Lymphocyte Activation Signals: Transduction

Gilbert J Kersh, *Emory University School of Medicine, Atlanta, Georgia, USA*

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Adaptive immunity depends on the transfer of information across the plasma membrane of T and B lymphocytes. This information takes the form of signal transduction pathways that are initiated by the recognition of antigens by specific transmembrane receptors, and result in a complex series of biochemical changes within the cell that ultimately alter cellular function.

Advanced article

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Introduction

B and T lymphocytes, the antigen-specific cells of the immune system, are generally quiescent and require antigen stimulation to progress from the G_0 stage of the cell cycle. The immune response to pathogens or foreign substances is initiated from clonotypic receptors on these cells that recognize the antigen and induce a series of signal transduction events. These signals are fundamental in both developmental decisions and the initiation of immune responses.

Antigen receptors on the lymphocytes consist of two, functional modules: a ligand-binding module and a signalling module. T and B cells possess structurally different, oligomeric receptors that recognize distinct forms of antigens. The antigen-specific receptor on T cells (T-cell receptor, TCR) is composed of clonotypic (TCR$\alpha\beta$ or TCR$\gamma\delta$) chains that are formed by deoxyribonucleic acid (DNA) recombination events during development. These clonotypic TCR chains, are noncovalently associated with the complex of invariant CD3γ, δ and ε chains and ζ family dimers. The clonotypic TCR$\alpha\beta$ or TCR$\gamma\delta$ chains are responsible for antigen recognition, but have a very small (five residues) cytoplasmic domain. The associated invariant CD3 and ζ chains have considerably larger cytoplasmic domains (40–113 residues), and are responsible for coupling to the intracellular signalling machinery. Clonotypic, antigen-recognizing receptors on B cells (BCR) are composed of membrane immunoglobulin (Ig) and are similarly expressed at the plasma membrane in a stable complex with the invariant disulfide-linked Igα and Igβ molecules, which are responsible for signal transduction. Antigen recognition by the BCR or TCR produces signals leading to multiple potential outcomes: proliferation, differentiation, activation, apoptosis or anergy of the B- and T-cell clones. In spite of the different receptor forms on T and B cells, the signal transduction events that result from the interaction of TCR or BCR with antigen are quite similar. Molecules involved in the signal transduction through antigen receptors on lymphocytes are described here (summarized in **Table 1**). **See also**: B Lymphocytes: Receptors; Lymphocytes: Antigen-induced Gene Activation; Signal Transduction: Overview; T-cell Receptors; T-lymphocyte Activation

Initial Events of Signal Transduction from Antigen Receptors

Protein tyrosine phosphorylation

When the antigen receptors on T or B cells bind to antigens with sufficient affinity, numerous intracellular events rapidly take place (**Figure 1**). It is not entirely clear how antigen binding is translated into intracellular responses. For both B and T cells, crosslinking of antigen receptors with antibodies results in signal transduction. For B cells, which bind soluble antigen via two antigen recognition sites per receptor, some form of crosslinking is likely to be an important trigger for signalling in response to antigen recognition. T cells, however, bind to antigens presented by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs) (**Figure 2**). Although crosslinking the TCR artificially with antibodies can be an effective stimulus for T cells, efforts to implicate some form of TCR multimerization as a prerequisite for signalling events have not been conclusive. Another possible mechanism for the TCR to indicate that it has bound ligand is for the TCR to undergo a conformational change after ligand binding. However, no direct evidence for this has been obtained, despite the availability of multiple crystal structures of TCR bound to ligand. More recently, a kinetic segregation model has been proposed, whereby the relatively small size of the TCR extracellular domain, when bound to ligand on the surface of an APC, forms a close contact that excludes larger transmembrane molecules from the vicinity of the TCR (Davis and van der Merwe, 2006). Larger transmembrane molecules such as CD45 and CD43 have intrinsic intracellular phosphatase activity. Exclusion of these molecules results in increased

Table 1 Summary of lymphocyte signalling molecules

Molecule	Lymphocyte specificity	Function	Substrate(s)
lck	T cells	Kinase	ITAMs, syk, zap-70, vav, c-Cbl
fyn	T cells	Kinase	ITAMs, syk, zap-70, vav, c-Cbl
lyn	B cells	Kinase	ITAMs, syk, vav, c-Cbl
zap-70	T cells	Kinase	Vav, LAT, slp-76, c-Cbl, Shc
syk	B cells and T cells	Kinase	Vav, LAT, BLNK, c-Cbl, Shc
itk	T cells	Kinase	PLCγ
rlk	T cells	Kinase	PLCγ
btk	B cells	Kinase	PLCγ
ERK	B cells and T cells	Kinase	Transcription factors (elk, fos, etc.)
JNK	B cells and T cells	Kinase	Transcription factors (jun, etc.)
p38	B cells and T cells	Kinase	Transcription factors (ATF-2, etc.)
Akt	B cells and T cells	Kinase	MAP3 K, IKK
Pak	B cells and T cells	Kinase	Actin
WASP	B cells and T cells	Adapter	Actin, Arp 2/3
PI3 kinase	B cells and T cells	Kinase	Phosphatidylinositol, PI(4)P, PI(4,5)P2
PKC	B cells and T cells	Kinase	btk, itk, rlk, CBM complex
IKK	B cells and T cells	Kinase	I-κB
SHIP	B cells and T cells	Phosphatase	PI(3,4,5)P3, Ins(1,3,4,5)P4
Shp-1	B cells and T cells	Phosphatase	lck, fyn, lyn
PTEN	B cells and T cells	Phosphatase	PI(3,4,5)P3, PI(3,4)P2
CD45	B cells and T cells	Phosphatase	lck
Calcineurin	B cells and T cells	Phosphatase	NFAT
Sos	B cells and T cells	GEF[a]	ras
RasGRP	B cells and T cells	GEF[a]	ras
Vav	B cells and T cells	GEF[a]	Rho GTPases, cdc42, rac
LAT	T cells	Adapter	Grb2, Shc, Gads, PLCγ
BLNK	B cells	Adapter	Grb2, vav, nck, PLCγ
Grb2	B cells and T cells	Adapter	Shc, LAT, BLNK, ITAMs, Sos
Slp-76	B cells and T cells	Adapter	vav, nck, Gads
Shc	B cells and T cells	Adapter	Grb2, LAT
Gads	B cells and T cells	Adapter	LAT, slp-76, BLNK
nck	B cells and T cells	Adapter	WASP, Pak, slp-76, BLNK
c-Cbl	B cells and T cells	Ubiquitin ligase	lck, fyn, lyn, syk, zap-70
PLCγ	B cells and T cells	Phospholipase	Phosphatidylinositol 4,5-biphosphate

[a]Guanine nucleotide exchange factor.

phosphorylation of molecules associated with the TCR, and this is the key event in initiating downstream signals.

Protein phosphorylation, particularly on tyrosine residues, is an important event in the initiation of cellular responses by antigen receptors on both B and T cells (Gold *et al*., 1990; Weiss and Littman, 1994). Crosslinking of antigen receptors and/or exclusion of phosphatases from the cytoplasmic area surrounding antigen receptors result in a rapid accumulation of phosphoproteins inside the cell. Since neither the TCR nor the BCR complex has intrinsic protein tyrosine kinase (PTK) activity, both activate non-receptor-type PTKs in the cytoplasm. **See also**: Protein Kinases

Antigen receptors consist of two functional modules: a ligand-binding module and a signalling module. Invariant CD3γ, δ and ε chains and ζ family dimers in the TCR complex and Ig-α/β subunits associated with the Ig receptor of the BCR are the signal-transducing modules. A 16 amino acid motif, termed the immunoreceptor tyrosine-based activation motif (ITAM), is present in each of the signalling subunits of the TCR (CD3γ, δ and ε and ζ chains) and BCR (Ig-α and Ig-β). The ITAM sequence consists of two tyrosine (Y) residues spaced 9–11 residues apart with isoleucine (I) or leucine (L) residues positioned three residues *C*-terminal to each tyrosine (YXXL/IX6-8YXXL/I). Upon recognition of antigen by the ligand-binding modules, ITAMs on the antigen-receptor signalling modules become phosphorylated, and phosphorylation of these ITAMs is required for all subsequent signalling events. Thus, the critical role of the antigen-receptor signalling modules is to be phosphorylated on tyrosine residues in the ITAMs by cytoplasmic PTKs. Phosphorylated ITAMs then serve as a docking site allowing the recruitment and activation of additional effector

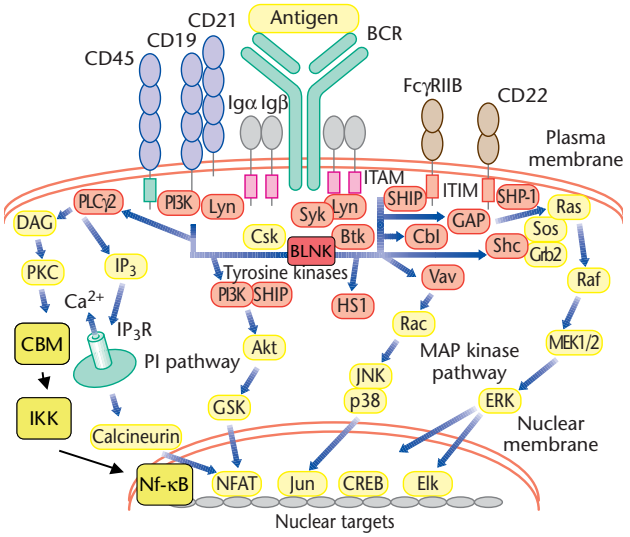

Figure 1 The signalling pathways following BCR stimulation. Molecules in red are major signalling components that are phosphorylated after BCR crosslinking. Arrows represent either phosphorylation induced after BCR ligation or connection between signalling molecules and downstream events or components. Coreceptors that play regulatory roles in BCR signalling are shown in colour.

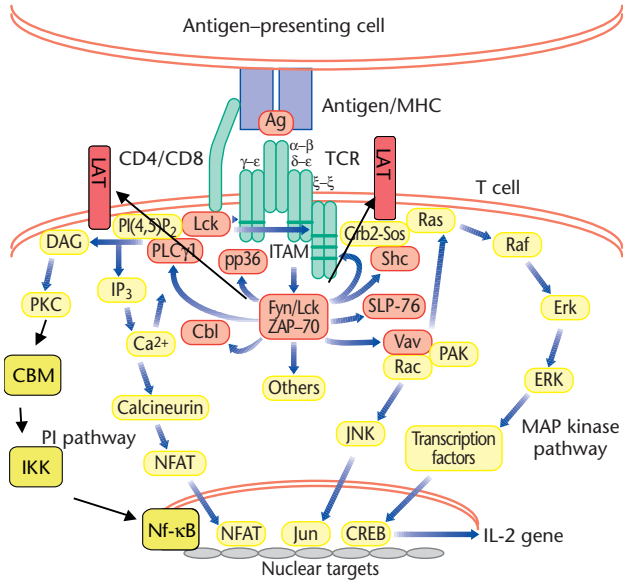

Figure 2 The signalling cascades activated by the TCR. The major signalling molecules which become tyrosine phosphorylated following TCR-CD4/CD8 crosslinking are shown in red.

molecules. **See also**: Antibodies; Antigen Recognition by Lymphocytes

Nonreceptor-type protein tyrosine kinases

Src family kinases are essential for signal transduction in both B and T cells. In B cells, Lyn, Blk and Fyn are activated and can phosphorylate the ITAMs of Igα/β. In T cells, Lck and Fyn are the kinases that phosphorylate ITAMs on the CD3 and ζ chains. The phosphorylated ITAMs then recruit kinases of the Syk/Zap-70 family. These kinases (Syk in B cells and Zap-70 in T cells) bind to phosphorylated ITAMs via an interaction between phosphotyrosine on the ITAMs and Src homology 2 (SH2) domains on Syk/Zap-70. Upon binding to the ITAMs syk/Zap-70 are phosphorylated by Src family kinases and become activated. Syk and Zap-70 have multiple substrates within the cell, but critical for the ability of these kinases to promote downstream signals is the ability to phosphorylate key adapter molecules. In B cells, the B-cell linker protein (BLNK) adapter is a crucial substrate for Syk, and in T cells the transmembrane adapter linker for activation of T cells (LAT) is a very important substrate for Zap-70.

Signals through another PTK, Btk, play a crucial role in B-cell development. Mutations in the gene encoding Btk result in X-linked agammaglobulinaemia (XLA) in humans and X-linked immunodeficiency (Xid) in mice. The former is characterized by a block in early B-cell development at the pro-B to pre-B transition, with increased numbers of pro-B cells and insufficient expansion and proliferation of pre-B cells. In Xid, early B-cell development appears not to be severely impaired, but numbers of mature B cells are decreased and the response to T-independent type II antigens is impaired. Btk is activated through phosphorylation by Src family PTKs upon crosslinking of the BCR, and its activity is negatively regulated by protein kinase C such as PKCβI/II. A critical step in Btk activation is its translocation from the cytosol to the inner leaflet of the plasma membrane where Btk binds to membrane phosphatidylinositol-3,4,5-trisphosphate through its pleckstrin homology (PH) domain. Targeting of PKCβI/II revealed a critical role for the Btk–PKCβI/II interaction, which indicates the regulatory role of PKCβI/II in the activation and translocation of Btk. Once activated, Btk phosphorylates phospholipase gamma 2 (PLCγ2) and is critical for its function. **See also**: Immunodeficiency, Primary: Affecting the Adaptive Immune System

Btk is part of a family of kinases known as the Tec kinases. In T cells, different Tec kinases play a similar role in signal transduction. The PTKs itk and rlk are part of the Tec family, and these molecules are activated after antigen recognition in T cells both by Src family kinases and association with phosphatidylinositol-3,4,5-trisphosphate. Similar to B cells, in T cells Tec family kinases are important for phosphorylation and activation of PLCγ1.

Control of cell morphology

Several proteins are involved in TCR and BCR signalling as adapter proteins. These molecules have SH2, Src homology 3 (SH3), proline-rich and PH domains that allow them to bind to molecules associated with the antigen-receptor signalling complex, and also bring in additional molecules important for signal transduction. Two adapter

proteins that are crucial in antigen-receptor activation signals are LAT in T cells and BLNK in B cells. LAT is a transmembrane molecule that has 10 tyrosines in the cytoplasmic domain. LAT is a substrate for Zap-70 and becomes highly phosphorylated after TCR-antigen recognition. LAT then serves as a docking site for numerous other molecules that are recruited based on interactions of SH2 domains with phosphorylated tyrosine. BLNK serves a function similar to LAT, but it is expressed in B cells. BLNK is located in the cytoplasm and contains 13 tyrosine residues, many of which become phosphorylated by Syk after BCR crosslinking, allowing molecules with SH2 domains to be recruited. The importance of LAT and BLNK is highlighted by the fact that mice deficient for LAT or BLNK have severe blocks in T-cell or B-cell development, respectively. Numerous downstream pathways are critically dependent on the presence of phosphorylated LAT and BLNK, including changes in cell morphology.

Vav is a 95-kDa protein that is tyrosine phosphorylated in response to various stimuli from the BCR, TCR and cytokine receptors. It contains a PH domain, one SH2, two SH3 domains and a guanine nucleotide exchange factor (GEF) domain. Thus, Vav acts as a GEF for the Rho family of small G protein GTPases. Vav is tyrosine phosphorylated by Src family PTKs and Syk/ZAP-70 upon stimulation of the BCR or TCR. Vav is brought into the proximity of the antigen receptor by association with LAT; Gads and slp-76 in T cells, and association with BLNK in B cells. Vav can then activate the Rho family GTPases cdc42 and Rac. In the active guanosine triphosphate (GTP)-bound form, cdc42 and Rac can activate the nonreceptor PTK p21 activated kinase (Pak) and also activate WASP (Wiskott–Aldrich syndrome protein). The activities of Pak and WASP then promote actin polymerization which can be important for changes in cell motility, cell polarization and the formation of the immunological synapse.

In T cells, TCR signalling results in the formation of a defined structure that involves close contacts between the membrane of the T cell and the APC. This structure is commonly called the immunological synapse (Grakoui *et al*., 1999). On the T-cell membrane, stimulated TCR become clustered into a small central area that is called the central supramolecular activation cluster or cSMAC. The corresponding peptide/MHC complexes cluster similarly on the APC membrane. Surrounding the central cluster of TCR is a ring of somewhat larger adhesion molecules that can include lymphocyte function-related antigen 1 (LFA-1) or other adhesion molecules, and this outer ring is called the peripheral SMAC (pSMAC). The small sizes of the TCR and MHC extracellular domains result in the two membranes residing very close together throughout the area of the synapse. There has been some debate about how formation of the synapse could facilitate T-cell activation. It does not seem to be important for initial events in T-cell signalling, as it requires 15–30 min for the synapse to form and most intracellular phosphorylation takes place in minutes. More likely, the synapse facilitates the directed secretion of cytokines and other effectors, as well as enables the efficient internalization of 'used' TCR.

Negative regulation of lymphocyte signalling

Although intracellular PTK activity is rapidly induced following antigen-receptor ligation, this activity is short-lived. Multiple negative feedback pathways function to limit the duration of kinase activity. The c-Cbl protein is expressed in numerous cell types and can act as a substrate for Src family kinases as well as for Syk/Zap-70. c- Cbl functions as an E3 ubiquitin ligase, and can bind to phosphorylated Src kinases and to Syk/Zap-70. This binding results in the ubiquitylation of these proteins leading to their subsequent degradation. Thus, antigen-receptor signalling is inhibited by the presence of c-Cbl.

Numerous phosphatases also play a role in reducing the activity of kinases following antigen-receptor signalling. Of note, the SH2 domain-containing phosphatase SHP-1 appears to play a critical role in modulating antigen-receptor signalling. SHP-1 is recruited to antigen-receptor signalling complexes by binding to phosphorylated tyrosine residues. However, in contrast to recruitment of Syk/Zap-70, SHP-1 is not recruited by phosphorylated ITAM sequences, but by a related sequence known as the immunoreceptor tyrosine-based inhibitory motif (ITIM). ITIMs (I/V/L)XYXX(L/V) are found in cytoplasmic domains of several transmembrane receptors that are known to function as inhibitors of antigen-receptor signalling. For example, in B cells, FcγRIIB, CD22 and paired Ig-like receptor B (PIR-B) contain ITIM sequences. In T cells, cytotoxic T-lymphocyte-associated antigen (CTLA4), programmed death 1 (PD-1), B- and T-lymphocyte attenuator (BTLA), and the signalling lymphocyte-activating molecule (SLAM) contain at least one ITIM sequence. After antigen-receptor ligation, tyrosines in ITIMs are phosphorylated by Src family kinases. SHP-1 is then recruited to the phospho-ITIM sequence and plays a negative role in signalling by dephosphorylation of Src family kinases and perhaps other substrates. Mutant mice lacking SHP-1 have a lowered threshold for both BCR and TCR signalling.

PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a lipid phosphatase that is also thought to play an inhibitory role in lymphocyte signalling. Mice with a deficiency of PTEN in T cells have hyperactive T cells, a breakdown in central tolerance and increased activity of Akt kinase and extracellular-regulated kinase (ERK). Similarly, mice with B-cell-specific PTEN deficiency have B cells that are very sensitive to stimulation. PTEN functions by regulating the turnover of inositol phospholipids, as described below. A second lipid phosphatase, SH2-containing inositol polyphosphate 5′-phosphatase or SHIP, is also involved in regulating turnover of inositol phospholipids. SHIP-deficient B cells have enhanced calcium flux and mitogen-activated protein kinase (MAPK) activation. SHIP-deficient T cells do not have major alterations in response to antigen-receptor

signalling, suggesting that SHIP plays a less prominent role in T cells.

Modulation of Antigen Receptor-mediated Signalling by Associating Coreceptors

In addition to the antigen receptors, many other surface molecules are reported to contribute to T- and B-cell activation; first, by functioning as coreceptors (e.g. CD4, CD8, CD19/CD21, CD22, FcγRIIB), which positively or negatively regulate the initiation of TCR or BCR signals; second, by increasing the avidity of the interaction with antigen or the APCs (e.g. LFA-1); and, third, by inducing separate signal transduction events that influence the cellular responses such as is the case for signals derived from the CD28/CTLA4 receptors on T cells and CD40 on B cells.

CD19 is a part of a complex containing CD21 (complement receptor 2, CR2) and CD81 (TAPA1). Coligation of the BCR with CD21 by antigen–antibody–complement complexes brings CD19 into close proximity with the BCR complex. Src family kinases such as Lyn phosphorylate cytoplasmic domains of CD19, and phosphatidyl inositol 3 kinase (PI3 kinase) is recruited to the phosphorylated CD19 through its SH2 domain and activated. Activation of PI3 kinase strongly augments B-cell activation and production of antibody by lowering the threshold for BCR-mediated signalling. Coligation of CD19 also promotes activation of Vav that augments signals as described above.

CD4 and CD8 function in two ways to enhance TCR-mediated signal transduction. The first is by binding to MHC molecules and thereby increasing the overall avidity of the TCR–ligand interaction. The second method is by associating with Src family kinases Lck and Fyn through motifs in the CD4 and CD8 cytoplasmic domains. This association facilitates recruitment of Lck and Fyn to the CD3 and ζ chain ITAMs after antigen recognition. The costimulatory molecule CD28 also augments TCR-signal transduction. A tyrosine in the cytoplasmic domain of CD28 can be phosphorylated by Src family kinases after antigen recognition and after binding of CD28 to one of its ligands (B7-1,B7-2). Phosphorylated CD28 can then recruit PI3 kinase, which activates the serine/threonine kinase, Akt. Activated Akt can contribute to changes in gene expression by promoting the activation of MAPK pathways. In B cells, the CD19 cytoplasmic domain can also recruit PI3 kinase after phosphorylation by Src family kinases. In B cells, active PI3 kinase can promote the recruitment of Btk and Vav.

The Phosphatidylinositol Pathway

PI3 kinase is part of a group of enzymes that controls the turnover of both membrane-associated and soluble inositol polyphosphates (Deane and Fruman, 2004). Also included in this group are the γ1 and γ2 isoforms of phospholipase C (PLC), SHIP and PTEN. Enhanced phosphoinositide turnover is linked with lymphocyte activation (**Figure 3**).

PLCγ functions in the cell by hydrolysing phosphatidylinositol 4,5- bisphosphate (PI(4,5)P2) to generate the signalling molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (Ins(1,4,5)P3). The second messenger DAG is important for activating multiple signalling pathways, and Ins(1,4,5)P3 binds IP3 receptors located on the endoplasmic reticulum (ER) and plasma membranes, leading to opening of calcium channels and an increase in intracellular calcium concentrations. **See also**: Phospholipases: Degradation of Membrane Phospholipids

BCR and TCR signalling induce PI(4,5)P2 hydrolysis by activating PLCγ1 and PLCγ2, the latter being more prevalent in B cells. The catalytic activity of the PLC isoforms is regulated by tyrosine phosphorylation. In B cells, this is mediated by Btk, whereas in T cells the Tec family kinases itk and rlk carry out this function. PLCγ1 and PLCγ2 can also can bind and be phosphorylated by Syk/Zap-70. Tec kinases and Syk/Zap-70 may be required to phosphorylate PLCγ2 on distinct sites and both may contribute to activation. Recruitment of PLCγ requires phospho-LAT and phospho-BLNK in T cells and B cells, respectively.

A second control of inositol phospholipids involves PI3 kinase, an enzyme that phosphorylates phosphatidylinositol, PI(4)P and PI(4,5)P2 on the D-3 portion of the myoinositol ring, yielding PI(3)P, PI(3,4)P2 and PI(3,4,5)P3, respectively. Activation of PI3 kinase resulting in elevated cellular levels of PI(3,4,5)P3 is a common response to the triggering of antigen receptors and costimulatory molecules, such as CD28 on T cells and CD19 on B cells. CD28 and CD19 have a cytoplasmic tyrosine that can be phosphorylated by Src family kinases. This can then serve as a docking site to recruit PI3 kinase via its SH2 domain. PI3 kinase can also be recruited to phosphorylated LAT and BLNK via an SH2 domain–phosphotyrosine interaction.

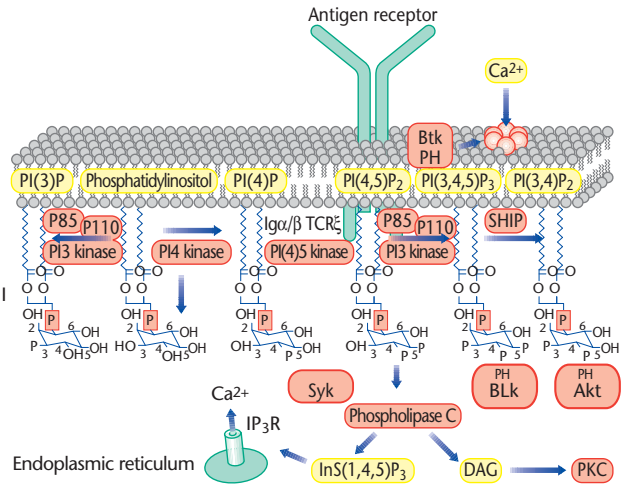

Figure 3 Putative phosphatidylinositol pathway and calcium flux induced by crosslinking of the BCR or TCR.

The PH domain acts as a membrane localization module through specific interaction with phosphoinositide phospholipids. Certain PH domains preferentially bind PI(3,4,5)P3 with high affinity, making proteins with PH domains direct targets for PI3 kinase regulation. The PH domain of Btk binds membrane PI(3,4,5)P3, suggesting that PI(3,4,5)P3 is a crucial second messenger in the activation of Btk by bringing it to the plasma membrane. Also, the proto-oncogenic serine/threonine kinase Akt is activated by PI3 kinase-mediated synthesis of phospholipid second messengers. In addition, Vav contains a PH domain and can be regulated by PI3 kinase activity.

In opposition to PI3 kinase, SHIP catalyses hydrolysis of the membrane phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3) to phosphatidylinositol 3,4-bisphosphate (PI(3,4)P2) and the soluble inositol polyphosphate Ins(1,3,4,5) P4 to Ins(1,3,4)P3. Thus, it acts to decrease the amounts of PI(3,4,5)P3 and to increase PI(3,4)P2. Recruitment of SHIP to the receptor complex results in the reduction of calcium influx from extracellular stores and negatively regulates signals from antigen receptors. Similarly, PTEN can dephosphorylate both PI(3,4,5)P3 and PI(3,4)P2 and thereby counteract many of the effects of PI3 kinase.

From the Membrane to the Nucleus

The large increases in protein phosphorylation, recruitment of molecules to the antigen-receptor complex and release of second messengers all serve to facilitate complex changes in gene expression that take place in response to lymphocyte activation. Here, three pathways will be highlighted that result in transcriptional changes: the activation of nuclear factor kappa B (NF-κB), nuclear factor of activated T cells (NFAT) and the MAP kinase pathway.

NF-κB pathway

The NF-κB family of transcription factors consists of five proteins that can exist in various hetero- and homodimeric forms (Schulze-Luehrmann and Ghosh, 2006). In resting cells, these dimers are retained in the cytoplasm by association with the inhibitory protein I-κB. After antigen-receptor activation, I-κB is phosphorylated. This leads to its subsequent ubiquitylation and degradation. After I-κB is degraded, NF-κB dimers are free to move to the nucleus and activate transcription. The phosphorylation of I-κB is mediated by the I kappa kinase (IKK) complex, which consists of the subunits IKKα and IKKβ, containing the kinase activity, and the structural and regulatory IKKγ subunit (also called NF-κB-essential-modulator (NEMO)).

Activation of IKK activity requires the presence of a multimolecular complex consisting of the Carma1, Bcl-10 and Malt1 molecules (the CBM complex). The intact CBM complex can cause the ubiquitylation of the IKK complex by a mechanism that includes the activities of TNF receptor-associated factor 6 (TRAF6), TRAF2 and ubiquitin-conjugating enzyme 13 (UBC13). Once IKK is polyubiquitylated, it becomes an active kinase and can phosphorylate I-κB and initiate its degradation. Activation of the CBM complex requires activity of protein kinase C (PKC). PKC is associated with the membrane in B cells (PKCβ) and T cells (PKCθ), and requires DAG and Ca^{2+} to be activated. Carma1 is phosphorylated by PKC and this results in activation of Bcl-10 and the formation of an oligomerized complex containing Carma1, Bcl-10 and Malt-1. Oligomerized CBM can then activate IKK. The reliance of PKC activation on DAG means that PLCγ activation is critical for NF-κB activation. This links nonreceptor PTKs that are activated immediately upon antigen-receptor binding to ligand to PLCγ activation and subsequent activation of the pathway leading to translocation of NF-κB to the nucleus and activation of transcription.

NFAT activation

The nuclear factor of activated T cells (NFAT) is also a transcription factor that is rendered inactive by retention in the cytoplasm (Macian, 2005). For NFAT, retention in the cytoplasm requires phosphorylation of NFAT on a key serine-rich motif. Removal of these phosphates results in exposure of a nuclear localization sequence and nuclear translocation of NFAT. In lymphocytes, the key phosphate group on NFAT is removed by the activity of the serine/threonine phosphatase calcineurin. Calcium elevation is required for the activity of calcineurin. Calcium does not directly promote calcineurin activity, but calcium complexed to the calcium-binding protein calmodulin activates calcineurin. The potent immunosuppressant drugs cyclosporin A and FK506 function by reducing the enzymatic activity of calcineurin, and thus prevent the nuclear translocation of NFAT.

The activation of NFAT-dependent gene expression depends entirely on elevation of cytoplasmic calcium concentration. Rapid calcium mobilization immediately following BCR or TCR stimulation has two phases. In the initial phase, a transient calcium release is induced from intracellular stores through IP3 receptor stimulation, followed by a continuous calcium influx from extracellular stores by the opening of the calcium channels present in the plasma membrane. Three types of IP3 receptors, coded by three distinct genes, have been identified. Most haematopoietic cells are reported to express at least two of the three types. Triple knockout of all three IP3 receptor isoforms in chicken DT40 B cells abolished the BCR-induced calcium mobilization from both internal and extracellular stores, whereas calcium mobilization is still observed after a single knockout of the receptors. Thus, the three IP3 receptor isoforms appear to be functionally redundant in BCR- or TCR-induced calcium mobilization. The BCR-induced apoptosis of mouse B-cell line WEHI-231 and proliferation of splenic B cells are inhibited by cyclosporin A, indicating the importance of calcineurin

and NFAT for these responses. BCR-induced apoptosis is also significantly inhibited by loss of all IP3 receptors, again suggesting an importance of intracellular calcium elevation and calcineurin in apoptosis induction via the antigen receptors.

Mitogen-activated protein (MAP) kinase cascades

Activation of signalling pathway driven by the small GTP-binding protein Ras following binding of antigen by the BCR or TCR has been extensively studied. Ras plays a key role in lymphocyte proliferation and differentiation. Ras activation is followed by the sequential stimulation of several cytoplasmic protein kinase signalling cascades, known as the mitogen-activated protein kinases (MAPKs). Ultimately, this phosphorylation cascade activates a set of transcription factors acting on early response genes and initiates cell proliferation. Thus, activation of the Ras and MAPK cascade is crucial for lymphocyte activation. Ras is maximally stimulated within 1–2 min after antigen-receptor ligation, as indicated by its transition from the RasGDP to the RasGTP state.

Activation of Ras requires a GEF to replace guanosine diphosphate (GDP) with GTP. In lymphocytes, there are two prominent Ras GEFs that can mediate Ras activation, Sos and RasGRP. Sos is activated when it is recruited to the TCR-signalling complex by the adapter molecule Grb2. Grb2 consists of one SH2 domain which can bind to tyrosine phosphorylated proteins, including Shc, LAT, BLNK and phosphorylated ITAMs, as well as two SH3 domains which interact with the proline-rich region in Sos. Sos is present in the cytosol in the resting state, and thus, it cannot efficiently activate membrane-bound Ras molecules. Upon antigen-receptor signalling, Grb2 recruits Sos to the antigen-receptor signalling complex. It is not entirely clear whether Grb2 recruits Sos by binding to LAT/BLNK, binding directly to phosphorylated ITAMs, or by binding to Shc, which can itself bind to phosphorylated ITAMs. Shc has one SH2 domain and thus can bind to the phosphorylated ITAMs in TCRζ chain or BCR-Igα and -Igβ. It is clear that Grb2 can bring Sos to the membrane, and membrane-localized Sos can then activate Ras. Thus, Grb2 is responsible for linking the receptor-associating PTKs to a GEF, Sos, consequently enabling Sos to activate membrane-bound Ras. This allows the TCR- or BCR-associating PTKs to modulate Ras activity. In lymphocytes, Grb2 and Sos are often unassociated prior to stimulation. Phosphorylation of Shc promotes the assembly of Grb2 and Sos. In summary, Ras activation in lymphocytes is dependent on the PTK-mediated membrane translocation of the GEF Sos by the Grb2 adaptor protein. **See also**: G Proteins; G Protein-coupled Receptors; Integrins: Signalling and Disease

A second GEF for activation of Ras in T cells is RasGRP1. RasGRP1 activity is dependent on DAG. Therefore, activation of PLCγ results in elevated RasGRP1 activity and increased Ras activation. There are at least four RasGRP family members, but RasGRP1 seems to be critical in T cells. RasGRP1-deficient mice have a severe impairment in T-cell development suggesting that RasGRP1 is the primary mechanism for Ras activation in the thymus. In B cells, development does not require RasGRP1 expression, but Ras activation is dependent on RasGRP1 and RasGRP3 in mature B cells. Thus, at some stages, MAPK activation in B cells is dependent on RasGRP family members.

Activation of Ras promotes a kinase cascade that ultimately results in activation of the MAPKs. MAPKs can be broadly grouped into three families: ERKs, JNKs and p38 type MAPK. All three families are activated by a similar cascade. The GTP-bound active form of Ras interacts with the *N*-terminal regulatory domain of a serine/threonine kinase referred to as MAP kinase kinase kinase (MAP3K). Activated MAP3K phosphorylates members of the MAPK/ERK kinase (MEK) family. After phosphorylation, MEKs activate the MAPKs (MAPK/ERK1,2) by threonine and tyrosine phosphorylation. The MEKs are part of the larger MAP kinase kinase (MKK) family that is comprised of several members, with different family members having a different ability to activate different MAP kinases.

MAPKs have been directly linked to the control of transcription via phosphorylation of certain transcription factors, including c-Jun, c-Fos and a group of E twenty-six (ETS) family members. MAPKs consist of a group of protein serine/threonine kinases. A number of distinct MAPKs have been identified, including extracellular signal-regulated kinase (ERK) 1/2, ERK3, ERK5, JNKs, stress-activated protein kinases (SAPKs), p38/RK/Mpk2, Fos-regulating kinase (FRK) and so on. In T cells, ERK1/2 are activated via Ras signals in response to TCR ligation. ERK1/2 activation is mediated by the MAPK/ERK kinases (MEK) 1/2, which are members of the MAPK kinase (MKK) gene superfamily. MEK1/2 themselves are activated through phosphorylation by distinct MAP3K, a family that includes Raf, c-Mos and Cot. **See also**: Regulatory Cascade; Regulatory Cascades: Function and Properties

Another family of small G proteins in lymphocytes is the Rho family of GTPases. This family includes Rho, Rac and Cdc42. As mentioned above, Rac and cdc42 are involved in coupling antigen-receptor signalling to actin polymerization. Rho family GTPases can also be involved in activation of MAP kinases. The Rho family GTPases are not activated by either the Sos or RasGRP1 GEFs. Instead, Vav contains a GEF domain and is responsible for activation of Rho family GTPases. Vav is activated by tyrosine phosphorylation by Src family PTKs and Syk/ZAP-70 after antigen-receptor ligation. Vav also contains a PH domain, an SH2 and two SH3 domains. Thus, Vav can interact with components of multiple signal transduction pathways, and is responsive to PI3 kinase dependent increases in PI(3,4,5)P3. Promotion of Rac-1 and other Rho family proteins to the active GTP-bound form, results in activation of JNK (Jun *N*-terminal kinase). Rho family

GTPases are not effective at activation of ERK MAP kinases. The importance of Vav in multiple signalling pathways is highlighted by the requirement for Vav in the positive selection of thymocytes. In the absence of Vav, T-cell development is severely impaired, that is poor proliferation of mature T cells and little interleukin 2 (IL-2) production in response to stimulation by TCR ligation, indicating that Vav plays a crucial role in the signal transduction pathway from the TCR.

Integration Signals

Our knowledge on the signalling pathways from antigen-receptors to the nucleus has grown dramatically in the past several years. The regulation of antigen-receptor signalling is central in determining the fate of immune cells. Cross-linking of the BCR or TCR by antigen produces signals leading to multiple potential outcomes: proliferation, differentiation, activation, apoptosis, tolerance or anergy of the B- and T-cell clones, suggesting that each reaction appears to be unique and requires the integration of distinct signalling pathways. As described in this article, many of the signalling pathways following TCR or BCR cross-linking resemble those found in other cell types, but diversity of the pathways is enormous. Final outcomes for the immune cells are the consequence of the integration of the positively and negatively regulated signals from antigen receptors, coreceptors, adhesion molecules and cytokine receptors. It is thus important to understand the molecular organization of the signalling cascades from various surface receptors and how these signal cascades interact and are integrated to govern the selection of downstream effector targets following the initial activation events driven by antigen receptors.

Structural or functional impairment of the signalling molecules often causes developmental arrest or unresponsiveness of the immune cells, resulting in various types of immunodeficiency states. However, recent studies clearly indicate that lymphocyte hyperreactivity is also associated with abnormally regulated signalling cascades. Such hyperactivated signals eventually give rise to autoimmune diseases or allergic states. Balancing the stimulatory pathways by signals from the inhibitory receptors has been shown to be responsible for determination of threshold for activation signals as well as termination of immune responses. In BCR-signalling cascades, the surface molecules, including CD22, PIR-B, killer Ig-like receptors (KIR), CD72 and FcγRIIB and the signalling molecules such as Lyn kinase and protein tyrosine phosphatases (PTPs), including SHP-1/2 and SHIP, play a crucial role in the maintenance of such balance. Signals from CTLA4 on T cells and KIR on natural killer cells seem to be crucial for negative regulation of the responses of these cell types. Further understanding of the molecular organization of signal transduction following antigen-receptor stimulation will provide new approaches for correction of various immunological disorders.

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