The T-cell-receptor signaling network

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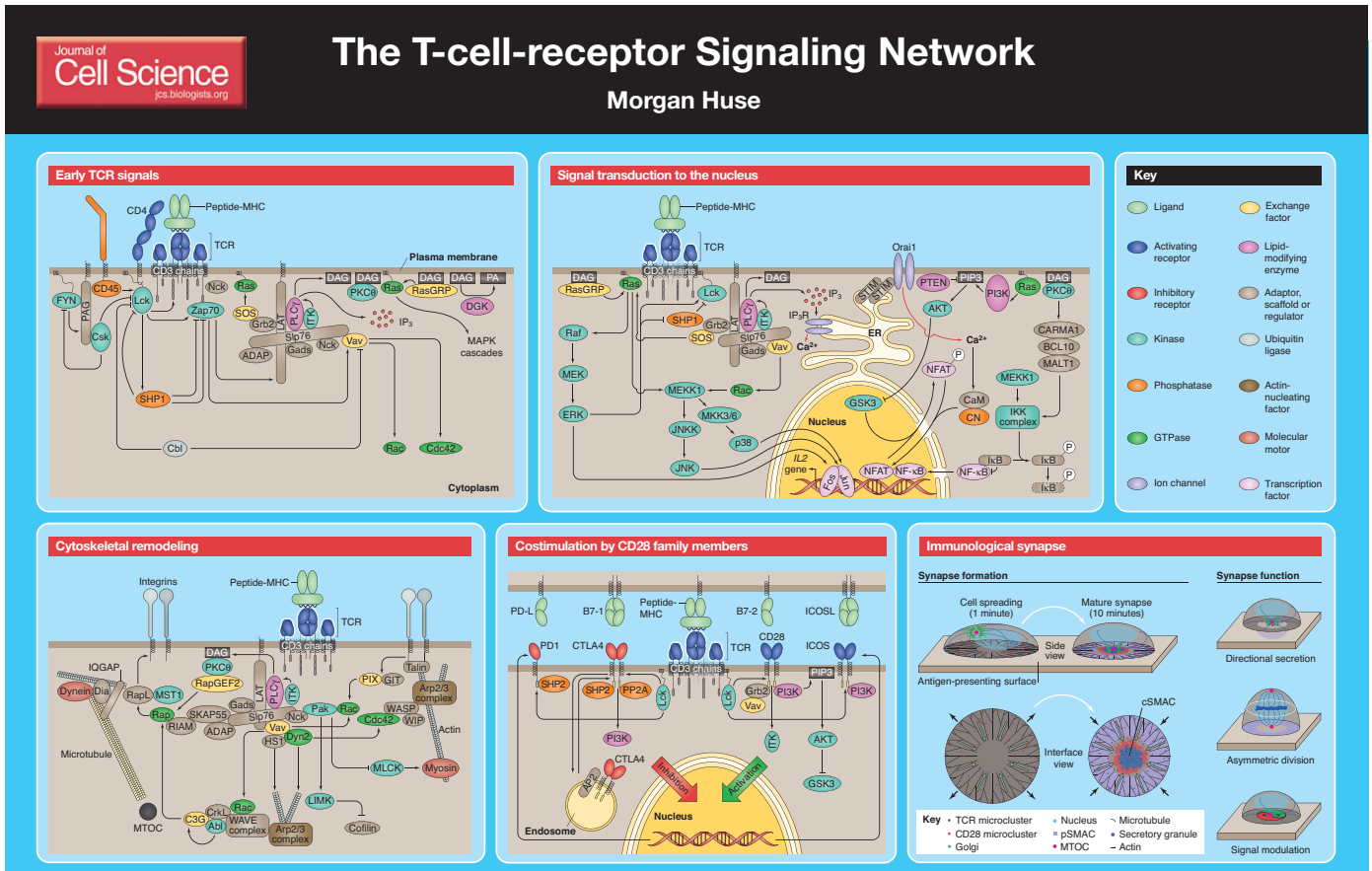
T-cell-receptor (TCR) signaling in response to antigen recognition has a central role in the adaptive immune response. Furthermore, the comprehensive nature of the TCR signaling network is such that it has become a model system for complex cellular responses ranging from gene regulation to cytoskeletal remodeling. Over the past five years, our understanding of TCR signaling has evolved on a number of fronts. Tremendous progress has been made in the characterization of pathways that link early signaling events to downstream transcriptional responses. There has also been significant improvement in our understanding of how TCR signals regulate the cytoskeleton. Our knowledge of how other cell-surface receptors modulate TCR signaling and T-cell effector functions has also continued to advance. Finally, the cell-biological context within which TCR signaling takes place has emerged as a fascinating area of study.

Here, I have schematized the core elements of the TCR signaling network in a series of panels, each of which encompasses one aspect of its function: early membrane-proximal signaling steps; the pathways that link TCR signaling to cytoskeletal reorganization; how early TCR signals are coupled to downstream pathways that alter gene expression; the costimulatory receptors of the CD28 family; and finally, how early TCR signaling is coupled to the formation of the immunological synapse – a stable and specialized junction between the T cell and the antigen-presenting cell (APC). For clarity, I have omitted a number of elements and connections from each of the panels.

Early TCR signals

The recognition of cognate antigenic peptide in the context of major histocompatibility complex (peptide-MHC) by the TCR is thought to induce conformational changes within the associated CD3 chains that facilitate their phosphorylation and association with downstream proteins (Alarcon et al., 2003). The CD3 δ-, γ-, ε- and ζ-chains all contain immunoreceptor tyrosine-based activation motifs (ITAMs), which are phosphorylated by the Src kinase leukocyte-specific tyrosine kinase (Lck)



Abbreviations: Abl, Abelson kinase; ADAP, adhesion- and degranulation-promoting adaptor protein; AP1, activator protein 1; AP2, adaptor protein 2; Arp2/3, actin-related protein 2/3; Bcl, B-cell lymphoma; CaM, calmodulin; CARMA1, caspase-recruitment domain-containing membrane-associated guanylate kinase protein-1; CD, cluster designation; CN, calcineurin; Csk, C-terminal Src kinase; cSMAC, central supramolecular activation cluster; CTLA4, cytotoxic T-lymphocyte antigen 4; DAG, diacylglycerol; DGK, diacylglycerol kinase; Dia, Diaphanous; Dyn2, dynamin 2; ER, endoplasmic reticulum; Erk, extracellular signal-regulated kinase; Gads, Grb2-related adapter protein 2; GIT, G-protein-coupled receptor kinase interactor; Grb2, growth-factor-receptor-bound protein 2; GSK3, glycogen synthase kinase 3; HS1, hematopoietic lineage cell-specific protein 1; ICOS, inducible costimulatory molecule; ICOSL, ICOS ligand; IκB, inhibitor of κB; IKK, IκB kinase; IP_3, inositol trisphosphate; IP_3R, IP_3 receptor; ITK, interleukin-2-inducible T-cell kinase; JNK, Jun kinase; JNKK, JNK kinase; LAT, linker for the activation of T cells; Lck, leukocyte-specific tyrosine kinase; LIMK, LIM kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; MAPK, mitogen-activated protein kinase; MEK, MAPK or Erk kinase; MEKK, MEK kinase; MHC, major histocompatibility complex; MKK, MAPK kinase; MLCK, myosin light chain kinase; MST1, mammalian sterile 20 kinase 1; MTOC, microtubule-organizing center; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; p38, protein of 38 kilodaltons; PA, phosphatidic acid; PAG, phosphoprotein associated with glycosphingolipid-enriched microdomains; PAK, p21 activated kinase; PD1, programmed cell death 1, PD-L, PD1 ligand; PI3K, phosphatidylinositol-3 kinase; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PIX, PAK-interacting exchange factor; PKCθ, protein kinase C-θ; PLCγ, phospholipase C-γ; PP2A, protein phosphatase 2A; pSMAC, peripheral supramolecular activation cluster; PTEN, phosphatase and tensin homolog; RapGEF, Rap guanine nucleotide exchange factor; RasGRP, RAS guanyl nucleotide-releasing protein; RIAM, Rap-GTP interacting adapter molecule; SHP, SH2-domain-containing phosphatase; SKAP55, Src kinase-associated phosphoprotein of 55 kilodaltons; Slp76, SH2-domain-containing leukocyte protein of 76 kilodaltons; SOS, son of sevenless; STIM, stromal interaction molecule; TCR, T-cell receptor; WAVE, WASP family verprolin-homologous protein; Zap70, zeta-activated protein 70 kilodaltons.

(See poster insert)

upon ligand recognition by the TCR (Kane et al., 2000). A significant proportion of Lck in the cell constitutively associates with the coreceptor CD4. Because CD4 also interacts with MHC molecules, it recruits Lck to regions that contain TCR complexes. Phosphorylated CD3 ITAMs recruit the Syk family kinase Zeta-activated protein 70 kDa (Zap70) via Src-homology-2 (SH2)-domain interactions. The adaptor protein Nck also associates directly with polyproline sequences within CD3ε, although the functional significance of this interaction remains controversial (Gil et al., 2002; Szymczak et al., 2005).

Upon localization to the TCR complex, Zap70 phosphorylates multiple tyrosine residues within linker for the activation of T cells (LAT), a membrane-associated scaffolding protein (Samelson, 2002). Phosphorylated LAT recruits a second molecular scaffold, SH2-domain-containing leukocyte protein of 76 kDa (Slp76), which binds to LAT via the intervening protein Gads (Grb2-related adapter protein 2 or GRAP2) (Koretzky et al., 2006). Slp76 is then phosphorylated by Zap70, and the resulting LAT-Slp76 complex acts as a platform for the recruitment of signaling effectors, many of which bind directly to phosphotyrosine-based motifs. One of the most important of these is phospholipase C-γ (PLCγ), which interacts directly with both LAT and Slp76. PLCγ transduces TCR signals by hydrolyzing phosphatidylinositol bisphosphate (PIP2) to yield diacylglycerol (DAG), a membrane-associated lipid, and inositol trisphosphate (IP_3), a diffusible second messenger. DAG recruits a number of downstream proteins to the membrane, among them protein kinase C-θ (PKCθ) and RasGRP (RAS guanyl nucleotide-releasing protein), which is a guanine nucleotide-exchange factor (GEF). RasGRP activates the small GTPase Ras, a crucial activator of mitogen-activated protein kinase (MAPK) signaling pathways in many cell types. Ras can also be activated by the exchange factor son of sevenless (SOS), which is recruited to LAT via the adaptor molecule Grb2 (growth-factor-receptor-bound protein 2).

Phosphorylated Slp76 binds directly to the Tec family kinase interleukin-2-inducible T-cell kinase (ITK). Together with Zap70 and Lck, ITK has an essential role in the phosphorylation and activation of PLCγ. In addition, Slp76 recruits the GEF Vav, which activates the small GTPases Rac and Cdc42. The adaptor proteins Nck and adhesion- and degranulation-promoting adaptor protein (ADAP) are also recruited into the complex. Recent evidence suggests that the LAT-Slp76 complex is a highly cooperative signalosome (Koretzky et al., 2006). Many of its constituent proteins interact with several partners, and the loss of any one protein disrupts signaling through other effectors. This cooperative behavior is probably important for coordinating and coupling different branches of the TCR signaling network.

Early membrane-proximal signaling steps are subject to inhibition on a number of levels (Cannons and Schwartzberg, 2004). The tyrosine phosphatase SH2-domain-containing phosphatase 1 (SHP1) dephosphorylates and deactivates both Zap70 and Lck. In addition, the E3 ubiquitin ligase Cbl targets several proteins for proteasomal degradation, including Lck, Zap70 and Vav (Duan et al., 2004). PLCγ-mediated signaling is attenuated by diacylglycerol kinases (DGKs), which phosphorylate DAG to yield phosphatidic acid (PA) (Zhong et al., 2008). Finally, the tyrosine kinase C-terminal Src kinase (Csk) inhibits proximal TCR signaling by phosphorylating a tyrosine motif in the C-terminal tail of Lck. Csk is recruited to the plasma membrane in a phosphotyrosine-dependent manner by the scaffolding molecule phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), which is maintained in a phosphorylated state by the Src kinase Fyn. In addition to targeting Lck, Csk also phosphorylates the inhibitory C-terminal tail of Fyn, which provides negative feedback by reducing PAG phosphorylation (Solheim et al., 2008).

Lck tail phosphorylation is removed by CD45, a tyrosine phosphatase, which restores TCR signaling. Under certain conditions, however, CD45 can inhibit Lck and other effectors by dephosphorylating phosphotyrosine residues that are required for their optimal activity (Thomas and Brown, 1999). The conditions that determine whether CD45 has an activating or inhibitory role remain poorly defined.

Cytoskeletal remodeling

TCR signaling induces dramatic changes in cytoskeletal architecture (Gomez and Billadeau, 2008). Antigen recognition by the T cell stimulates a burst of actin polymerization at the immunological synapse, generating a lamellapodial sheet structure that spreads over the surface of the APC. The actin-related protein 2/3 (Arp2/3) complex, which stimulates the growth of branched actin arrays, has a central role in this process. Arp2/3 is coupled to the LAT-Slp76 signalosome through Vav, which activates Cdc42 and Rac. Cdc42 triggers Arp2/3 activation by recruiting and activating the Wiskott-Aldrich syndrome protein (WASP), whereas Rac activates Arp2/3 through the WAVE (WASP family verprolin-homologous protein) complex. Actin polymerization is also stimulated by the cortactin homolog HS1 (hematopoietic lineage cell-specific protein 1) as well as the GTPase dynamin 2 (Dyn2), both of which interact with Vav.

TCR-stimulated actin polymerization is temporally correlated with an increase in integrin-mediated adhesion, which occurs via an ‘inside-out’ signaling mechanism (Gomez and Billadeau, 2008). The upregulation of the function of integrins, primarily of the αLβ2 integrin LFA1 (lymphocyte function-associated antigen 1) is directly affected by Vav, PLCγ and other components of the LAT-Slp76 complex. Vav-dependent actin polymerization can induce integrin activation via recruitment of the cytoskeletal linker talin, which binds directly to integrin tails. PLCγ, for its part, activates integrins via the small GTPase Rap (Gomez and Billadeau, 2008; Mor et al., 2007). This occurs through the generation of DAG by PLCγ, which stimulates Rap by recruiting a protein complex containing PKCθ and the Rap exchange factor RapGEF2 (Letschka et al., 2008). Rap can also be activated by the exchange factor C3G (RapGEF1), which is recruited together with the tyrosine kinase Abl to the WAVE complex (Nolz et al., 2008). Once Rap is loaded with GTP, it associates with LAT-Slp76 through a protein complex that contains ADAP, Src kinase-associated phosphoprotein of 55 kDa (SKAP55) and Rap-GTP-interacting adapter molecule (RIAM). The precise mechanism by which Rap-GTP mediates integrin activation remains elusive, but probably involves the adaptor Rap ligand (RapL) and the kinase mammalian sterile 20 kinase (MST1) (Katagiri et al., 2006).

Integrin activation promotes enhanced adhesion of the T cell to the APC, facilitating the establishment of a long-lived

T-cell–APC contact. Activated integrins also induce intracellular signals that promote further cytoskeletal remodeling. For example, the exchange factor p21-activated kinase (PAK)-interacting exchange factor (PIX), which is associated with the adaptor G-protein-coupled receptor kinase interactor (GIT), is activated downstream of integrin adhesion (Phee et al., 2005). PIX-mediated activation of Rac in this context stimulates the kinase activity of PAK, which phosphorylates LIM kinase (LIMK) and myosin light chain kinase (MLCK). PAK phosphorylation activates LIMK, which promotes actin polymerization by phosphorylating and inhibiting the actin-severing protein cofilin. Phosphorylation of MLCK inhibits its kinase activity, and thereby its ability to promote myosin-based contraction. Taken together, these effects promote the growth and maintenance of actin-based structures in the cell.

TCR signaling also induces the polarization of the microtubule-organizing center (MTOC) to the immunological synapse (Gomez and Billadeau, 2008). MTOC reorientation appears to depend on the negatively directed microtubule motor dynein. Microtubules radiate from the MTOC with positive ends facing outwards and negative ends facing inwards. Therefore, dynein that is localized at the immunological synapse can bind to microtubule tips and 'reel' the MTOC in towards itself. However, precisely how dynein is recruited to the immunological synapse remains poorly understood. A recent study suggested that dynein binds directly to ADAP, but this observation appears to hold true only in human T cells (Combs et al., 2006). Certain cytoskeletal regulators of actin, such as the formin Diaphanous (Dia) and the scaffolding molecule IQ-motif-containing GTPase-activating protein homolog (IQGAP), have also been implicated in MTOC reorientation (Gomez and Billadeau, 2008). Further studies will be required to define the mechanistic roles of these and other proteins in the polarization process.

Signal transduction to the nucleus

TCR stimulation leads to profound changes in gene expression. Many of these changes are mediated by the transcription factors activator protein 1 (AP1, a heterodimer of Fos and Jun), nuclear factor of activated T cells (NFAT) and nuclear factor-κB (NF-κB). These three factors act together to activate transcription of the interleukin-2 gene.

The activation of Fos and Jun occurs as a downstream event of three MAPK signaling pathways (Rincon et al., 2001). Each pathway consists of an effector MAPK [extracellular signal-regulated kinase (Erk), Jun kinase (JNK) and protein of 38 kDa (p38)], an upstream MAPK kinase [MAPK or ERK kinase (MEK), JNK kinase (JNKK) and MAPK kinase 3/6 (MKK3/6)] and a MAPK kinase kinase [MEK kinase 1 (MEKK1) and Raf]. The Erk pathway is stimulated by the association of active Ras with Raf, whereas the JNK and p38 pathways respond to activated Rac in addition to Ras. MAPK signaling cascades stimulate AP1 activity via the upregulation of *Fos* and *Jun* transcription, and also by direct phosphorylation of the Fos and Jun proteins. In addition, Erk engages in positive feedback by phosphorylating Lck. This phosphorylation event blocks inhibitory interactions between Lck and SHP1 (Stefanova et al., 2003).

NFAT activity is regulated by the concentration of intracellular Ca^{2+} (Oh-hora and Rao, 2008). When Ca^{2+} levels are low, phosphorylation by the kinase glycogen synthase kinase 3 (GSK3) induces the nuclear export of NFAT. Increases in intracellular Ca^{2+} lead to the dephosphorylation and nuclear import of NFAT. NFAT dephosphorylation is mediated by the phosphatase calcineurin (CN), which is activated by its association with the Ca^{2+}-binding protein calmodulin (CaM). Cytoplasmic Ca^{2+} levels are coupled to TCR activation through PLCγ. The production of IP_3 by PLCγ stimulates the opening of Ca^{2+}-permeable ion channels known as IP_3 receptors (IP3Rs) in the endoplasmic reticulum (ER). This leads to the depletion of Ca^{2+} from the ER, which induces the aggregation of the Ca^{2+} sensors stromal interaction molecule 1 (STIM1) and STIM2 in regions of close ER–plasma-membrane apposition. These STIM clusters are thought to trigger the opening of Orai1 channels in the cell membrane, leading to a large and sustained influx of Ca^{2+} into the cytoplasm. This second, Orai1-dependent, rise in Ca^{2+} drives NFAT into the nucleus.

NFAT translocation is also regulated by phosphatidylinositol 3-kinase (PI3K) (Okkenhaug et al., 2007), which is activated downstream of several TCR signaling effectors, including Ras. PI3K phosphorylates PIP2 to yield PIP3, a phospholipid that recruits a variety of cytoplasmic proteins to the cell membrane. One of the most important of these is the kinase AKT, which promotes cell survival via several distinct pathways. AKT phosphorylates GSK3, thereby inhibiting the phosphorylation of NFAT and promoting its nuclear translocation. PI3K signaling is regulated by the opposing activity of the phosphatase and tensin homolog (PTEN).

Under resting conditions, NF-κB is sequestered in the cytoplasm by inhibitor of κB (IκB). Phosphorylation of IκB by the IκB kinase (IKK) complex leads to the ubiquitylation and degradation of IκB, allowing NF-κB to translocate to the nucleus. IKK is activated by MEKK1 and also by a protein complex comprising the adaptors caspase recruitment domain-containing membrane-associated guanylate kinase protein 1 (CARMA1), B-cell lymphoma 10 (Bcl10) and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) (Thome, 2008). This complex functions downstream of PKCθ, which is recruited to the cell membrane by DAG. Thus, both NFAT and NF-κB rely on different branches of the PLCγ signaling pathway for their activation.

Costimulation by CD28 family members

Optimal T-cell stimulation that leads to proliferation and other effector functions requires that a second, 'costimulatory' signal be delivered through a distinct cell-surface receptor. Although several transmembrane proteins, including LFA1 and CD2, can provide costimulation in certain contexts, the archetypal costimulatory receptor is CD28. CD28 binds to B7-1 (also known as CD80) and B7-2 (also known as CD86), which are highly expressed by professional APCs such as dendritic cells. Ligand binding of CD28 induces the phosphorylation of tyrosine-containing sequences in its cytoplasmic tail by Src-family kinases. This leads to the recruitment of several downstream proteins, including PI3K, Grb2, Vav and ITK. As each of these proteins is also recruited to the activated TCR complex, this suggests that CD28 stimulation does not activate qualitatively distinct pathways, but rather that it

enhances TCR signaling quantitatively (Acuto and Michel, 2003).

The inhibitory receptor cytotoxic T-lymphocyte antigen 4 (CTLA4) is closely related to CD28 (Valk et al., 2008). CTLA4 also binds to B7-1 and B7-2, but with significantly higher affinity than CD28. In resting T cells, almost all CTLA4 is sequestered in intracellular compartments such as endosomes via a mechanism that depends on the sorting adaptor AP2 (adaptor protein 2). TCR stimulation induces the trafficking of CTLA4 to the cell surface, where it can bind to its ligand and trigger signals that attenuate TCR signaling. Similarly to CD28, CTLA4 is phosphorylated by Src kinases at tyrosine residues in its cytoplasmic tail. The phosphatases protein phosphatase 2A (PP2A) and SHP2 both bind to phosphorylated CTLA4, as does PI3K. PP2A and SHP2 might inhibit TCR signaling by dephosphorylating membrane-proximal effectors, although it is also possible that CTLA4 mediates its inhibitory effects by competing with CD28 for binding to B7 ligands that are common to both receptors, which would crowd CD28 out of the immunological synapse.

TCR signaling stimulates the expression of two other CD28 family members known as inducible costimulatory molecule (ICOS) and programmed cell death 1 (PD1) (Greenwald et al., 2005). After trafficking to the surface, both of these proteins can regulate the sustained phase of T-cell signaling when activated by their respective ligands. ICOS enhances T-cell effector functions but, unlike CD28, does not stimulate proliferation. By contrast, PD1 is a potent inhibitor of TCR signalling, similarly to CTLA4. It appears to act in different contexts than CTLA4, however, because PD1 ligand (PD-L) is expressed by different cell types than those that express B7-1 and B7-2. The precise physiological roles of these receptors and the molecular mechanisms by which they act are areas of active interest.

The immunological synapse

The initial detection of cognate peptide-MHC by the TCR induces actin-dependent spreading over the surface of the APC. The TCR and its associated membrane-proximal effectors (such as Zap70, Slp76 and Vav) accumulate in microclusters at the cell-cell interface (Saito and Yokosuka, 2006). These microclusters almost immediately begin to traffic inward, driven by retrograde actin flow. Over time, the lamellapodium retracts somewhat and the central TCR microclusters coalesce into a larger structure that is known as the central supramolecular activation cluster (cSMAC). By concentrating activated TCRs together with intracellular signaling molecules such as Zap70 and PI-3 kinase, the cSMAC is thought to amplify signals initiated by TCR ligands of low abundance or low affinity (Cemerski et al., 2008). However, the cSMAC is also the site of TCR internalization, which is a process that functions to attenuate signals from highly abundant or high-affinity antigens (Varma et al., 2006). Hence, by balancing recruitment with internalization, the cSMAC exerts a moderating influence on TCR signaling, amplifying the effects of weak ligands and diminishing those of strong ligands.

As the cSMAC forms, integrins such as LFA1 accumulate in the region around it, forming an adhesion ring that is known as the peripheral SMAC (pSMAC). This ‘bulls-eye’ pattern of pSMAC surrounding cSMAC, which has been called the ‘mature immunological synapse’, can persist for hours (Bromley et al., 2001). During this sustained phase, microclusters that contain activated TCR complexes continue to form in the pSMAC and are trafficked towards the center. Signals that are derived from these peripheral microclusters are thought to be essential for promoting sustained TCR signaling that lead to full T-cell activation (Varma et al., 2006).

Immunological synapse formation is accompanied by the polarization of the T-cell MTOC to the region just beneath the APC. This sets up an axis of polarity that plays a crucial role in T-cell function (Huse et al., 2008). MTOC reorientation enables the T cell to release cytotoxic factors and cytokines toward the APC in a directional manner, thereby maintaining the specificity of secretory responses. In addition, recent work has suggested that MTOC reorientation is required to orient the T cell for asymmetric cell division, which might be important for the development of T-cell memory (Chang et al., 2007). Hence, the immunological synapse profoundly affects both TCR signals as well as the effector responses that result from those signals.

Our understanding of immunological synapse structure and function is still in flux. For example, recent work has indicated that CD28 and PKCθ accumulate in a previously uncharacterized area of the cSMAC that might have an important role in costimulation (Yokosuka et al., 2008). As additional synapse subdomains are identified and characterized in the coming years, we will no doubt have to revise our current notions of immunological synapse function.

Perspectives

Over the past 20 years, we have made tremendous progress in our understanding of the signals that regulate T-cell activation. Most of the relevant signaling effectors have been identified and organized into defined pathways. Despite this progress, however, it remains unclear precisely how these pathways work together to elicit the complex cellular responses we observe.

Future progress will require higher resolution mechanistic experiments that focus on both the cell biology and biochemistry of cellular responses. The recent discovery of signaling microclusters provides a good example of how quantitative image analysis, when applied appropriately, can transform the field. These studies, however, must be coupled with computational methods to model T-cell responses. The TCR signaling network is far too complex to be understood intuitively. Models that account for this complexity will be instrumental, not only in helping us make sense of what we see, but also in devising testable predictions of how the system might act under different conditions.

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