma\delta$ TCR-expressing T cells⁵³. Nonetheless, VAV1-deficient **$\gamma\delta$ T cells** are unable to proliferate or secrete cytokines normally in response to TCR stimulation.

In the absence of the RhoGEF LSC, thymocyte numbers are increased, most probably because of defective apoptosis⁵⁴. This might be due to the reduced apoptosis of LSC-deficient thymocytes in response to thromboxane A₂ (TXA₂), a prostanoid which signals

through the $G_{\alpha 12}$ - and $G_{\alpha 13}$ -coupled thromboxane prostanoid receptor (TPR) leading to cell death (Figure 5). LSC selectively couples to only a subset of TPR signalling pathways. So whereas TXA_2 -induced RHOA activation, actin polymerization and cofilin phosphorylation were all reduced in the absence of LSC, there was no effect on TXA_2 -induced activation of ERK or p38 MAP kinase pathways⁵⁴. By contrast, lack of LSC had no effect on TCR-induced apoptosis, demonstrating selective coupling of GEFs to specific receptors.

DBS is a GEF for RHOA and CDC42, which is expressed throughout thymocyte development. Transgenic mice expressing an activated form of DBS had larger numbers of CD4⁺CD8⁻ DN thymocytes, but increased apoptosis in later DP and SP thymocyte subsets⁵⁵.

DOCK2-deficient mice have impaired positive and negative selection of DP thymocytes⁵⁶. In particular, there is a large reduction in the number of CD1d-restricted NKT cells expressing a V α 14⁺ TCR. It is unclear why this subset is affected disproportionately⁵⁷.

Finally, in mice deficient in IBP (also known as SLAT and DEF6), a SWAP70-related GEF that acts on both RAC1 and CDC42^{58–60}, one study found that T cell development was normal⁶¹, whereas a second report showed that lack of IBP results in a smaller thymus due to reduced numbers of the earliest DN1 cells⁶².

T cell activation

RAC2-deficient T cells have a partial defect in TCR-induced activation and proliferation⁶³. This is accompanied by a small decrease in TCR-induced calcium flux and TCR-induced activation of ERK and p38 MAP kinase pathways. It is notable that this phenotype is much milder than that seen in the absence of the RacGEF VAV1, implying that in the absence of RAC2, other GTPases might have an important role. In view of the marked redundancy between RAC1 and RAC2 in T cell development^{43,44}, it is probable that the same is true for T cell activation.

The RHOH GTPase is expressed predominantly in the haematopoietic system and is atypical in that it mainly binds GTP. RHOH-deficient T cells are defective in TCR-induced activation and proliferation^{64,65}. In the absence of RHOH, TCR-induced calcium flux and ERK activation are reduced, as is the recruitment of the tyrosine kinase ZAP70 (ζ -chain-associated protein kinase of 70 kDa) to the plasma membrane, most probably because RHOH acts as an adaptor (Figure 7). RHOH contains two tyrosines, which are phosphorylated following TCR engagement and function as binding sites for the SH2 domains of ZAP70⁶⁵. Thus, in the absence of RHOH, there is less ZAP70 recruitment to the plasma membrane and hence less signal transduction to downstream components such as ERK. This may account for the marked developmental block in the thymus of RHOH-deficient mice at both the pre-TCR and positive selection checkpoints, resembling that seen in ZAP70-deficient mice^{64,65}. These studies exemplify another emerging theme where Rho GTPases or their regulators have functions that are unrelated to their canonical GTPase roles.

Stimulation of T cells through the TCR results in rapid tyrosine phosphorylation of VAV1, which activates its GEF activity towards RAC1, CDC42 and RHOA^{66–69}. VAV1-deficient

T cells proliferate less well and secrete less IL-2 in response to stimulation through the TCR47,49,70–72. In the absence of VAV1, there is a reduction of TCR-induced calcium flux, and less activation of ERK and AKT and the transcription factors nuclear factor of activated T cells (NFAT), NF- κ B and serum response factor (SRF)70,71,73–75 (Figure 7). Similar changes were also seen in a VAV1-deficient Jurkat T cell line76. Defects in both calcium flux and ERK activation in the absence of VAV1 seem to be secondary to a failure to activate PLC γ 1, which results in decreased production of InsP₃ and DAG52. Whereas InsP₃ is required for calcium flux, DAG activates RASGRP (a RasGEF) and therefore RAS, B-RAF, MEK and ERK51. The defective TCR-induced activation of AKT in VAV1-deficient T cells is probably due to reduced PI3K activation, and in turn results in reduced phosphorylation and nuclear translocation of the transcription factor FOXO1, and thus less induction of the cell cycle inhibitor p27^{Kip1} (REFS 52,75). However one study has failed to find a role for VAV1 in TCR-induced activation of AKT77. VAV1 may control the activation of NF- κ B by associating with PKC θ , and recruiting it to the plasma membrane78,79. Interestingly, caspase 3-mediated cleavage of VAV1 has been proposed to contribute to unresponsiveness to TCR stimulation in anergic T cells80.

VAV1 is required to transduce inside-out signals leading to the activation of LFA1, and hence to adhesion between T cells and antigen-presenting cells (APCs)81,82. In the absence of VAV1 there is less actin polymerization, less clustering of TCR and lipid rafts at the immunological synapse and reduced movement of the **microtubule organizing centre** towards the T cell interface with the APC71,81–84. VAV1 may control the reorganization of the actin cytoskeleton through its GEF activity on RAC1, or through its association with dynamin 2 or the EZH2 methyltransferase85,86. Finally, VAV1 and RAC1 have been shown to transduce TCR signals leading to the dephosphorylation and inactivation of ezrin, radixin and moesin (**ERM**) **proteins**, which act as linkers between the actin cytoskeleton and the plasma membrane, thereby leading to a transient increase in cell deformability and more efficient conjugate formation87.

A recent study using T cells from mice with a knock-in mutation that eliminates the GEF activity of VAV1, but leaves its other domains intact, has shown that the GEF activity is required for TCR-induced activation of AKT, but not for calcium flux or activation of the ERK MAP kinase pathway, demonstrating that VAV1 must have GEF-independent functions (A. Saveliev and V. Tybulewicz, unpublished observations). VAV1 could act as an adaptor protein that controls the formation of a signalling complex that is required to activate PLC γ 152,88,89. Some evidence for this has come from studies showing that overexpression of a GEF-inactive VAV1 in Jurkat T cells results in an increase in NFAT-dependent gene transcription90. Furthermore, mutation of the calponin homology domain interferes with the ability of VAV1 to activate calcium flux in Jurkat T cells, suggesting that this domain may be involved in the adaptor function of VAV176,91. This work provides another example of a GTPase regulator whose function is in part unrelated to its canonical role as a GEF.

Most VAV1 is localized to the cytoplasm in naive unstimulated T cells, and is recruited to the plasma membrane following TCR stimulation, however VAV1 might also have a role in the nucleus92. In a surprising finding, CD28 stimulation in T cells was shown to result in the methylation of VAV1 on an arginine residue93. The significance of this modification

remains unknown, but, interestingly, methylated VAV1 is selectively localized to the nucleus.

Mice deficient in α PIX have reduced numbers of mature T cells, and these show defective TCR-induced proliferation and cytokine secretion²⁶. Analysis showed that many TCR proximal signalling events were not disturbed by the absence of α PIX, including TCR-induced activation of both RAC1 and CDC42, showing that α PIX does not transduce TCR signals to the activation of these GTPases. However α PIX-deficient T cells have defective TCR-induced phosphorylation of PAK1 and PAK2 and less recruitment of these kinases to the immunological synapse, probably through a GIT- α PIX-PAK1 complex^{26,94}. They are also less efficient at forming antigen-specific conjugates with APCs, which may be due to defective inside-out activation of LFA1, and reduced recruitment and clustering of LFA1 at the immunological synapse²⁶.

DOCK2-deficient T cells have defects in TCR-induced proliferation⁵⁶. Analysis of signalling showed that in the absence of DOCK2, TCR-induced calcium flux and the activation of LCK, ZAP70, JNK, PYK2, AKT and PLC γ 1 were all unaffected. However, DOCK2-deficient T cells had reduced TCR-induced activation of RAC1, RAC2 and ERK, as well as impaired immunological synapse formation. Although DOCK2-deficient T cells formed antigen-specific conjugates normally with APCs, and showed the expected polarization of LFA1 and PKC θ to the synapse, there was greatly reduced recruitment of both the TCR and of lipid rafts to the T cell–APC interface⁵⁶. It remains unknown how DOCK2 contributes to this process. It is curious that two GEFs, VAV1 and DOCK2, are both required for TCR-induced RAC1 activation. It could be that they function together, a possibility suggested by the observation that DOCK2 and VAV1 bind to each other⁹⁵.

IBP-deficient CD4⁺ T cells have reduced TCR-induced proliferation, at high doses of TCR stimulation, but increased proliferation at low doses^{61,62,96}. IBP is required for TCR-induced calcium flux, ERK activation and nuclear translocation of the NFAT transcription factor^{62,97}. The defect in NFAT nuclear translocation seen in IBP-deficient T cells can be rescued either by a membrane-tethered domain of IBP containing the GEF activity or by a constitutively active form of CDC42. Constitutively active CDC42 also restores the TCR-induced calcium flux. Taken together these results suggest that the GEF activity of IBP regulates NFAT activation through CDC42 and an increase in intracellular calcium (Figure 7).

In mice deficient for RHOGDI α and RHOGDI β , there are decreased numbers of T cells and these have reduced TCR-induced proliferation⁹⁸. These mutant T cells have decreased levels of RHOA, RAC1 and CDC42 proteins, perhaps because of increased proteolysis, although a larger fraction of the remaining GTPases is in the active GTP-bound state. It is likely that the phenotype of these mice is a complex superposition of partial disruptions to the function of the multiple GTPases that bind GDIs.

While most analysis has been carried out on VAV1, it is clear that other GEFs (α PIX, DOCK2 and IBP) also transduce crucial signals during T cell activation and development. The interplay between these GEFs remains unclear.

T cell differentiation and function of effector subsets

RAC2 is expressed at higher levels in T helper 1 (T_H1) cells than in T_H2 cells⁹⁹. This expression is important as RAC2-deficient T cells have selective defects in T_H1 cell differentiation and interferon- γ (IFN γ) production⁹⁹. However a subsequent study showed only a minor skewing of the T cell response towards the T_H2 cell lineage in RAC2-deficient mice challenged with *Leishmania major*¹⁰⁰.

In vitro culture of VAV1-deficient T cells under neutral or T_H2-cell-polarizing conditions resulted in reduced expression of IL-4, suggesting that VAV1 is required for normal T_H2 cell differentiation¹⁰¹. Furthermore, VAV1 is required in CD4⁺ T cells for IL-4 production *in vivo*, which could account for the defective T-cell-dependent antibody responses seen in VAV1-deficient mice⁹. However, VAV1 is also involved in T_H1 and/or T_H17 cell responses, as VAV1-deficient mice are resistant to **experimental autoimmune encephalomyelitis**, showing inefficient priming and expansion of antigen-specific T cells and reduced production of IL-2, IL-4 and IFN γ ¹⁰².

VAV1 is important for primary antiviral CD8⁺ cytotoxic T cell responses, which are reduced in the absence of VAV1⁷². However secondary recall responses are normal, demonstrating that the loss of VAV1 only partially disrupts T cell activation. Finally, mice deficient in either VAV1 alone or VAV1 and VAV2 show long-term survival of MHC-mismatched cardiac transplants, most probably due to defective allogeneic T cell responses¹⁰³.

DOCK2-deficient mice develop an allergic disease that is characterized by increased numbers of T_H2 cells¹⁰⁴. In the absence of DOCK2, internalized IL-4R α is re-routed from lysosomes to recycling endosomes, and is therefore not degraded, but re-expressed on the cell surface, thereby promoting T_H2 cell differentiation. DOCK2 may control this trafficking by regulating microtubule dynamics through phosphorylation and inactivation of stathmin¹⁰⁴.

Loss of the SWAP70-like GEF IBP results in a reduction of both T_H1 and T_H2 cell responses⁶². *In vitro* culture of IBP-deficient CD4⁺ T cells in polarizing conditions showed decreased production of both IFN γ and IL-4, which may be due to defects in TCR-induced activation of NFAT transcription factors⁶². However, another study found no defects in IL-4 production and reported reduced IFN γ secretion in T cells cultured in non-polarizing conditions⁶¹. Female IBP-deficient mice over 5 months of age develop autoantibodies and an accumulation of effector/memory T cells and IgG1⁺ B cells⁶¹. Interestingly, when the same genetic mutation was crossed to transgenic mice expressing a monoclonal MHC class II-restricted TCR, the mice spontaneously develop an inflammatory arthritic disease characterized by hyperresponsive T cells producing increased quantities of IL-17 and IL-21, and an apparent skewing of T cell differentiation to the T_H17 cell lineage⁹⁶. This effect seems to be due to the regulation of the transcription factor interferon-regulatory factor 4 (IRF4) by IBP, which binds IRF4 and inhibits its function⁹⁶. It is unclear whether this inhibitory function requires the GEF activity of IBP. In the absence of IBP, IRF4 activates the expression of IL-17 and IL-21, as well as retinoic acid receptor-related orphan receptor- γ t (ROR γ t), a transcription factor that is required for the development of T_H17 cells. Thus

IBP contributes to T helper cell differentiation, and at least some of its functions may be GEF independent.

T cell migration

GTPases of the RHOA subfamily have an important role in the regulation of integrin-mediated adhesion. Transgenic expression of constitutively active V14RHOA results in increased adhesion of both thymocytes and T cells through $\beta 1$ and $\beta 2$ integrins, whereas expression of C3 transferase, the RHOA/B/C inhibitor causes decreased adhesion¹⁰⁵. Thymocytes expressing C3 transferase also show reduced chemotaxis in response to CXCL12 and CCL25 when migrating across membranes coated in fibronectin, a ligand for $\beta 1$ integrins.

RAC2-deficient T cells show defects in chemotaxis to the lymphoid chemokines CCL19 and CCL21, and a mildly reduced ability to home to lymph nodes¹⁰⁰. The partial nature of these phenotypes suggests that there might be redundancy between RAC2 and other GTPases (such as RAC1) in transducing chemokine receptor signals. Studies in human T cells show that both RAC1 and RHOA are required to transduce chemokine receptor signals to the activation of LFA1 via PLD and PIP5K¹⁰⁶ (Figure 5).

In the Jurkat T cell line, mutagenesis of RHOH showed that it is required for the maintenance of LFA1 in a non-adhesive state¹⁰⁷ (Figure 5). A similar result was found using RNA interference to knockdown expression of RHOH in primary human T cells, which resulted in increased LFA1-mediated adhesion. These opposing functions of RHOA and RHOH in integrin regulation illustrate the complexity of Rho GTPase function.

Stimulation of human T cells with CXCL12 causes VAV1 to become phosphorylated and to relocalize to the leading edge of the cell¹⁰⁸. Expression of a mutant form of VAV1 in these cells impairs CXCL12-induced cell polarization and migration, implying that VAV1 may be important for transducing signals from CXCR4 (the receptor for CXCL12). However, VAV1-deficient mouse T cells have normal chemotactic responses to CXCL12¹⁰⁸. This difference may be due to functional redundancy with VAV2 and VAV3.

DOCK2 is required for the *in vitro* polarization and migration of T cells in response to CCL21 and CXCL12, and for their efficient homing to lymph nodes and splenic white pulp *in vivo*^{29,30}. Furthermore, intravital microscopy showed that DOCK2-deficient T cells had reduced interstitial movement within lymph nodes³¹. In the absence of DOCK2, T cells have normal CXCL12-induced calcium flux and activation of AKT and ERK, but reduced RAC1 activation and actin polymerization. In contrast to the requirement for DOCK2 in B cells for chemokine-induced adhesion to integrin ligands, DOCK2-deficient T cells adhere normally *in vitro*³⁰. Furthermore, lack of DOCK2 does not affect either the rolling and arrest of T cells in HEVs of lymph nodes, or their subsequent transmigration through the endothelial cell layer^{30,109}. However DOCK2 is required for the lateral mobility of T cells on both the apical and basal endothelial surfaces, and this could explain the inefficient homing of T cells to peripheral lymphoid organs in the absence of DOCK2¹⁰⁹. DOCK2 is also required for S1P-dependent egress of T cells from secondary lymphoid organs³¹.

Finally, α PIX-deficient T cells migrate more efficiently both in the presence and absence of chemokines, and T cells deficient in both RHOA and RHOA β migrate poorly in response to CXCL12, CCL19 and CCL21^{26,98}.

Lymphocyte polarization is crucial for *in vivo* migration. Polarized T cells are characterized by an actin-dependent leading edge, and a uropod and MTOC at the rear. Rho GTPase signalling is compartmentalized in these distinct regions, with RAC1 and CDC42 acting at the leading edge through WASP and WAVE, and Rho at the rear acting through ROCK1¹⁰ (Box 1). Lymphocyte polarity has not been analysed in GTPase-deficient mice, however expression of dominant negative and constitutively active GTPases demonstrates that RAC1, RHOA and CDC42 are required to establish polarity in T cell lines^{111–113}. T cells deficient for the RAC1 GEF T-cell lymphoma invasion and metastasis 1 (TIAM) show reduced polarization and chemotaxis to CXCL12. In addition to activating RAC1, TIAM1 may function as a scaffold protein, associating with active RAP1 which activates the PAR polarity complex through CDC42¹¹⁴. Genetic dissection of the proposed associations of TIAM1 with RAP1 and the polarity complex would be informative. In an assay of T cell shape regulation, α PIX and PAK1 were shown to be important for CXCL12-induced migration through restrictive barriers¹¹⁵. Imaging analysis of GTPase activity in distinct subcellular localizations within lymphocytes would greatly improve our understanding of the roles of individual GTPases in T cell polarization.

In summary, a complex picture is emerging of the roles of Rho GTPases and their regulators in T cell biology. The multiplicity of processes perturbed by deficiencies of these proteins support the idea that they are involved in cross-talk between multiple signalling pathways.

Future Directions and Implications

Analysis of the lymphoid system in mice deficient in Rho GTPases or their regulators has revealed important roles for these proteins in signalling through antigen receptors, chemokine receptors and integrins, with critical functions in development, activation, differentiation and migration.

Several themes have emerged from these studies. First, there is often redundancy between related proteins (for example, RAC1 and RAC2). Second, the GTPases seem to lie at focal points of crosstalk between signalling pathways, which may explain the large number of GEFs and GAPs compared to GTPases. Finally, in many cases proteins in these families have functions unrelated to the regulation of GTPases. For example, VAV1 and IBP may have GEF-independent functions and RHOH acts as an adaptor protein.

Several techniques will be essential to the resolution of these issues. Tissue-specific or inducible gene inactivation will be an essential tool in establishing cell-intrinsic roles for proteins, and for avoiding lethality and/or developmental blocks, both of which can preclude use of conventional knockout mice. The use of gene targeting to “knock-in” point mutations will be an invaluable technique to dissect the role of different signalling inputs and outputs from the same GTPase¹¹⁶. This can also be used to distinguish different functions within the same signaling molecule, for example determining which aspects of a GEF’s function

require its enzymatic activity, and which might be due to adaptor function. It is possible that some of the GTPases or their regulators are compartmentalized in several intracellular pools, which would have distinct functions. Resolution of such pools will require careful biochemical or imaging analysis with higher resolution techniques than are currently available.

We note that at least 90 of these proteins are expressed in lymphocytes (Supplementary Table 1) and, for the majority, their roles in lymphocyte development and function have not yet been analysed. In many cases, suitable genetically modified mice have not been generated. Clearly, the analysis of the *in vivo* function of this key family of signal transducers has only just begun and far more remains to be discovered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

prenyl group

An isoprenoid lipid, either farnesyl or geranylgeranyl. Most Rho proteins are modified by the addition of a prenyl group at their C termini. This modification anchors Rho GTPases in membranes.

follicular B cell

A recirculating mature B-cell subset that populates the follicles of secondary lymphoid organs such as the spleen, lymph nodes and Peyer's patches.

Marginal zone B cell

A non-circulating B-cell subset found predominantly in the marginal zone of the spleen which is located between the non-lymphoid red pulp and the lymphocyte-rich white pulp. These cells are thought to mount early responses to blood-borne antigens.

secondary lymphoid organs

Organs in which naïve B and T cells reside such as spleen, lymph nodes, Peyer's patches.

B1 cell

A cell belonging to the B-cell lineage that populates the peritoneal and pleural cavities and secretes polyspecific, low-affinity IgM.

transitional B cell

An immature B-cell subset found in the spleen, recently arrived from the bone marrow. These cells are short-lived, with half-lives of 2–4 days, which mature into follicular or marginal zone B cells.

immunological synapse

A region that can form between two cells of the immune system in close contact. The immunological synapse originally referred to the interaction between a T cell and an antigen-presenting cell. It involves adhesion molecules, as well as antigen receptors and cytokine receptors. The immunological synapse is characterized by polarization of the lymphocyte resulting, for example, in directed secretion towards the antigen presenting cell.

G-protein-coupled receptor

(GPCR). One of a large group of receptors that bind a diverse set of molecules, including chemokines, complement components, biologically active amines and neurotransmitters. GPCRs are seven-transmembrane-spanning receptors and are coupled to heterotrimeric, GTP-regulated signalling proteins.

sphingosine 1-phosphate

(S1P). A sphingolipid involved in signalling. In the immune system, S1P induces egress of lymphocytes from lymphoid organs by binding to S1P receptors on the cells.

Intravital microscopy

This is used for examination of biological processes, such as leukocyte–endothelial-cell interactions, in living tissue. In general, translucent tissues are used, such as the mesentery or cremaster muscle, or lymph nodes, which can be exposed and mounted for microscopic observation.

high endothelial venules

(HEVs). Specialized venules with a cuboidal endothelial lining. They are found in peripheral lymph nodes and Peyer's patches. HEVs are the sites of entry of lymphocytes into lymphoid tissues from the bloodstream.

lamellipodia

Flattened, sheet-like protrusions that are supported by a meshwork of F-actin and extend at the leading edge of crawling cells.

uropods

Slender protrusions that form at the rear end of migrating leukocytes.

positive selection

A process that leads to the survival of immature thymocytes that express a T-cell receptor that binds with an appropriate affinity to self MHC.

negative selection

The process in the thymus that eliminates thymocytes that express T-cell receptors with high affinity for self antigens.

 $\gamma\delta$ T cells

T cells that express the $\gamma\delta$ T-cell receptor. These T cells are present in the skin, vagina and intestinal epithelium as intraepithelial lymphocytes (IELs). Although the exact function of $\gamma\delta$ T cells is unknown, it has been suggested that mucosal $\gamma\delta$ T cells are involved in innate immune responses.

microtubule-organizing centre

A region of the cell from which microtubules grow.

ERM proteins

Ezrin, radixin and moesin (ERM) proteins act as general cytolinkers between the cortical actin-filament network and the plasma membrane, and are thought to function mainly through their ability to bind both F-actin and the cytoplasmic regions of integral membrane proteins.

Experimental autoimmune encephalomyelitis

(EAE). An experimental model of the human disease multiple sclerosis. EAE is an autoimmune disease mediated by CD4⁺ T helper 1 (T_H1) cells and interleukin-17-producing T_H17 cells reactive to components of the myelin sheath that infiltrate the nervous parenchyma, release pro-inflammatory cytokines and chemokines, promote leukocyte infiltration and contribute to demyelination.

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Box 1**Effector proteins of Rho GTPases that regulate the actin cytoskeleton.**

Regulation of the actin cytoskeleton downstream of the Rho GTPases CDC42 (cell division cycle 42), RAC1, RHOV and RHOA is mediated by several effector proteins (see figure). CDC42 activates WASP (Wiskott–Aldrich syndrome protein), which, via ARP2 (actin-related protein 2 homologue)-ARP3, leads to actin polymerization from the sides of existing filaments, creating branched structures. RAC1 activates the same pathway through the WASP-related WAVE (WASP-family verprolin homologous protein) family of proteins. Both CDC42 and RAC1 activate mDIA2, leading to the nucleation of unbranched actin filaments. The same two GTPases also activate the PAK family kinases, which phosphorylate and activate LIMK (LIM domain kinase), a kinase which in turn phosphorylates and inhibits cofilin. Cofilin promotes severing of actin filaments and depolymerization, hence its inhibition leads to increased stability of polymerized actin. RHOV inhibits PAK activation¹¹⁷. LIMK is also activated by ROCK (RHO-associated coiled-coil-containing protein kinase), a kinase effector that is downstream of RHOA. ROCK increases phosphorylation of myosin light chain (MLC) by phosphorylating and inhibiting myosin light chain phosphatase (MLCP), and might also directly phosphorylate MLC. Phosphorylation of MLC leads to its increased association with actin filaments. Finally, PAK counteracts ROCK function by inhibiting myosin light chain kinase (MLCK), thereby reducing phosphorylation of MLC. Interestingly, in migrating T cells, there is spatial segregation of these pathways, with MLCK activated predominantly at the leading edge and ROCK activated in the trailing edge¹¹⁸. Many of the pathways illustrated here have been identified in non-lymphoid cells and it is not known if they exist in B or T cells.

Online summary

- In mammals, the Rho family of GTPases has 23 members, which are regulated by 79 guanine nucleotide exchange factors (GEFs), 65 GTPase activating proteins (GAPs) and 3 guanine nucleotide dissociation inhibitors (GDIs). About half of these are expressed in lymphocytes.
- RAC1 and RAC2 have overlapping redundant roles in both B and T cell development, activation and migration.
- RHOH has an important role in T cell activation as an adaptor protein recruiting ζ -chain-associated protein kinase of 70 kDa (ZAP70) to the plasma membrane.
- The VAV1, VAV2 and VAV3 GEFs have overlapping redundant roles in both B and T cell development, and activation but not migration. VAV1 has GEF-independent functions, possibly as an adaptor protein.
- The DOCK2 GEF is a crucial transducer of chemokine receptor signals in both B and T cells, and is also required for T cell activation.
- The SWAP70 GEF is required for polarization of B cells and transmigration across high endothelial venules. The related IBP GEF regulates T helper cell differentiation, in part by binding the transcription factor interferon-regulatory factor 4, a function that may be GEF independent.

Figure 1.

Mouse Rho GTPases. Phylogenetic tree of 23 mouse Rho GTPases, showing how they cluster into different subfamilies, for example the Rac, RhoA/RhoB/RhoC and Rnd subfamilies. We note that it has been proposed that MIRO1, MIRO2 and RHOB3 do not belong to the Rho GTPase family¹²⁰.

Figure 2.

Regulation of Rho GTPases. This figure shows a generic Rho GTPase anchored to the membrane with a prenyl group near the C-terminus (zigzag line). The GTPase binds either GDP or GTP. Guanine nucleotide-exchange factors (GEFs) catalyse the release of GDP from the GTPase, allowing GTP to bind. GTPase-activating proteins (GAPs) increase the intrinsic GTPase activity of the Rho proteins, causing GTP to be hydrolysed to GDP and phosphate (P_i). GEFs and GAPs are often constitutively or inducibly associated with membranes. GDP-bound Rho proteins can be sequestered by Rho guanine nucleotide dissociation inhibitors (GDIs), which bind to the lipid modification and thereby inhibit membrane binding of the GTPase. GTP-bound Rho proteins transduce signals by binding to effector proteins.

Figure 3.

Role of Rho GTPases and their regulators in B cell development and function. The earliest B cell progenitors in the bone marrow, pro-B cells, rearrange immunoglobulin heavy chain genes. If they successfully generate a heavy chain, this assembles into the pre-B cell receptor (pre-BCR). Signals from the pre-BCR allow cells to differentiate into pre-B cells and proliferate. Pre-B cells rearrange light chain genes, and if this process is successful, the light chain pairs with the heavy chain to generate cell surface-bound BCR in the form of IgM. These cells, known as immature B cells, exit the bone marrow and migrate through the circulation to the red pulp of the spleen, where we have recently described them as transitional type 0 (T0) cells (R. Henderson and V. Tybulewicz, unpublished observations). Chemokine-induced migration of T0 cells into the white pulp is accompanied by maturation into transitional type 1 (T1) and then type 2 (T2) cells, and finally into either follicular or marginal zone (MZ) B cells. Follicular B cells recirculate between secondary lymphoid organs, including spleen, lymph node, Peyer's patches and bone marrow. B1 cells, a distinct lineage of B cells, which predominate in the peritoneal and pleural cavities, are derived from foetal progenitors (not shown). Activation of naïve follicular B cells (thick arrow) results in their movement into germinal centres (GCs) where, as GC B cells, with T cell help, they undergo somatic hypermutation, affinity maturation and class switching. Eventually the cells mature into antibody-secreting plasma cells and memory B cells. Points where roles for Rho

GTPases or their regulators have been identified are indicated, and discussed more fully in the main text.

Figure 4.

Signal transduction from the B cell receptor. Binding of antigen to the B cell receptor (BCR) leads to activation of the tyrosine kinase LYN, phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domains of Igα and Igβ, recruitment and activation of the tyrosine kinase SYK (spleen tyrosine kinase), phosphorylation of the adaptor protein BLNK (B cell linker) and assembly of a complex including BTK (Bruton's tyrosine kinase), VAV1 and PLCγ2 (phospholipase Cγ2). Activation of PLCγ2 leads to hydrolysis of the lipid phosphatidylinositol-4,5-bisphosphate (Ptd(4,5)InsP₂) to the second messengers inositol-3,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG). Ptd(4,5)InsP₂ is synthesized by sequential phosphorylation of phosphatidylinositol (PtdIns) to phosphatidylinositol-4-phosphate (Ptd(4)InsP) and then to Ptd(4,5)InsP₂ by phosphatidylinositol-4-kinase (PI4K) and phosphatidylinositol-4-phosphate-5-kinase (PIP5K) respectively. Both of these latter kinases are activated by RHOA. VAV1 may contribute to the activation of PLCγ2 through an adaptor function, and via RAC1 lead to increased synthesis of PtdIns(3,4,5)P₃, potentially by activating phosphatidylinositide 3-kinase (PI3K). Increased levels of InsP₃ lead to an increase in intracellular Ca²⁺ and eventually to activation of nuclear factor of activated T cells (NFAT). DAG activates the RAS exchange factor RASGRP, leading to activation of RAS and the

RAF, MEK, ERK kinase cascade. Ca^{2+} and DAG may also contribute to activation of nuclear factor- κB (NF- κB) via PKC β . PtdIns(3,4,5) P_3 leads to activation of the kinase AKT which signals cell survival through inhibition of the FOXO transcription factors. Signalling from the BCR also activates αPIX , and hence RAC1 and PAK.

Figure 5.

Signal transduction from G-protein-coupled receptors and integrins. Receptors for sphingosine 1-phosphate (SIP) and thromboxane A₂ (TXA₂) are members of the G-protein-coupled receptor (GPCR) family, and signal through Gα12 and Gα13 proteins to LSC and RHOA activation. LSC inhibits Gα12/13 function through its RGS domain. Chemokine receptors signal through Gαi2 to DOCK2 and other guanine nucleotide-exchange factors (GEFs) leading to the activation of RAC1 and RHOA. These GTPases transduce signals to integrin activation through phospholipase D (PLD) and PIP5K1C. Integrin signalling is inhibited by RHOH through an unknown mechanism. Integrin signalling leads to the activation of the αPIX GEF and thus the activation of RAC1 and PAK. In myeloid cells, integrin signalling also leads to activation of SRC family kinases which phosphorylate immunoreceptor tyrosine-based activation motif (ITAM)-bearing receptor subunits, such as Fc receptor γ-chain (FcRγ), leading to recruitment and activation of spleen tyrosine kinase (SYK). SYK in turn phosphorylates SH2 domain-containing leukocyte protein of 76 kDa (SLP76) leading to assembly of a complex including PLCγ1, NCK and VAV1, and hence to activation of RAC1 and CDC42. It is not known if this latter pathway operates in lymphocytes.

Figure 6.

Role of Rho GTPases and their regulators in T cell development and function. The earliest T cell progenitors, CD4⁺CD8⁺ double negative (DN) thymocytes, rearrange T cell receptor β (TCRβ) chain genes. If they successfully generate a TCRβ chain, this assembles into the pre-TCR. Signals from the pre-TCR allow cells to differentiate into CD4⁺CD8⁺ double positive (DP) cells and proliferate. DP thymocytes rearrange TCRα chain genes, and if this process is successful, the TCRα chain pairs with TCRβ to generate cell-surface-bound TCR. Signalling from the TCR on DP cells results in positive or negative selection depending on avidity of binding to self-peptide MHC complexes. DP cells with a MHC class I-restricted TCR differentiate into CD4⁺CD8⁺ single positive (CD8 SP) cells, whereas MHC class II-restricted cells are directed into CD4⁺CD8⁺ single positive (CD4 SP) cells. SP thymocytes exit the thymus, migrate through the blood to secondary lymphoid organs, and become either CD4⁺ or CD8⁺ T cells. Activation of naïve CD4⁺ or CD8⁺ T cells (thick arrow) results in their proliferation and differentiation. CD4⁺ T cells can become T helper 1 (T_H1), T_H2 or T_H17 cells, T regulatory (T_{Reg}) cells or memory T cells. CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTL) and memory T cells. Points where roles for Rho GTPases or their regulators have been identified are indicated and discussed more fully in the main text.

Figure 7.

Signal transduction from the T cell receptor. Binding of peptide:MHC complex to the T cell receptor (TCR) leads to activation of the tyrosine kinases LCK and ZAP70 (ζ -chain-associated protein kinase of 70 kDa), phosphorylation of the adaptor protein LAT (linker for activation of T cells) and assembly of a complex including the adaptors GADS, SLP76 and NCK, the ITK and PAK kinases, VAV1 and PLC γ 1. Activation of PLC γ 1 leads to hydrolysis of PIP₂ to the second messengers InsP₃ and DAG. VAV1 may contribute to the activation of PLC γ 2 through an adaptor function, and, via RAC1 to increased synthesis of PIP₃, potentially by activating phosphatidylinositol-3-kinase (PI3K). Increased levels of InsP₃ lead to a rise in intracellular Ca²⁺ and eventually to activation of the NFAT transcription factor. DAG activates the Ras exchange factor RASGRP, leading to activation of RAS and the RAF, MEK, ERK kinase cascade. DAG may also contribute to activation of NF κ B via PKC θ . PIP₃ leads to activation of the AKT kinase which signals cell survival via

inhibition of the FOXO1 transcription factor. TCR signalling via IBP leads to CDC42 activation and increased intracellular Ca^{2+} .

Table 1

Expression of some Rho GTPases and their regulators in lymphocytes. Expression data is compiled from NCBI Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) under accession numbers GSE6259 and GSE6506 119. Only those genes that have been targeted for deletion in mice are listed; a full list of all Rho GTPases and their regulators is shown in Supplementary Table 1. +++, high level expression; ++, intermediate level expression; + low level expression; -, not expressed.

Gene name	B cell	CD4 ⁺ T cell	CD8 ⁺ T cell	NK cell
Rho GTPases				
<i>Rac1, Rac2, Rhog, Cdc42</i>	+++	+++	+++	+++
<i>Rac3</i>	-	-	-	-
<i>Rhoh</i>	+++	+++	+++	++
<i>Rhob</i>	+++	++	++	++
<i>Rhoc</i>	-	+	-	++
RhoGEFs				
<i>Abr</i>	-	++	++	++
<i>Als2</i>	++	+	+	++
<i>Arhgef1</i>	+++	+++	+++	++
<i>Arhgef6, Arhgef18, Sos1, Sos2, Dock2, Def6, Vav1</i>	+++	+++	+++	+++
<i>Arhgef9</i>	-	-	+	-
<i>Arhgef12</i>	++	++	++	+
<i>Arhgef15, Kalrn, Mcf2, Ngef, Prex2, Rasgrf1, Rasgrf2, Tiam1, Dock3, Dock7</i>	-	-	-	-
<i>Bcr</i>	+	-	-	-
<i>Ect2</i>	+	-	-	++
<i>Fgd2</i>	+++	-	-	-
<i>Itsn1</i>	+	+	+	+
<i>Trio</i>	+	+	+	-
<i>Vav2</i>	+++	++		++
<i>Vav3</i>	++	++	++	+++
<i>Dock1</i>	-	+	+	+
<i>Dock5</i>		++	++	+++
<i>Swap70</i>	+++	++	-	++
RhoGAPs				
<i>Abr, Chn2</i>	-	++	+	++
<i>Arhgap1, Inpp5b, Pik3r1, Tagap1</i>	+++	+++	+++	+++
<i>Arhgap5</i>	-	+	+	-
<i>Arhgap6</i>	++	+	-	++
<i>Arhgap12, Srgap2</i>	++	++	++	+++
<i>Bcr</i>	+	-	-	-
<i>Chn1, Dlc1, Grit, Ocl1, Ophn1</i>	-	-	-	-
<i>Pik3r2</i>	+	+	-	-

Gene name	B cell	CD4 ⁺ T cell	CD8 ⁺ T cell	NK cell
<i>Ptpdc1</i>	+	+	+	+
<i>Racgap1</i>	–	–	++	++
<i>Ralbp1</i>	++	++	++	++
<i>RhoGDI</i>s				
<i>Arhgdia</i> , <i>Arhgdib</i>	+++	+++	+++	+++
<i>Arhgdig</i>	–	–	–	–