T-Cell receptor and signalling

T- lymphocytes respond to peptide fragments of protein antigens that are displayed by the Antigen Presenting Cells(APCs). These responses require.

1.Specific antigen recognition by the T-cells

2.Stable adhesion of T-cells to the APCs.

3.Transduction of activating signals to the Tcells.

TCR has its invariant proteins called CD3 and Zeta which are noncovalently linked to the antigen receptor to form the TCR Complex. T cells also express other membrane proteins. These are collectively called accessory molecules. These are CD2, CD4/CD8, CD28, Integrin.

Each of these events is mediated by a set of proteins in the form of a receptor. The receptor that performs these functions is known as T-cell Receptor (TCR).

TypesofTCR:
1.αβTcell Receptor 2.γδTcell Receptor. CD4+ helper T cell and CD8+ cytolytic T Lymphocytes (CTLs) have this kind of TCR.

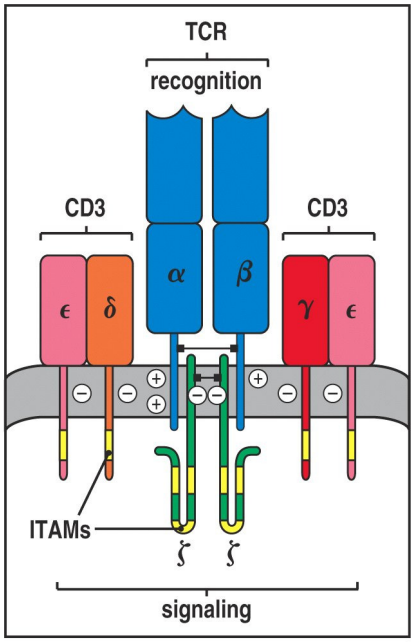

Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

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) 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 GTPaseRas, 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 GTPasesRac 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

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 PLCγsignaling pathway for their activation.