Signaling in Lymphocyte Activation

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SUMMARY

The fate of T and B lymphocytes, the key cells that direct the adaptive immune response, is regulated by a diverse network of signal transduction pathways. The T- and B-cell antigen receptors are coupled to intracellular tyrosine kinases and adaptor molecules to control the metabolism of inositol phospholipids and calcium release. The production of inositol polyphosphates and lipid second messengers directs the activity of downstream guanine-nucleotide-binding proteins and protein and lipid kinases/phosphatases that control lymphocyte transcriptional and metabolic programs. Lymphocyte activation is modulated by costimulatory molecules and cytokines that elicit intracellular signaling that is integrated with the antigen-receptor-controlled pathways.

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1 INTRODUCTION

The adaptive immune response is directed by B and T lymphocytes. These cells express specific receptors that recognize pathogen-derived antigens: the B-cell antigen receptor (BCR) and the T-cell antigen receptor (TCR), respectively. B lymphocytes have two principal roles: to produce and secrete specific antibodies/immunoglobulins, and to function as antigen-presenting cells (APCs). T cells have multiple roles in adaptive immune responses. In this context, peripheral T cells can be subdivided on the basis of whether they express CD8 or CD4, receptors that recognize class I and class II major histocompatibility complex (MHC) molecules, respectively. CD8⁺ T cells differentiate to cytolytic effectors that directly kill virus- or bacteria-infected cells. CD4⁺ T cells are referred to as “helper” T cells because they produce regulatory cytokines and chemokines that mediate autocrine or paracrine control of T-cell differentiation and/or regulate the differentiation of B cells and/or direct the activity of macrophages and neutrophils (O’Shea and Paul 2010). At least five major subpopulations of mature CD4⁺ cells exist with distinct functions that are tailored to deal with different pathogens. Th1 cells, characterized by interferon (IFN)γ production; Th2 cells, characterized by interleukin 4 (IL4) and IL13 production; Th17 cells, which produce the proinflammatory cytokines IL17 and IL22; regulatory T (Treg) cells that function to restrain autoimmunity and strong inflammatory responses; and follicular helper T (Tfh) cells, a class of effector CD4⁺ T cells that regulate the development of antigen-specific B-cell immunity.

The paradigm of the adaptive immune response is that a primary response to an antigen causes clonal expansion of antigen-reactive T or B cells and produces a large number of effector lymphocytes that cause clearance of the pathogen. Once the pathogen is cleared there is a contraction phase of the immune response characterized by loss of effector lymphocytes and the emergence of long-lived memory cells capable of mounting rapid secondary responses to reinfection with the original pathogen.

The proliferation and differentiation of mature lymphocytes in adaptive immune responses are directed by antigen receptors, costimulatory molecules, adhesion molecules, cytokines, and chemokines. These extrinsic stimuli are coupled to a diverse network of signal transduction pathways that control the transcriptional and metabolic programs that determine lymphocyte function. At the core of lymphocyte signal transduction is the regulated metabolism of inositol phospholipids and the resultant production of inositol polyphosphates and lipids such as polyunsaturated diacylglycerols (DAGs). These second messengers direct the activity of protein and lipid kinases

and guanine-nucleotide-binding proteins that control lymphocyte proliferation, differentiation, and effector function. Below, I outline both the unique and the conserved aspects of signaling in lymphocytes, focusing on signaling pathways controlled by antigen receptors and how these responses are subsequently shaped and modulated by cytokines and chemokines.

2 ANTIGEN-RECEPTOR STRUCTURE AND FUNCTION

The TCR and BCR are multiprotein complexes comprising subunits containing highly variable antigen-binding regions linked noncovalently to invariant signal transduction subunits. In both cases, rearrangements of the DNA sequences that encode the antigen-binding region create a diversity in antigen-receptor structures. A key feature of T- and B-cell populations is that each individual lymphocyte will express multiple copies of a unique antigen receptor with a single antigen specificity (defined by three complementarity-determining regions [CDRs]). It is the selectivity of antigen receptors that underpins immune specificity by ensuring that only those lymphocytes that recognize a specific pathogen are activated by it.

The BCR is composed of a highly variable membrane-bound immunoglobulin of either the IgM or IgD subclass in a complex with the invariant also known as Igα and Igβ (CD79a and CD79b) heterodimer (Tolar et al. 2009). Immunoglobulin subunits are highly variable because the genes that encode these proteins undergo rearrangements and somatic hypermutation during B-cell development, which produces a high degree of protein diversity ($\geq 10^{11}$ different receptors) (Schatz and Ji 2011).

The TCR is also characterized by highly variable antigen-binding subunits, either an αβ or a γδ dimer (Davis 2004; Krogsgaard and Davis 2005; Xiong and Raulet 2007). These are coupled to the invariant CD3 subunits γε, δε, and ζζ, which are essential for trafficking and stability of the γδ and αβ subunits at the plasma membrane. CD3 antigens also transmit signals into the cell across the plasma membrane. Like the BCR immunoglobulin sequences, the TCR-αβ or γδ dimers are highly variable because the genes that encode them undergo rearrangements (but not hypermutation) during their development. Indeed, there is potential for the production of $\sim 10^{18}$ different TCR-αβ receptor complexes. This is compared with to a minimal estimate of 10^{11} BCR complexes. The salient feature is that each T cell only expresses an αβ or a γδ receptor complex with a single specificity.

T cells that express TCR-γδ complexes are found predominantly at epithelial barriers (e.g., in the skin and gut epithelia). The ligands for TCR-γδ complexes are not well

defined but can be bacterial phosphoantigens, alkylamines, and aminobisphosphonates (Hayday 2009). T cells that express TCR- $\alpha\beta$ complexes typically recirculate between the blood, secondary lymphoid organs (spleen and lymph nodes), and the lymphatic system. The ligands for TCR- $\alpha\beta$ complexes are not antigens per se but rather pathogen- (or transplantation-antigen)-derived peptides bound to MHC molecules, a group of molecules that display the short, approximately nine-residue peptides on the surface of APCs. TCR- $\alpha\beta$ -expressing T cells are thus not triggered by soluble pathogen-derived peptides but only by peptide-MHC complexes on the surface of dendritic cells, B cells, and other cells that can function as APCs (Krogsgaard and Davis 2005).

3 IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS

The antigen-receptor subunits that mediate signal transduction are the invariant chains CD3 γ , δ , ϵ , ζ in T cells, Ig α and Ig β in B lymphocytes, and the FcR γ chain in mast cells (see below). These signaling subunits have no intrinsic signaling capacity, but all contain a YxxL/I-X₆₋₈-YxxL/I motif referred to as an immunoreceptor tyrosine-based activation motif (ITAM) (Abram and Lowell 2007; Love and Hayes 2010). The CD3 γ , δ , and ϵ subunits each contain a single ITAM, and there are three ITAMs in the CD3 ζ chain. The minimal TCR complex thus has 10 ITAMs. These couple the TCR to intracellular tyrosine kinases (see below). ITAM motifs are a defining feature of antigen-receptor complexes. Ig α and Ig β , the signaling subunits of the BCR, both have a single ITAM.

ITAM motifs are not restricted to the TCR and BCR. For example, mast cells comprise an important group of lymphocytes whose fate is determined by antigen-specific immunoglobulin. These cells respond to antigen because they express a high-affinity receptor for IgE. This receptor, termed Fc ϵ R1, binds to the immunoglobulin IgE with high affinity. When Fc ϵ R1-IgE complexes are cross-linked by polyvalent antigen they can trigger mast cell degranulation and the release of cytokines and allergic mediators. The Fc ϵ R1 is assembled from three subunits: the α subunit that binds to the Fc region of IgE, a β subunit that provides important accessory signaling, and the FcR γ chain, which is a signaling subunit that contains a single ITAM (Beaven and Metzger 1993; Abram and Lowell 2007; Samelson 2011).

TCR/BCR/Fc ϵ R1 signaling is initiated by the tyrosine phosphorylation of ITAMs by Src-family tyrosine kinases such as Lck and Fyn in T cells, Lyn in B cells, and Fyn in mast cells (Salmond et al. 2009). When both tyrosine residues are phosphorylated, the ITAM forms a high-affinity binding site for Syk-family tyrosine kinases; generally in T cells this is Zap-70 (Wang et al. 2010), whereas in B cells and

mast cells Syk is recruited (Chu et al. 1998). Zap-70 and Syk contain tandem SH2 domains that bind with high affinity to the doubly phosphorylated ITAM (Chu et al. 1998). The activation of Zap-70 or Syk is initiated by binding to phosphorylated ITAMs. This is proposed to release Syk/Zap-70 from an autoinhibited conformation and expose regulatory tyrosine residues for phosphorylation by Src-family kinases (Au-Yeung et al. 2009). The phosphorylation of tyrosine residues in the activation loop in the Zap-70/Syk catalytic domain, as well as two residues in the adjacent linker region, then further stimulates their catalytic activity. Antigen-receptor control of Syk-family tyrosine kinases is fundamental for lymphocyte activation and underpins the ability of antigen receptors to transduce signals from pathogen-derived antigens to the interior of lymphocytes (Mocsai et al. 2010; Wang et al. 2010).

How the Src-family kinases such as Lck are regulated is central to antigen-receptor signal transduction (Salmond et al. 2009). The activity of Lck is regulated by phosphorylation and dephosphorylation of a carboxy-terminal tyrosine (Y505) by the ubiquitously expressed kinase carboxy-terminal Src kinase (CSK), as well as autophosphorylation of the activation loop tyrosine residue, Y394. Phosphorylated Y505 forms an intramolecular binding site for the Lck SH2 domain, thereby locking the kinase into an autoinhibited state. The key to initiating the activation of Lck and its relatives is to dephosphorylate the carboxy-terminal tyrosine and relieve autoinhibition of the kinase. This is mediated by transmembrane-receptor-like tyrosine phosphatases, such as CD45 and CD148 (Hermiston et al. 2009; Zikherman et al. 2010). Hence in T cells, the Lck activation threshold is set by the balanced activity of the kinase-phosphatase pair CSK, which phosphorylates Y505, and CD45, which dephosphorylates this residue (Zikherman et al. 2010).

It is frequently assumed that triggering antigen receptors stimulates Src kinase family activity, and antigen receptors are often depicted as molecular switches that are either on or off. In reality, antigen receptors are always signaling and it is the intensity of the signal that changes. The assembly of antigen receptors at the plasma membrane is thus proposed to mediate low-level signaling and the engagement with high-affinity ligands (antigen or antigen-MHC) increases the intensity. Indeed Src-family kinases such as Lck are constitutively active before antigen-receptor engagement and cause low-level ITAM phosphorylation (Nika et al. 2010). The levels of ITAM phosphorylation are limited by tyrosine phosphatases, and the increases in ITAM phosphorylation that follow antigen-receptor engagement probably result from spatial constraints on the ITAM-phosphatase interaction (van der Merwe and Dushek 2011).

How are these spatial constraints regulated to explain how ligand occupancy triggers TCR signaling? Surprisingly,

we do not know, although there is no shortage of theories. Current models range from the ligand-induced conformational change to the idea that the TCR is a mechanosensor that converts the mechanical energy generated by antigen binding into a biochemical signal (Kim et al. 2009). One other idea well supported by experimental data is that binding of the TCR to peptide-MHC complexes on the surface of APCs causes spatial segregation of TCR complexes (which have small ectodomains) away from receptor tyrosine phosphatases such as CD45 and CD148 (which have very large ectodomains). This might locally perturb the kinase–phosphatase balance sufficiently to favor ITAM phosphorylation and Zap-70 recruitment (van der Merwe and Davis 2003; van der Merwe and Dushek 2011). Note that the MHC-binding coreceptors CD4 and CD8 are also thought to play a role in perturbing the kinase-phosphatase balance in localized areas of the T-cell membrane. CD4 and CD8 can thus promote TCR signaling by stabilizing interactions between the TCR and peptide-MHC ligands. However, the cytoplasmic domains of CD4 and CD8 constitutively bind Lck and hence facilitate the recruitment of this kinase to ligand-engaged TCR complexes (Artyomov et al. 2010).

What about the BCR and FcεR1? In quiescent B cells, the BCR may exist in an oligomeric autoinhibited state, and ligand occupancy could drive the dissociation of these oligomers into monomers that interact more effectively with downstream tyrosine kinases (Yang and Reth 2010a,b). For the FcεR1, the opposite is probably the case. This receptor binds IgE but is only effectively triggered when antigen oligomerizes the receptor (Beaven and Metzger 1993).

4 ADAPTOR MOLECULES FOR ANTIGEN RECEPTORS

The immediate substrates for tyrosine kinases activated by TCRs/BCRs/FcεR1s are specialized adaptor proteins that coordinate the localization and activation of key effector enzymes. In T cells and mast cells, the adaptors LAT and SLP76 are substrates for Zap-70 and Syk, respectively (Jordan and Koretzky 2010; Samelson 2011). In B cells, the adaptor coupling Syk to effector enzymes is B-cell linker protein (BLNK), also known as SLP65 (Koretzky et al. 2006).

LAT is an integral membrane protein with a cytoplasmic tail containing nine tyrosine residues. When phosphorylated, these act as docking sites for effector enzymes containing SH2 domains. For example, phosphorylated Y132 of LAT recruits phospholipase Cγ (PLCγ), a critical molecule for lymphocyte activation. The subsequent tyrosine phosphorylation of PLCγ activates the enzyme, resulting in the hydrolysis of its substrate phosphatidylinositol 4,5-bisphosphate (PIP₂). LAT not only recruits PLCγ but also

plays a complex role as a scaffold that ensures PLCγ activation. Phosphorylated Y171, Y191, and Y226 in LAT can thus bind to the SH2 domain of Grb2 family members such as Gads, which recruits SLP76 to the LAT complex.

SLP-76 contains three key tyrosine residues, a central SH3-binding proline-rich domain and a carboxy-terminal SH2 domain. The SLP76 proline-rich domain binds to the SH3 domain of Gads; the SLP76-Gads complex is then recruited to LAT via binding of the Gads SH2 domain binding to tyrosine-phosphorylated LAT.

Tyrosine-phosphorylated SLP76 can recruit a number of effector molecules into the LAT complex, notably the Tec-family tyrosine kinase Itk, which phosphorylates PLCγ, leading to its activation. The SH2 domain of SLP76 is also important because it binds to the cytosolic adaptor ADAP, which links SLP76 to the regulation of integrin-mediated cell adhesion. The LAT-SLP76 complex thus nucleates and organizes multiple TCR-dependent signaling pathways in T cells. Indeed, LAT and SLP76 are essential for TCR function: there are multiple defects in thymus T-cell development and peripheral T-cell function in the absence of these adaptors.

LAT and SLP-76 are equally important for mast cell function, coupling Syk to signaling pathways downstream from the FcεR1 (Alvarez-Errico et al. 2009; Kambayashi et al. 2009). However, neither LAT nor SLP76 is expressed in B cells; there, the predominant adaptor molecule is BLNK (Kurosaki and Hikida 2009). BLNK is a Syk substrate and contains nine tyrosine residues that are rapidly phosphorylated following BCR triggering. Its recruitment to the plasma membrane requires association with CIN85, and the BLNK-CIN85 complex coordinates recruitment of effectors such as PLCγ and Grb2-family adaptors (Oellerich et al. 2011). BLNK is essential for normal B-cell development and for peripheral B-cell function (see Fig. 1).

5 CALCIUM AND DIACYLGLYCEROL SIGNALING

A major function for antigen-receptor-coupled tyrosine kinases and adaptors is to regulate intracellular calcium levels and control DAG-mediated signaling (Oh-hora and Rao 2008; Matthews and Cantrell 2009). Inositol 1,4,5-trisphosphate (IP₃) produced by PLCγ binds to IP₃ receptors on endoplasmic reticulum (ER) membranes, initiating release of calcium from stores and an increase in cytosolic calcium concentration (Bootman 2012). This in turn triggers calcium entry across the plasma membrane via activation of highly selective store-operated calcium-release-activated calcium (CRAC) channels. Stromal interaction molecules 1 and 2 (STIM1 and STIM2) sense depletion of the ER stores and relocate to ER–plasma-membrane junctions. There they bind to the CRAC channel protein Orai1, which

Figure 1. Signaling downstream from immune receptors bearing immunoreceptor tyrosine-based activation motifs (ITAMs; yellow rectangles). T-cell receptors, B-cell receptors, and FcεR1s all contained ITAMs that can be tyrosine phosphorylated (red circles) by Src-family kinases such as Fyn and Lck. This creates docking sites for the recruitment and activation of the tyrosine kinases Zap-70 and Syk. These in turn phosphorylate adaptor complexes that recruit numerous additional signaling molecules that control phospholipid, calcium, small G protein, and kinase signaling.

activates the channels to allow entry of extracellular calcium to promote a sustained increase in intracellular calcium levels. This coupling of antigen receptors to CRAC channels allows lymphocytes to sustain high levels of intracellular calcium concentrations during an immune response (Hogan et al. 2010).

6 DOWNSTREAM FROM CALCIUM SIGNALING IN LYMPHOCYTES

Increases in intracellular calcium concentration in lymphocytes initiate signaling by the calcium/calmodulin-dependent protein kinase kinases (CaMKKs) (Matthews and Cantrell 2009). The best-studied role for calcium signaling

in both B and T lymphocytes, however, is control of calcineurin (also known as protein phosphatase 2B, PP2B), a protein phosphatase that controls the intracellular localization of members of the NFAT (nuclear factor of activated T cells) family of transcription factors (Im and Rao 2004; Muller and Rao 2010). These are key regulators of cytokine gene expression in B and T lymphocytes, in which they control expression of IL2, IL4, TNF, and IFNγ. In quiescent lymphocytes, before antigen-receptor engagement, NFATs are constitutively phosphorylated via the actions of NFAT kinases that include CK1 and GSK3. This phosphorylation of NFATs causes their nuclear exclusion as a result of binding to 14-3-3 proteins, thus maintaining them inactive in the cytosol. NFATs remain inactive until triggering of antigen

receptors raises intracellular free calcium levels, which activates calcineurin, which then dephosphorylates NFATs, allowing their translocation to the nucleus.

In the nucleus, NFATs form complexes with other transcription factors, bind to target genes and modulate gene transcription. In the context of *IL2* expression, NFAT–AP1 complexes act as positive regulators of *IL2* production, whereas complexes containing NFAT with the Foxp3 transcription factor appear to repress cytokine gene expression (Im and Rao 2004; Muller and Rao 2010). The impact of NFAT translocation to the nucleus on the T-cell transcriptional program thus depends on cellular context and the available NFAT-binding partners. Nevertheless, the rate-limiting step for NFAT activation is antigen-receptor-regulated increases in intracellular calcium and the resultant activation of calcineurin.

The importance of calcium/calcineurin signaling for T-cell activation is emphasized by the clinical efficacy of drugs based on the compound cyclosporin A or FK506 that prevent calcineurin activation and NFAT dephosphorylation (Gallo et al. 2006). These are potent T-cell immunosuppressants used for the prevention of organ transplant rejection and for the treatment of chronic T-cell-mediated autoimmune diseases, such as ectopic eczema.

7 DIACYLGLYCEROL SIGNALING IN LYMPHOCYTES

Multiple species of DAG are produced as intermediates in phospholipid resynthesis pathways. Consequently, quiescent lymphocytes have high levels of DAG before immune activation. However, antigen-receptor stimulation induces further production of polyunsaturated DAG by triggering PLC γ -mediated hydrolysis of PIP $_2$; in particular, triggering localized increases in DAG levels in membrane microdomains (Spitaler et al. 2006; Quann et al. 2009). DAG binds with high affinity to proteins that contain a conserved cysteine-rich domain (CRD) (H-X $_{12}$ -C-X $_2$ -C-X $_{13/14}$ -C-X $_2$ -C-X $_4$ -H-X $_2$ -C-X $_7$ -C). In lymphocytes, these proteins include the Ras/Rap guanyl-releasing protein (GRP) family of guanine nucleotide exchange factors (GEFs), which activate Ras and Rap GTPases, and the serine/threonine kinases protein kinase C (PKC) and protein kinase D (PKD).

8 PKC AND LYMPHOCYTES

Lymphocytes express multiple PKC isoforms, including α , β I, β II, δ , ϵ , η , and θ , and these have key roles in lymphocyte activation (Matthews and Cantrell 2009). They are important regulators of lymphocyte transcriptional programs and, in particular, control expression of genes encoding cytokines and cytokine receptors. DAG/PKC signaling also

plays a key role in controlling integrin-mediated cell adhesion and lymphocyte polarity. The direct substrates for PKCs include PKDs (Matthews et al. 2010). Lymphocytes predominantly express PKD2 and activation of this kinase requires *trans*-phosphorylation of conserved serine residues within the enzyme's catalytic domain (S701 and S711). These sites are substrates for both conventional and novel PKCs, and their phosphorylation is essential for efficient TCR-induced cytokine production and for optimal antibody production by B lymphocytes. Other PKC substrates include scaffolding proteins such as Carma1 and GEFs for the GTPases Ras and Rap1 (Matthews and Cantrell 2009). In particular, PKC-mediated phosphorylation of RapGEF2 is critical for activation of the GTPase Rap1, which controls the activity of the integrin LFA1 (also known as integrin $\alpha_L\beta_2$) and hence lymphocyte adhesion (Kinashi 2005).

The coordination of integrin-mediated cell adhesion by PKC and GTPases is essential to allow T cells, B cells, and natural killer (NK) cells to form tight contacts with APCs or target cells via a structure known as the immunological synapse (Dustin et al. 2010; Springer and Dustin 2011). These are formed between naïve T cells and APCs or effector cytolytic T cells and pathogen-infected target cells. B cells can also form immunological synapses with APCs in a process that potentiates antigen binding and processing of even membrane-tethered antigens (Harwood and Batista 2011). Immunological synapses are highly ordered structures characterized by the segregation of receptors and signaling molecules into distinct areas known as supramolecular activation clusters (SMACs). Stable immune synapses are arranged in concentric zones: antigen receptors accumulate in the center (cSMAC), whereas integrins segregate to the periphery (pSMAC). One common misconception is that the immune synapse is involved in the initiation of antigen-receptor signaling. The reality is that immune synapses are formed as a downstream consequence of antigen-receptor engagement. Immunological synapses provide a focus for DAG signaling following antigen-receptor engagement (Spitaler et al. 2006). Moreover, formation of immunological synapses is associated with the polarization of the microtubule-organizing center (MTOC) toward the target cell. This MTOC polarization is coordinated by calcium and DAG signaling pathways, with PKC family members playing a crucial role. The reorientation of the MTOC controls the ability of lymphocytes to direct cytokine secretion and to direct the exocytosis of secretory or lytic granules. For example, in cytotoxic T cells the immunological synapse directs the secretion of the granules that contain cytolytic effector molecules such as perforin and granzymes toward the target cell (Jenkins and Griffiths 2010).

One of the best characterized roles for PKCs in lymphocytes is the control of gene expression via the transcription

factor NF- κ B1 (also known as p50) (Oeckinghaus et al. 2011; Gerondakis and Siebenlist 2012). In quiescent lymphocytes, NF- κ B1 is sequestered in the cytosol in a complex with inhibitor of NF- κ B (I κ B). The activation of PKC results in the assembly of a complex comprising the scaffolding protein Carma1, Bcl10, and MALT1 (Blonska and Lin 2009). This PKC-induced Carma1-Bcl10-MALT1 complex subsequently binds to and activates the I κ B kinase (IKK) complex, which then phosphorylates I κ B, triggering its rapid ubiquitylation by the E3 ligase SCF- β TrCP and degradation by the proteasome. The removal of I κ B unmasks the nuclear localization sequence of NF- κ B1 and permits its translocation to the nucleus, where it stimulates the transcription of target genes. This mechanism is common to all lymphocytes but there is redundancy between PKC isoforms: in B lymphocytes, PKC β isoforms are involved, whereas in T cells PKC ϵ and θ are essential.

9 Ras SIGNALING AND LYMPHOCYTES

In quiescent lymphocytes Ras GTPases are predominantly inactive. Engagement of antigen receptors stimulates Ras proteins to accumulate in a GTP-bound state. This allows Ras to bind to the serine/threonine kinase Raf1, which in turn activates the kinase MEK1 that phosphorylates and activates the MAP kinases (MAPKs) ERK1 and ERK2 (Morrison 2012). Two major classes of GEFs couple antigen receptors to Ras activation: the Ras GRPs and SOS. Ras GRPs are activated by DAG and PKC-mediated phosphorylation. RasGRP1 acts downstream from antigen receptors in T cells, whereas RasGRP1 and RasGRP3 function in B cells, and RasGRP4 functions in mast cells. SOS is activated independently of DAG/PKC via a tyrosine-kinase-dependent pathway. It thus binds constitutively to the SH3 domains of the adaptor Grb2 and is recruited to the plasma membrane when the SH2 domain of Grb2 binds to tyrosine-phosphorylated adaptors such as LAT in T cells or Shc in B cells. Note that Ras is also activated by members of the common cytokine-receptor γ chain (γ_c) family of cytokines (see below), such as IL2. Receptors for these cytokines recruit SOS to the plasma membrane via Grb2 and the adaptor Shc (Harrison 2012).

The prototypical role for Ras in lymphocytes is to control gene transcription via ERK1 and ERK2 (Matthews and Cantrell 2009). These phosphorylate and regulate a number of key substrates, including the ternary complex factor (TCF) subfamily of ETS-domain transcription factors. They also control the activity of the RSK serine/threonine kinases that are known to have important functions in lymphocyte development and peripheral lymphocyte function. The initiating step for RSK activation is thus ERK1/2-mediated phosphorylation of S369, T365, and T577 in the

carboxy-terminal catalytic domain of the kinase. The activated carboxy-terminal catalytic domain of RSK then phosphorylates S386 intramolecularly to create a docking site for the kinase PDK1, which then phosphorylates S227 in the amino-terminal RSK kinase domain, thereby activating the enzyme (Finlay and Cantrell 2011a).

A full list of ERK1/2 substrates in lymphocytes is beyond our scope here but there have been some unexpected insights into the complexity of ERK signaling pathways in lymphocytes that warrant discussion. Flow cytometric-based assays that assess ERK activity at the single-cell level have shown that, when lymphocytes respond to an increasing strength of antigen-receptor stimulus, ERK activation is a digital (all or nothing) rather than an analog response (Chakraborty et al. 2009; Das et al. 2009). In this digital response, the frequency of cells within a population that activate ERK changes, each cell activating it to an equivalent level. This means, in practice, that even a strong antigen-receptor stimulus can only trigger a proportion of lymphocytes to activate ERKs at any one time. The digital nature of this ERK response creates signaling heterogeneity within the responding lymphocyte population.

10 COSTIMULATORY MOLECULES, CYTOKINES, AND LYMPHOCYTE ACTIVATION

Lymphocyte responses both prior and subsequent to antigen-receptor engagement are modulated by multiple costimulatory and coinhibitory receptors. Signaling via Toll-like receptors (TLRs) is also a major factor influencing the fate of lymphocytes during an immune response. Because T and B lymphocytes respond to antigens presented to them by APCs, lymphocyte activation can be regulated by the adhesion molecules and costimulatory molecules expressed by the APC. Note also that many of the cytokines that control lymphocyte fate are produced in response to TLR-mediated activation of dendritic cells and macrophages (Newton and Dixit 2012). Hence, the nature of the pathogen challenge to the innate immune system, and the resultant cytokine milieu modulate the adaptive immune response.

For T cells, key coreceptor molecules include the MHC receptors CD4 and CD8, and proteins such as CD28 (a positive coregulator) and CTLA4 and PD-1 (negative coregulators) (Artyomov et al. 2010; Francisco et al. 2010; Bour-Jordan et al. 2011; Walker and Sansom 2011). In B cells, molecules such as CD19 and the CD21 receptor for complement component C3d are essential (Carter and Fearon 1992; Depoil et al. 2008; Elgueta et al. 2009; Mackay et al. 2010) as are the TNF receptor family members CD40 and receptor for B-cell-activating factor (BAFFR) (Watts 2005; Elgueta et al. 2009; Karin and Gallagher 2009).

A full review of lymphocyte regulation by costimulatory factors is beyond our scope here but there are some general themes. Costimulatory molecules frequently work as adaptors to recruit signaling molecules to the plasma membrane and hence amplify antigen-receptor-mediated signaling. For example, CD4 and CD8 in T cells recruit Lck to the plasma membrane. Similarly, CD28 in T cells and CD19 in B cells both have cytoplasmic domains that can be tyrosine phosphorylated and thus can act as docking sites for SH2-domain-containing adaptors and enzymes. The CD19 cytoplasmic tail contains nine tyrosine residues with the potential to be phosphorylated and interact with signaling molecules including lipid kinases, Vav-family GEFs, and adaptor proteins such as Grb2. Other important examples of molecules that recruit key adaptor molecules to the plasma membrane are the lymphocytic activation molecule (SLAM) family of receptors and associated intracellular adaptors of the SLAM-associated protein (SAP) family (Veillette 2010).

The engagement of CD40 by its ligand (CD40L) leads to signals via adaptor proteins known as TNFR-associated factors (TRAFs), which activate signaling pathways, including MAPKs and NF- κ B (Lim and Staudt 2012).

The plethora of costimulatory molecules that can contribute to lymphocyte activation can be confusing, particularly because all seem to activate similar signal transduction pathways. The key message is that these receptors function at different times and in different contexts. For example, CD28 binds to the B7 family members CD80 and CD86, which are mainly expressed on APCs responding to TLR signaling. The ligand for CD40 is produced transiently by antigen-activated T cells and plays a key role in promoting specific T cell “help” to B cells by ensuring integration of signals between CD40-expressing B cells and antigen-primed T cells. In contrast, BAFF is mainly produced by neutrophils, monocytes, and macrophages and hence allows crosstalk between B cells and these cells of the innate immune system.

11 CYTOKINE SIGNALING IN LYMPHOCYTES

Cytokines that signal via the Janus tyrosine kinases (JAKs) (Harrison 2012), such as the γ_c family of cytokines, IFNs, and cytokines such as IL12 and IL23, are particularly important to the adaptive immune system (Rochman et al. 2009). For example, CD4-expressing $\alpha\beta$ T cells differentiate during immune responses to produce distinct effector subpopulations (O’Shea and Paul 2010) and the specification of these CD4⁺ T-cell subsets is controlled by cytokines that direct the combinatorial action of multiple chromatin regulators and key lineage-specifying transcription factors. For example, IL12 drives Th1 T-cell differentiation and IL6,

IL21, and IL23 drive Th17 cell differentiation. Moreover, cytokines have pleotropic roles. IL2 is important for the differentiation of antigen-primed CD8⁺ T cells to effector cytotoxic T cells (CTLs) but is also required for optimal Th1 T-cell differentiation and for the development of Treg cells.

One striking feature of lymphocyte biology is that the ability of cells to respond to cytokines (i.e., to express particular cytokine receptors) can be shaped by antigen-receptor triggering. Cytokine production by cells of the immune system is, in turn, controlled by triggering of antigen receptors in T and B cells or by receptors of the innate immune system. A prototypical example is IL2, which is only produced by antigen-receptor-activated T cells and B cells or pathogen-triggered dendritic cells. Moreover, expression of the IL2 receptor (IL2R) is tightly controlled by immune activation. The IL2R receptor complex consists of a γ_c , a β subunit (CD122), and an α subunit (CD25). The expression of CD25 is rate limiting as it determines the ability of the receptor to bind IL2 with high affinity. Importantly, CD25 is not expressed on naïve CD4 and CD8 T cells but only on activated T cells. In addition, the expression of CD25 is transient and its sustained expression requires constant immune stimulation. IL2 responsiveness is thus tightly linked to antigen-receptor triggering to ensure the tight control of T cells by IL2. IL12 receptors are similar: these are only expressed on activated T cells. Furthermore, IL12 receptor expression needs to be sustained by IL2 and there is tight control of IL12 secretion by pathogen-activated dendritic cells and macrophages. Such dynamic regulation of cytokine and cytokine-receptor expression during immune activation ensures the immune specificity of cytokine action (i.e., only lymphocytes that have been primed by antigen-receptor triggering can respond to IL12). Note the production of cytokines is also limited to either pathogen-activated innate immune cells or antigen-activated lymphocytes (Fig. 2).

Cytokines that activate JAKs regulate the function of SH2-domain-containing transcription factors known as STATs (signal transducers and activators of transcription) (Ghoreschi et al. 2009; Harrison 2012). There are four JAKs (JAK1, JAK2, JAK3, and Tyk2) and 7 STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6). A single JAK, or combination of JAKs, associates selectively with the cytoplasmic domains of the cytokine receptors. The model for JAK activation is that ligand occupancy of cytokine-receptor dimers results in JAK transphosphorylation and activation. The type I IFN receptors signal via JAK1 and Tyk2; IL12 and IL23 receptors signal via JAK2 and Tyk2. The IFN γ receptor activates JAK1 and JAK2, whereas γ_c -containing receptors, which include the receptors for IL2, IL4, IL7, IL9, IL15, and IL21, use JAK1 and JAK3. JAK activation results in phosphorylation of tyrosine residues

Figure 2. Signaling by interleukin (IL) receptors. Many cytokines signal via receptors linked to Janus tyrosine kinases (JAKs), which regulate the SH2-domain-containing transcription factors STATs. The different ILs produced by different cell types activate receptors coupled to different combinations of JAKs and STATs.

within the cytoplasmic tails of cytokine-receptor subunits that act as docking sites for the SH2 domains of the STATs. The recruitment of STATs leads to their phosphorylation by the JAKs. The STATs then form homodimers via SH2 domain interactions and translocate to the nucleus to bind STAT-response elements in DNA. STATs control lymphocyte transcriptional programs by working as transcriptional activators but they can also function as gene repressors (O'Shea and Paul 2010).

The specificity of STAT activation is determined by the selectivity of STAT SH2 domains for the STAT-recruitment motifs in the different cytokine-receptor subunits. For example, IL2 predominantly activates STAT5, because tyrosine-phosphorylated IL2R β subunits contain a high-affinity binding site for STAT5. The IL4 receptor, which comprises γ_c and a unique IL4 receptor α chain, activates STAT6 because tyrosine-phosphorylated IL4 receptors selectively bind STAT6. Figure 2 summarizes current information

about the JAK/STAT signaling combinations that function downstream from the major cytokine receptors.

It should be stressed that although the activation of STATs is pivotal for cytokine actions it is usually not sufficient to mimic the effects of cytokines. Indeed, cytokines can regulate other signal transduction pathways, some of which are shared with other receptors (e.g., IL2 and IL15 also activate Ras/ERK signaling) (Cantrell 2003). Moreover, many cytokines induce accumulation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃), a product of phosphoinositide 3-kinases (PI3Ks) (Okkenhaug and Fruman 2010; Finlay and Cantrell 2011).

12 PI3K-MEDIATED SIGNALING IN LYMPHOCYTES

PI3K signaling is important for lymphocyte activation and integrates multiple receptor inputs. For example, in naïve T cells, low basal levels of PIP₃ are maintained by IL7

signaling; these increase strikingly in response to triggering of the antigen-receptor complex and are then sustained by stimuli from costimulatory molecules such as CD28. Cytokines such as IL2 and IL15 can then further sustain intracellular concentrations of PIP₃. Similarly, in B cells, cytokines such as BAFF and low-level signaling by non-antigen-engaged BCRs maintain a low level of PIP₃ (Srinivasan et al. 2009). The levels of PIP₃ increase following BCR activation, and costimulatory molecules such as CD19 and cytokines such as IL4 can also sustain levels of this lipid.

Antigen receptor and cytokines control PIP₃ metabolism in lymphocytes via class I PI3Ks, which typically exist in a complex comprising a p110 catalytic subunit and an 85-kDa SH2-domain-containing regulatory/adaptor subunit. Four p110 isoforms exist (α , β , γ , and δ) and two p85 subunits (α and β) exist. These different isoforms function in distinct pathways in lymphocytes, and expression of p110 δ is restricted to hematopoietic cells. p110 δ produces the PIP₃ that is generated in response to many antigen receptors and cytokines, whereas p110 γ , which heterodimerizes with the p101 regulatory subunit rather than a p85-type subunit, is involved in chemokine receptor signaling (Okkenhaug and Fruman 2010).

The production of PIP₃ requires recruitment of PI3K to the plasma membrane. There are two possible mechanisms: binding of the SH2 domain of p85 to phosphorylated tyrosine residues in receptor cytoplasmic domains or membrane-localized adaptors; and direct recruitment of p110 by Ras. In the case of the BCR, CD19 recruits PI3K to the plasma membrane via binding of p85 to its tyrosine-phosphorylated cytoplasmic domain. Tyrosine-phosphorylated cytokine receptors similarly recruit PI3K by binding p85. Surprisingly, how TCR and CD28 signaling induces PIP₃ accumulation is not known, but direct recruitment to tyrosine-phosphorylated CD28 does not occur, and it is more likely that adaptors such as LAT or SLP76 are important.

PIP₃ binds to pleckstrin homology (PH) domains in other signaling proteins to control their activity and subcellular localization. In lymphocytes, these include Tec-family tyrosine kinases such as Itk and Btk, GEFs for Rho family GTPases, and the kinases PDK1 and Akt (also known as PKB) (Hemmings and Restuccia 2012). Akt is activated by PDK1-mediated phosphorylation of T308 within its catalytic domain. This is PIP₃ dependent probably because the binding of PIP₃ to the Akt PH domain causes a conformational change that allows PDK1 to phosphorylate T308. PDK1 also has a PIP₃-binding PH domain, but this promotes translocation of the enzyme to the plasma membrane (where it can colocalize with Akt) rather than enzyme activation (Finlay and Cantrell 2011).

Once activated, Akt phosphorylates a number of critical signaling molecules. For example, it phosphorylates and

inactivates the Rheb GAP TSC2, causing accumulation of Rheb-GTP complexes, which play a role in activating the mTORC1 complex (mammalian target of rapamycin complex 1) (Laplanche and Sabatini 2012). Akt also phosphorylates the transcription factors Foxo1/3 and Foxo4A. These Foxo family transcription factors are nuclear and active in quiescent cells but, when phosphorylated, they exit the nucleus and form a complex with 14-3-3 proteins in the cytosol, which terminates their transcriptional activity.

Akt is fundamentally important in many cells because it controls nutrient uptake and cellular metabolism. In particular, activated lymphocytes up-regulate glucose, amino acid and iron uptake, and switch their metabolism to glycolysis (see Ward and Thompson 2012). This increases cellular energy production and nutrient uptake to support the increased biosynthetic demands of rapid cell proliferation. Note, however, that it is difficult to ascribe a universal function for Akt that holds for all lymphocyte subpopulations. For example, Akt is important for metabolism and cell survival in peripheral B lymphocytes (Srinivasan et al. 2009) and in T lymphocyte progenitors in the thymus, but is not essential for metabolism or for the survival of peripheral or effector cytotoxic T cells (Finlay and Cantrell 2011). Moreover, the Akt/Foxo pathway has a critical role controlling expression of the recombinase genes responsible for antigen-receptor diversity in B cells (Kuo and Schlisel 2009) but there is no evidence for such a role in T cells. The molecular basis for these differences is not understood but probably reflects redundancies with other kinases that have similar substrate specificities (e.g., SGK1).

Akt/Foxo signaling is also uniquely linked to the regulation of the expression of key cytokine and chemokine receptors and adhesion molecules in lymphocytes (Hedrick 2009; Lorenz 2009; Macintyre et al. 2011). Hence, when Akt is inactive in quiescent lymphocytes, nonphosphorylated Foxo1, Foxo3, Foxo3A, and Foxo4 are found in the nucleus, where they drive transcription of genes encoding the receptor for IL7, an essential homeostatic cytokine for lymphocytes. Moreover, Foxo transcription factors also drive expression of the transcription factor KLF2; this directly regulates transcription of adhesion molecules and chemokine receptors that together control lymphocyte entry and egress from secondary lymphoid tissues and lymphocyte positioning in lymphoid tissue. The activation of Akt thus causes lymphocytes to change their trafficking program around the body. Akt activation also changes the cytokine-receptor profile of T cells and hence the ability of cytokines to determine T-cell fate.

In many cells, a key role for Akt is to control the activity of the mammalian target of rapamycin complex 1 (mTORC1) (Laplanche and Sabatini 2012). Rapamycin is a powerful immunosuppressant that is used in the clinic to

prevent rejection of organ transplants. mTORC1 coordinates inputs from nutrients and antigen and cytokine receptors to control T-cell differentiation (Powell and Delgoffe 2010). The molecular mechanisms used by mTORC1 to control T-cell differentiation are not fully understood; neither are the signaling processes that activate mTORC1. There is, however, evidence that mTORC1 controls expression of genes encoding effector cytokines and cytolytic molecules. Moreover, mTORC1 directs the tissue-homing properties of T cells by regulating the expression of chemokine and adhesion receptors (Sinclair et al. 2008).

13 INHIBITORY SIGNALS AND LYMPHOCYTE ACTIVATION

Signals from APCs and other immune cells can also deliver inhibitory signals to lymphocytes to ensure immune homeostasis. Indeed these are vital for a balanced immune response because a failure to limit immune responses results in excessive inflammation and potentially autoimmunity. Examples of signaling molecules that mediate key negative-feedback pathways in lymphocytes include SHIP, a lipid phosphatase with specificity for the 5' position of PIP₃ (Parry et al. 2010). SHIP is recruited to the plasma membrane by the binding of its SH2 domain to a tyrosine-phosphorylated immune cell tyrosine-based inhibitory motif (ITIM) located in the cytosolic domain of cell-surface receptors and dampens production of PIP₃. A prototypical example of this feedback process occurs in B cells when coligation of the BCR with the FcγRIIB by antigen-antibody complexes results in tyrosine phosphorylation of the ITIM in FcγRIIB (Daëron and Lesourne 2006). SHIP binds to the phosphorylated ITIM, thereby recruiting this inositol 5' phosphatase into the BCR-FcγRIIB complex. SHIP dephosphorylates PIP₃ to produce PI(3,4)P₂ and, accordingly, diminishes the BCR-dependent elevation of intracellular PIP₃ levels. There are many other examples of ITIM-containing receptors that play an important role in immune homeostasis. For example, an extensive family of sialic acid-binding immunoglobulin-like lectins, siglecs, responds to sialylated glycans to regulate lymphocyte function (Nitschke 2009; Cao and Crocker 2011). Siglecs are key regulators of B cell, NK cell, and macrophage biology.

In T cells, transmembrane receptors such CTLA4 and PD1 are critical for limiting T-cell function during immunity and tolerance (Veillette et al. 2002; Francisco et al. 2010; Bour-Jordan et al. 2011). The purpose of PD1 signaling is to limit the expansion of effector T cells during an immune response and hence to limit the pathology and tissue damage associated with effector CD8⁺ T-cell-mediated tissue destruction. However, the failure to control chronic viral infections such as HIV results from inhibitory-receptor-

driven exhaustion of antigen-specific T cells, demonstrating how the balancing of positive- and negative-feedback signaling needs to be finely tuned to ensure a favorable outcome. The impact of any imbalance of these pathways on human health is enormous: a failure of feedback control leads to autoimmunity; too much feedback control can limit the ability of the immune system to clear the pathogen.

B lymphocytes express the siglec family member CD22 (also known as siglec2), which inhibits B-cell signaling and B-cell-mediated autoimmunity by recruiting SHP1 (Lorenz 2009; Nitschke 2009). CD22 interacts with ligands carrying α2–6-linked sialic acids both in *cis* and in *trans* to modulate the BCR signaling threshold. The importance of SHP1 is strikingly illustrated by the phenotype of the moth-eaten (me/me) mouse, which lacks SHP1 tyrosine phosphatase activity and displays a variety of hematopoietic and immune disorders that result in death two or three weeks after birth (Lorenz 2009).

There are additional key negative regulator receptors in which there is either no classical ITIM or controversy as to the importance of the recruitment of phosphatases. CTLA4 is an example. It is an essential negative regulator of T-cell-mediated immune responses: CTLA4-deficient mice show a fatal lymphoproliferative disorder. CTLA4 binds the same two ligands (CD80 and CD86) the costimulatory molecule CD28 binds. The engagement of CD28 by CD80 or CD86 results in T-cell costimulation, whereas CTLA4 engagement results in inhibition of T-cell activation. CTLA4 might deliver a negative signal to the T cell by recruiting tyrosine phosphatases to the plasma membrane. However, two other models exist. One proposes that CTLA4 activates T cells to increase their motility and that this prevents T cells from making stable contacts with APCs (Rudd 2008). The other proposes that CTLA4 competes with CD28 for ligand but binds to CD80/86 with higher avidity than does CD28. Indeed CTLA4 has now been shown to capture its ligands CD80 and CD86 by *trans*-endocytosis (Qureshi et al. 2011). It could thus inhibit CD28 costimulation by depleting CD28 ligands. These models are not necessarily mutually exclusive, and how CTLA4 and the other inhibitory molecules exert essential feedback control is still the subject of much debate.

14 CONCLUDING REMARKS

In lymphocytes, signal inputs generated by specific pathogens regulate the activity of evolutionarily conserved signaling pathways. Antigen receptors direct the immune response but lymphocyte signaling is also controlled by cytokines and chemokines that are not antigen specific. These antigen-specific and -nonspecific elements of lymphocyte signal transduction are tightly coupled because

antigen-receptor signaling controls the repertoire of cytokine and chemokine receptors and adhesion molecules expressed by lymphocytes. Antigen receptors also direct lymphocyte trafficking between the blood, peripheral tissues, and secondary lymphoid organs and hence control the cytokine milieu available to these cells. This coordination of antigen receptor and cytokine signaling ensures the immune specificity of lymphocyte activation and is fundamental for adaptive immune responses.

REFERENCES

*Reference is also in this collection.

- Abram CL, Lowell CA. 2007. The expanding role for ITAM-based signaling pathways in immune cells. *Sci STKE* **2007**: re2.
- Alarcón B, Swamy M, van Santen HM, Schamel WW. 2006. T-cell antigen-receptor stoichiometry: Pre-clustering for sensitivity. *EMBO Rep* **7**: 490–495.
- Alvarez-Errico D, Lessmann E, Rivera J. 2009. Adapters in the organization of mast cell signaling. *Immunol Rev* **232**: 195–217.
- Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. 2010. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proc Natl Acad Sci* **107**: 16916–16921.
- Au-Yeung BB, Deindl S, Hsu LY, Palacios EH, Levin SE, Kuriyan J, Weiss A. 2009. The structure, regulation, and function of ZAP-70. *Immunol Rev* **228**: 41–57.
- Beaven MA, Metzger H. 1993. Signal transduction by Fc receptors: The FcεRI case. *Immunol Today* **14**: 222–226.
- Blonska M, Lin X. 2009. CARMA1-mediated NF-κB and JNK activation in lymphocytes. *Immunol Rev* **228**: 199–211.
- * Bootman MD. 2012. Calcium signaling. *Cold Spring Harb Perspect Biol* **4**: a011171.
- Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. 2011. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol Rev* **241**: 180–205.
- Cantrell DA. 2003. GTPases and T cell activation. *Immunol Rev* **192**: 122–130.
- Cao H, Crocker PR. 2011. Evolution of CD33-related siglecs: Regulating host immune functions and escaping pathogen exploitation? *Immunology* **132**: 18–26.
- Carter RH, Fearon DT. 1992. CD19: Lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* **256**: 105–107.
- Chakraborty AK, Das J, Zikherman J, Yang M, Govern CC, Ho M, Weiss A, Roose J. 2009. Molecular origin and functional consequences of digital signaling and hysteresis during Ras activation in lymphocytes. *Sci Signal* **2**: t2.
- Chow LM, Veillette A. 1995. The Src and Csk families of tyrosine protein kinases in hemopoietic cells. *Semin Immunol* **7**: 207–226.
- Chu DH, Morita CT, Weiss A. 1998. The Syk family of protein tyrosine kinases in T-cell activation and development. *Immunol Rev* **165**: 167–180.
- Daéron M, Lesourne R. 2006. Negative signaling in Fc receptor complexes. *Adv Immunol* **89**: 39–86.
- Das J, Ho M, Zikherman J, Govern C, Yang M, Weiss A, Chakraborty AK, Roose JP. 2009. Digital signaling and hysteresis characterize ras activation in lymphoid cells. *Cell* **136**: 337–351.
- Davis MM. 2004. The evolutionary and structural “logic” of antigen receptor diversity. *Semin Immunol* **16**: 239–243.
- Depoil D, Fleire S, Treanor BL, Weber M, Harwood NE, Marchbank KL, Tybulewicz VL, Batista FD. 2008. CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nat Immunol* **9**: 63–72.
- Dustin ML, Chakraborty AK, Shaw AS. 2010. Understanding the structure and function of the immunological synapse. *Cold Spring Harb Perspect Biol* **2**: a002311.
- Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. 2009. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev* **229**: 152–172.
- Finlay D, Cantrell D. 2011a. The coordination of T-cell function by serine/threonine kinases. *Cold Spring Harb Perspect Biol* **3**: a002261.
- Finlay D, Cantrell D. 2011b. Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol* **11**: 109–117.
- Francisco LM, Sage PT, Sharpe AH. 2010. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* **236**: 219–242.
- Gallo EM, Canté-Barrett K, Crabtree GR. 2006. Lymphocyte calcium signaling from membrane to nucleus. *Nat Immunol* **7**: 25–32.
- Gerondakis S, Siebenlist U. 2012. Roles of the NF-κB pathway in lymphocyte development and function. *Cold Spring Harb Perspect Biol* **2**: a000182.
- Ghoreschi K, Laurence A, O’Shea JJ. 2009. Janus kinases in immune cell signaling. *Immunol Rev* **228**: 273–287.
- Harwood NE, Batista FD. 2011. The cytoskeleton coordinates the early events of B-cell activation. *Cold Spring Harb Perspect Biol* **3**: a002360.
- * Harrison DA. 2012. The JAK/STAT pathway. *Cold Spring Harb Perspect Biol* **4**: a011205.
- Hayday AC. 2009. γδ T cells and the lymphoid stress-surveillance response. *Immunity* **31**: 184–196.
- Hedrick SM. 2009. The cunning little vixen: Foxo and the cycle of life and death. *Nat Immunol* **10**: 1057–1063.
- * Hemmings BA, Restuccia DE. 2012. PI3K-PKB/Akt pathway. *Cold Spring Harb Perspect Biol* **4**: a011189.
- Hermiston ML, Zikherman J, Zhu JW. 2009. CD45, CD148, and Lyp/Pep: Critical phosphatases regulating Src family kinase signaling networks in immune cells. *Immunol Rev* **228**: 288–311.
- Hogan PG, Lewis RS, Rao A. 2010. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* **28**: 491–533.
- Im SH, Rao A. 2004. Activation and deactivation of gene expression by Ca²⁺/calineurin-NFAT-mediated signaling. *Mol Cells* **18**: 1–9.
- Jenkins MR, Griffiths GM. 2010. The synapse and cytolytic machinery of cytotoxic T cells. *Curr Opin Immunol* **22**: 308–313.
- Jordan MS, Koretzky GA. 2010. Coordination of receptor signaling in multiple hematopoietic cell lineages by the adaptor protein SLP-76. *Cold Spring Harb Perspect Biol* **2**: a002501.
- Kambayashi T, Larosa DE, Silverman MA, Koretzky GA. 2009. Cooperation of adapter molecules in proximal signaling cascades during allergic inflammation. *Immunol Rev* **232**: 99–114.
- Karin M, Gallagher E. 2009. TNFR signaling: Ubiquitin-conjugated TRAF6 signals control stop-and-go for MAPK signaling complexes. *Immunol Rev* **228**: 225–240.
- Kim ST, Takeuchi K, Sun ZY, Touma M, Castro CE, Fahmy A, Lang MJ, Wagner G, Reinherz EL. 2009. The αβ T cell receptor is an anisotropic mechanosensor. *J Biol Chem* **284**: 31028–31037.
- Kinashi T. 2005. Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol* **5**: 546–559.
- Koretzky GA, Abtahian F, Silverman MA. 2006. SLP76 and SLP65: Complex regulation of signalling in lymphocytes and beyond. *Nat Rev Immunol* **6**: 67–78.
- Krogsgaard M, Davis MM. 2005. How T cells “see” antigen. *Nat Immunol* **6**: 239–245.
- Kuo T, Schlissel MS. 2009. Mechanisms controlling expression of the RAG locus during lymphocyte development. *Curr Opin Immunol* **21**: 173–178.
- Kurosaki T, Hikida M. 2009. Tyrosine kinases and their substrates in B lymphocytes. *Immunol Rev* **228**: 132–148.
- * Laplante M, Sabatini DM. 2012. mTOR signaling. *Cold Spring Harb Perspect Biol* **4**: a011593.

- * Lim K-H, Staudt LM. 2013. Toll-like receptor signaling. *Cold Spring Harb Perspect Biol* 5: a011247.
- Lorenz U. 2009. SHP-1 and SHP-2 in T cells: Two phosphatases functioning at many levels. *Immunol Rev* 228: 342–359.
- Love PE, Hayes SM. 2010. ITAM-mediated signaling by the T-cell antigen receptor. *Cold Spring Harb Perspect Biol* 2: a002485.
- Macintyre AN, Finlay D, Preston G, Sinclair LV, Waugh CM, Tamas P, Feijoo C, Okkenhaug K, Cantrell DA. 2011. Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. *Immunity* 34: 224–236.
- Mackay F, Figgett WA, Saulep D, Lepage M, Hibbs ML. 2010. B-cell stage and context-dependent requirements for survival signals from BAFF and the B-cell receptor. *Immunol Rev* 237: 205–225.
- Matthews SA, Cantrell DA. 2009. New insights into the regulation and function of serine/threonine kinases in T lymphocytes. *Immunol Rev* 228: 241–252.
- Matthews SA, Navarro MN, Sinclair LV, Emslie E, Feijoo-Carnero C, Cantrell DA. 2010. Unique functions for protein kinase D1 and protein kinase D2 in mammalian cells. *Biochem J* 432: 153–163.
- Mocsai A, Ruland J, Tybulewicz VL. 2010. The SYK tyrosine kinase: A crucial player in diverse biological functions. *Nat Rev Immunol* 10: 387–402.
- * Morrison DK. 2012. MAP kinase pathways. *Cold Spring Harb Perspect Biol* 4: a011254.
- Muller MR, Rao A. 2010. NFAT, immunity and cancer: A transcription factor comes of age. *Nat Rev Immunol* 10: 645–656.
- * Newton K, Dixit VM. 2012. Signaling in innate immunity and inflammation. *Cold Spring Harb Perspect Biol* 4: a006049.
- Nika K, Soldani C, Salek M, Paster W, Gray A, Etzensperger R, Fugger L, Polzella P, Cerundolo V, Dushek O, et al. 2010. Constitutively active Lck kinase in T cells drives antigen receptor signal transduction. *Immunity* 32: 766–777.
- Nitschke L. 2009. CD22 and Siglec-G: B-cell inhibitory receptors with distinct functions. *Immunol Rev* 230: 128–143.
- Oeckinghaus A, MS Hayden, Ghosh S. 2011. Crosstalk in NF- κ B signaling pathways. *Nat Immunol* 12: 695–708.
- Oellerich T, Bremes V, Neumann K, Bohnenberger H, Dittmann K, Hsiao HH, Engelke M, Schnyder T, Batista FD, Urlaub H, et al. 2011. The B-cell antigen receptor signals through a preformed transducer module of SLP65 and CIN85. *EMBO J* 30: 3620–3634.
- Oh-hora M, Rao A. 2008. Calcium signaling in lymphocytes. *Curr Opin Immunol* 20: 250–258.
- Okkenhaug K, Fruman DA. 2010. PI3Ks in lymphocyte signaling and development. *Curr Top Microbiol Immunol* 346: 57–85.
- O'Shea JJ, Paul WE. 2010. Mechanisms underlying lineage commitment and plasticity of helper CD4⁺ T cells. *Science* 327: 1098–1102.
- Parry RV, Harris SJ, Ward SG. 2010. Fine tuning T lymphocytes: A role for the lipid phosphatase SHIP-1. *Biochim Biophys Acta* 1804: 592–597.
- Powell JD, Delgoffe GM. 2010. The mammalian target of rapamycin: Linking T cell differentiation, function, and metabolism. *Immunity* 33: 301–311.
- Quann EJ, Merino E, Furuta T, Huse M. 2009. Localized diacylglycerol drives the polarization of the microtubule-organizing center in T cells. *Nat Immunol* 6: 627–635.
- Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, et al. 2011. Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332: 600–603.
- Rochman Y, Spolski R, Leonard WJ. 2009. New insights into the regulation of T cells by γ_c family cytokines. *Nat Rev Immunol* 9: 480–490.
- Rudd CE. 2008. The reverse stop-signal model for CTLA4 function. *Nat Rev Immunol* 8: 153–160.
- Salmond RJ, Filby A, Qureshi I, Caserta S, Zamoyska R. 2009. T-cell receptor proximal signaling via the Src-family kinases, Lck and Fyn, influences T-cell activation, differentiation, and tolerance. *Immunol Rev* 228: 9–22.
- * Samelson LE. 2011. Immunoreceptor signaling. *Cold Spring Harb Perspect Biol* 3: a011510.
- Schatz DG, Ji Y. 2011. Recombination centres and the orchestration of V(D)J recombination. *Nat Rev Immunol* 11: 251–263.
- Sinclair LV, Finlay D, Feijoo C, Cornish GH, Gray A, Ager A, Okkenhaug K, Hagenbeek TJ, Spits H, Cantrell DA. 2008. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. *Nat Immunol* 9: 513–521.
- Spitaler M, Emslie E, Wood CD, Cantrell D. 2006. Diacylglycerol and protein kinase D localization during T lymphocyte activation. *Immunity* 24: 535–546.
- Springer TA, Dustin ML. 2011. Integrin inside-out signaling and the immunological synapse. *Curr Opin Cell Biol* 24: 107–115.
- Srinivasan L, Sasaki Y, Calado DP, Zhang B, Paik JH, DePinho RA, Kutok JL, Kearney JF, Otipoby KL, Rajewsky K. 2009. PI3 kinase signals BCR-dependent mature B cell survival. *Cell* 139: 573–586.
- Tolar P, Sohn HW, Liu W, Pierce SK. 2009. The molecular assembly and organization of signaling active B-cell receptor oligomers. *Immunol Rev* 232: 34–41.
- van der Merwe PA, Davis SJ. 2003. Molecular interactions mediating T cell antigen recognition. *Annu Rev Immunol* 21: 659–684.
- van der Merwe PA, Dushek O. 2011. Mechanisms for T cell receptor triggering. *Nat Rev Immunol* 11: 47–55.
- Veillette A. 2010. SLAM-family receptors: Immune regulators with or without SAP-family adaptors. *Cold Spring Harb Perspect Biol* 2: a002469.
- Veillette A, Latour S, Davidson D. 2002. Negative regulation of immunoreceptor signaling. *Annu Rev Immunol* 20: 669–707.
- Walker LS, Sansom DM. 2011. The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. *Nat Rev Immunol* 11: 852–863.
- Wang H, Kadlec TA, Au-Yeung BB, Goodfellow HE, Hsu IY, Freedman TS, Weiss A. 2010. ZAP-70: An essential kinase in T-cell signaling. *Cold Spring Harb Perspect Biol* 2: a002279.
- * Ward PS, Thompson CB. 2012. Signaling in control of cell growth and metabolism. *Cold Spring Harb Perspect Biol* 4: a006783.
- Watts TH. 2005. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 23: 23–68.
- Xiong N, Raulet DH. 2007. Development and selection of $\gamma\delta$ T cells. *Immunol Rev* 215: 15–31.
- Yang J, Reth M. 2010a. The dissociation activation model of B cell antigen receptor triggering. *FEBS Lett* 584: 4872–4877.
- Yang J, Reth M. 2010b. Oligomeric organization of the B-cell antigen receptor on resting cells. *Nature* 467: 465–469.
- Zikherman J, Jenne C, Watson S, Doan K, Raschke W, Goodnow CC, Weiss A. 2010. CD45-Csk phosphatase-kinase titration uncouples basal and inducible T cell receptor signaling during thymic development. *Immunity* 32: 342–354.

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