Cell Mediated Immunity: T-Cell Signal Transduction

ZCT – 210 [Topic No: 5] Lecture No: 1

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The idea that cells may have specific surface receptors that can be triggered by external ligands came from one of the founders of modern immunology.

Paul Ehrlich, in his "side chain theory," published in 1897, conceived of antibodies on the surface of immune cells that recognize antigens and instruct the immune cell to secrete more of the same antibody.

Cell surface receptors serve two major functions — the induction of intracellular signaling and the adhesion of one cell to another or to the extracellular matrix.

Signal transduction broadly refers to the intracellular biochemical responses of cells after the binding of ligands to specific receptors. Most but not all signaling receptors are located on the plasma membrane.

- Signaling initiated by these receptors typically involves an **initial cytosolic phase** when the receptor or proteins that interact with the receptor may be post-translationally modified. This often leads to the activation or nuclear translocation of transcription factors that are silent in resting cells, followed by a **nuclear phase** when transcription factors orchestrate changes in gene expression (Fig. 7-1).

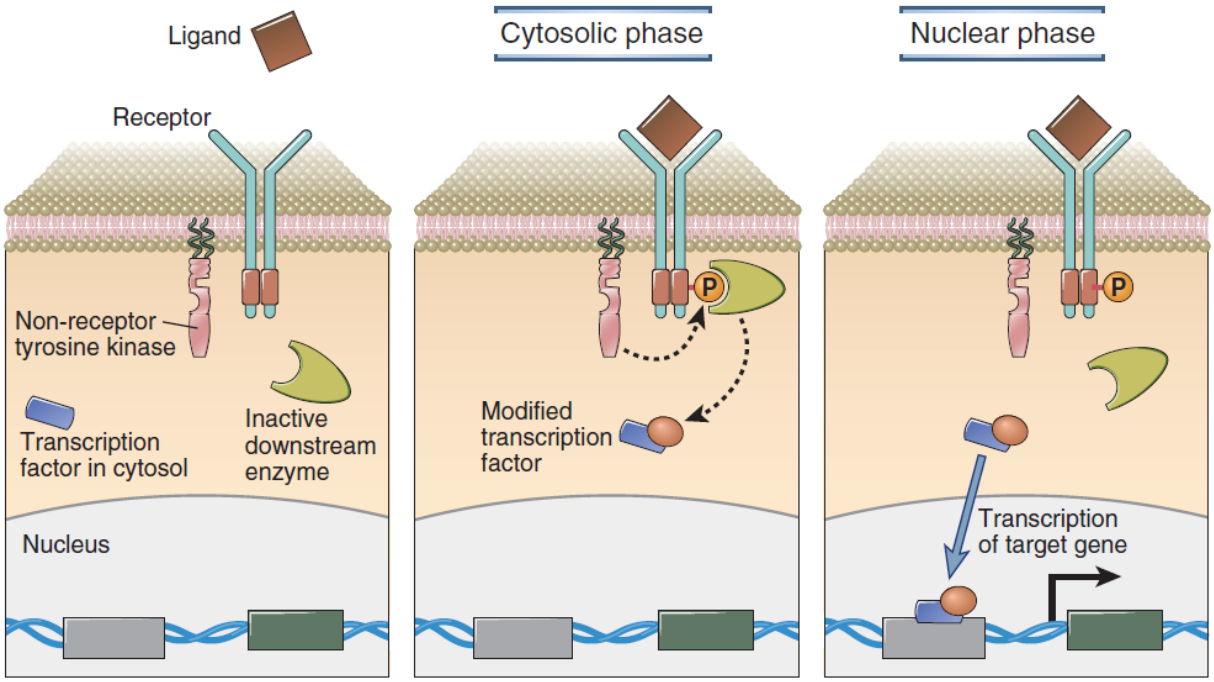


FIGURE 7–1 Signaling from the cell surface involves cytosolic and nuclear phases. A generic receptor that activates a nonreceptor tyrosine kinase after it binds ligand is shown. In the cytosolic signaling phase, the non-receptor kinase phosphorylates a key tyrosine residue on the cytoplasmic tail of the receptor, as a result of which the phosphotyrosine-containing receptor tail is able to recruit a downstream enzyme that is activated once it is recruited. In the cytosolic phase, this activated downstream enzyme post-translationally modifies a specific transcription factor that is located in the cytoplasm. In the nuclear phase, this modified transcription factor enters the nucleus and induces the expression of target genes that all have a binding site in the promoter or in some other regulatory region that can bind to this modified transcription factor and facilitate transcription.

Some signal transduction pathways stimulate cell motility or activate granule exocytosis from the cytoplasm independent of a nuclear phase.

Signal transduction can result in a number of different consequences for a cell, including acquisition of new functions, induction of differentiation, commitment to a specific lineage, protection from cell death, initiation of proliferative and growth responses, and induction of cell cycle arrest or of death by apoptosis. Antigen receptors on B and T lymphocytes are among the most sophisticated signaling machines known, and they will form a large part of the focus of this topic.

AN OVERVIEW OF SIGNAL TRANSDUCTION

Receptors that initiate signaling responses are generally integral membrane proteins present on the plasma membrane, where their extracellular domains recognize soluble secreted ligands or structures that are attached to the plasma membrane of a neighboring cell or cells.

One distinct category of receptors, nuclear receptors, are actually transcription factors that are functionally activated by lipid-soluble ligands that can easily cross the plasma membrane.

The initiation of signaling from a cell surface receptor may require ligand induced clustering of the receptor, known as receptor cross-linking, or may involve a conformational alteration of the receptor that is induced by its association with ligand.

Both mechanisms of signal initiation typically result in the creation of a novel geometric shape in the cytosolic portion of the receptor that promotes interactions with other signaling molecules.

This change in receptor geometry may sometimes result from enzymatic addition of a bulky phosphate residue on a key tyrosine, serine, or threonine side chain on the cytosolic portion of a receptor component or on a distinct adaptor protein.

The enzymes that add phosphate groups onto amino acid side chains are called **protein kinases**.

- Many of the initiating events in lymphocyte signaling depend on protein kinases that phosphorylate key tyrosine residues, and these enzymes are therefore called **protein tyrosine kinases**.
- Other protein kinases that are involved in distinct signaling pathways are **serine / threonine kinases**, enzymes that phosphorylate protein substrates on serine or threonine residues.

- Some enzymes activated downstream of signaling receptors phosphorylate lipid substrates; they are therefore known as **lipid kinases**.

For every type of phosphorylation event, there is a specific **phosphatase**, an enzyme that can remove a phosphate residue and thus modulate signaling.

These phosphatases play important, usually inhibitory roles in signal transduction. Phosphorylation of proteins is not the only post-translational modification that drives signal transduction. Many other modifications are known to facilitate signaling events. Some transcription factors as well as histones can be regulated by **acetylation** and **methylation**, for instance.

A type of modification that we will describe later in this chapter is protein **ubiquitination**, the addition of ubiquitin molecules that either target proteins for degradation or drive signal transduction in many cells, including lymphocytes. Many important signaling molecules are modified by the addition of lipids that may help localize the protein in the plasma membrane, or sometimes to a specialized region of the plasma membrane that is rich in signaling molecules.

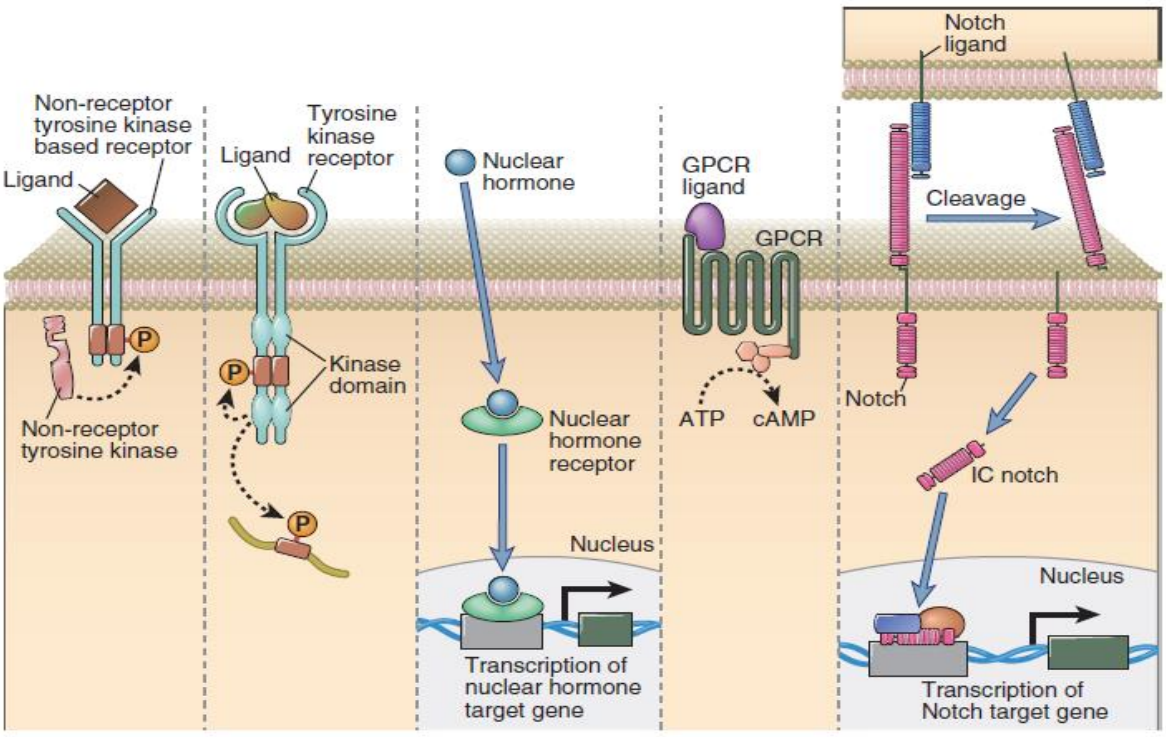


FIGURE 7–2 Major categories of signaling receptors in the immune system. Depicted here are a receptor that uses a non-receptor tyrosine kinase, a receptor tyrosine kinase, a nuclear receptor that binds its ligand and can then influence transcription, a seven-transmembrane receptor linked to heterotrimeric G proteins, and Notch, which recognizes a ligand on a distinct cell and is cleaved, yielding an intracellular fragment (IC Notch) that can enter the nucleus and influence transcription of specific target genes.

Cellular receptors are grouped into several categories based on the signaling mechanisms they use and the intracellular biochemical pathways they activate (Fig.7-2):

- Receptors that use **non-receptor tyrosine kinases**.
    - In this category of membrane receptors the ligand binding chains have no intrinsic catalytic activity, but a separate intracellular tyrosine kinase, known as a non-receptor tyrosine kinase, participates in receptor activation by phosphorylating specific motifs on the receptor or on other proteins associated with the receptor (see Fig. 7-1).
    - A family of receptors called immune receptors, some of which recognize antigens while others recognize the Fc portions of antibodies, all use non-receptor tyrosine kinases to initiate signaling.
    - Apart from the immune receptor family, some cytokine receptors, also use non-receptor tyrosine kinases.
    - Integrins, key adhesion receptors in the immune system, also signal by activating non-receptor tyrosine kinases.
- **Receptor tyrosine kinases (RTKs).**
    - Are integral membrane proteins that activate an intrinsic tyrosine kinase domain (or domains) located in their cytoplasmic tails when they are cross-linked by multivalent extracellular ligands (see Fig. 7-2).

- An example of an RTK relevant to blood cell formation is the c-Kit protein. This RTK has extracellular Ig domains that bind to a ligand known as stem cell factor.
- Interaction with stem cell factor leads to dimerization of c-Kit and activation of the cytosolic kinase domains of the dimerized receptor.
- Signaling through c-Kit contributes to the initiation of hematopoiesis and lymphopoiesis.
- Other examples of RTKs include the insulin receptor, the epidermal growth factor receptor, and the platelet derived growth factor receptor.

- **Nuclear receptors.**
  - The binding of a lipid-soluble ligand to its nuclear receptor (see Fig. 7-2) results in the ability of the latter either to induce transcription or to repress gene expression.
  - **Nuclear hormone receptors**, such as the vitamin D receptor and the glucocorticoid receptor, can influence events that range from the development of the immune system to the modulation of cytokine gene expression.
- **Seven-transmembrane receptors**
  - Are polypeptides that traverse the plasma membrane seven times, because of which they are sometimes called serpentine receptors (see Fig. 7-2).
  - Because these receptors function by activating associated GTP-binding proteins (G proteins), they are also commonly called **G protein–coupled receptors (GPCRs)**.
  - A conformational change induced by the binding of ligand to this type of receptor permits the activation of an associated heterotrimeric G protein, which initiates downstream signaling events.

- Examples of this category of receptors that are relevant to immunity and inflammation include receptors for leukotrienes, prostaglandins, histamine, complement fragments C3a and C5a, bacterial f-met-leu-phe peptide, and all chemokines.
- Different types of G proteins linked to distinct GPCRs may activate or inhibit different downstream effectors.
- **The two major enzymes that GPCRs activate** are **adenylate cyclase**, which converts ATP to the effector molecule cAMP, capable of activating numerous cellular responses, and **phospholipase C**, which also triggers multiple signals as discussed later.

- **Other classes of receptors.**
  - Other categories of receptors have long been known to be important in embryonic development and in certain mature tissues, and their functions in the immune system have more recently begun to emerge.
  - Receptor proteins of the **Notch** family (see Fig. 7-2) are involved in development in a wide range of species. The association of specific ligands with receptors of this family leads to proteolytic cleavage of the receptor and the nuclear translocation of the cleaved cytoplasmic domain (intracellular Notch), which functions as a component of a transcription complex.
  - Notch proteins contribute to cell fate determination during lymphocyte development and may also influence the activation of mature lymphocytes.
  - A group of ligands called **Wnt** proteins can influence lymphopoiesis. Signaling through transmembrane receptors for these proteins can regulate the levels of **β-catenin**, which facilitates the transcriptional activity of proteins that contribute to B and T cell development.

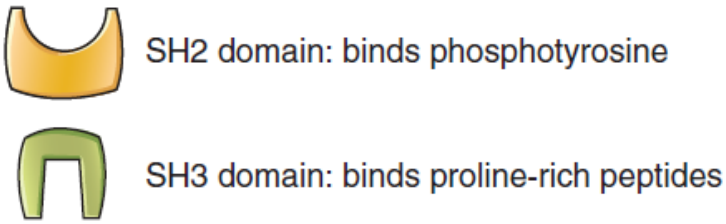
Modular Signaling Proteins and Adaptors

Signaling molecules are often composed of distinct modules, each with a specific binding or catalytic function.

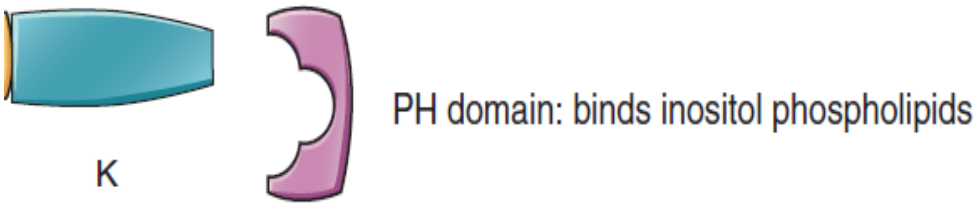
- The discovery of tyrosine phosphorylation represented a major breakthrough in the study of cellular signaling pathways.
- It was subsequently discovered that the sequence surrounding specific phosphorylated tyrosine residues contributes to the interaction of tyrosinephosphorylated proteins with other signaling molecules.

An appreciation that signaling molecules contain modules or domains that each have defined functions was obtained from the study of non-receptor tyrosine kinases.

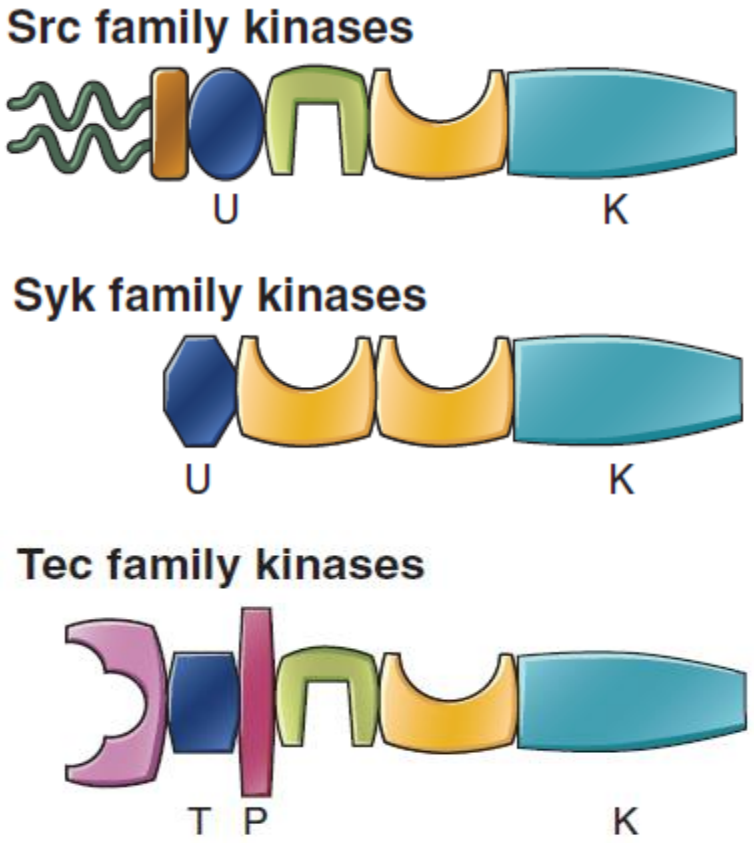
- The cellular homologue of the transforming protein of the Rous sarcoma virus, called c Src, is the prototype for an immunologically important family of non-receptor tyrosine kinases known as **Src family kinases**.
    - c-Src contains unique domains, including **Src homology 2 (SH2)** and **Src homology 3** (SH3) domains described later.



    - It also contains a catalytic tyrosine kinase domain (K) and an N-terminal lipid addition domain that facilitates the covalent addition of a myristic acid molecule to the protein (PH).



- The myristate helps target Src family kinases to the plasma membrane. The modular structures of three families of tyrosine kinases that are important in the immune system are depicted in Figure 7-3.



- **SH2 domains** are composed of about 100 amino acids folded into a particular conformation, and they recognize specific phosphotyrosine-containing peptides.
- In antigen receptor signaling, Src family kinases phosphorylate tyrosine residues present in particular motifs in the cytoplasmic tails of proteins that are part of the receptor complex (described later).
- These phosphotyrosine motifs in the antigen receptor complex are then recognized by SH2 domains present in tyrosine kinases of the Syk family, such as Syk and ZAP-70 (see Fig. 7-3).

- The recruitment of a Syk family kinase to an antigen receptor by means of a specific SH2 domain – phosphotyrosine interaction is a key step in antigen receptor activation.
- **SH3 domains** are also about 100 amino acids in length, and they help mediate protein-protein interactions by binding to proline-rich stretches in certain proteins.
- Another type of modular domain, called a **pleckstrin homology (PH) domain,** can recognize specific phospholipids.
- The PH domains in a number of signaling molecules including the TEC family tyrosine kinase Btk, recognize phosphatidylinositol trisphosphate (PIP3), a lipid moiety on the inner leaflet of the plasma membrane.

Adaptor proteins function as molecular hubs that physically link different enzymes and promote the assembly of complexes of signaling molecules.

- Adaptors may be integral membrane proteins like **LAT (linker for the activation of T cells)** (Fig. 7-4), or they may be cytosolic proteins such as **BLNK (B cell linker)**, **SLP-76 (SH2 domain–containing linker protein of 76 kD),** and **GADS (Grb-2–related adaptor protein downstream of Shc).**

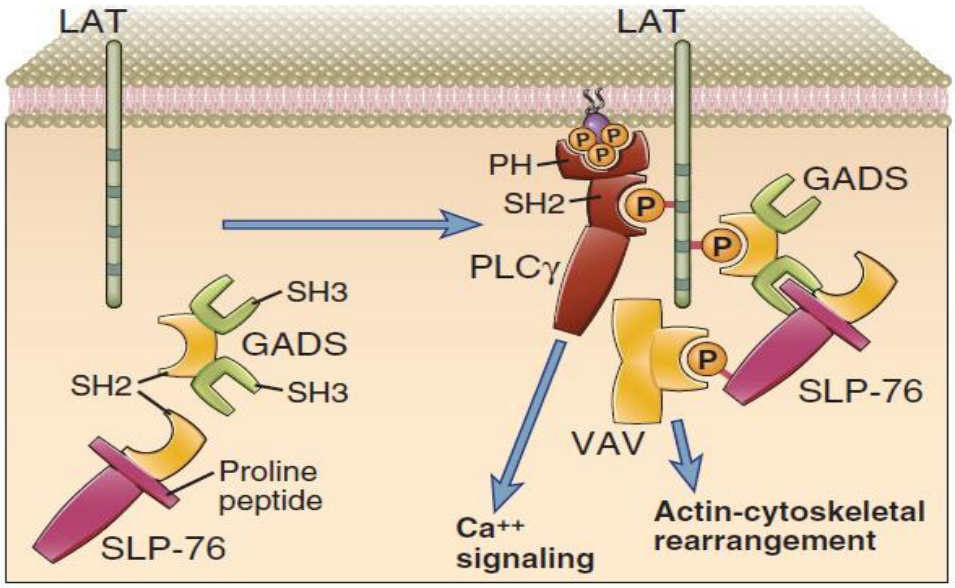


FIGURE 7–4 selected adaptors that participate in lymphocyte activation. On the left, LAT, an integral membrane protein that functions as an adaptor, and two cytosolic adaptors, GADS and SLP-76, are shown in a nonactivated T cell. On the right, after T cell activation, LAT is tyrosine phosphorylated and is shown to have recruited PLCγ and the GADS adaptor, both of which contain SH2 domains. A proline-rich amino acid stretch in SLP-76 associates with an SH3 domain of GADS, and tyrosine-phosphorylated SLP-76 recruits Vav.

- A typical adaptor may **contain a few specific domains that mediate protein-protein interactions**, such as SH2 and SH3 domains, among others (there are many more types of modular domains not mentioned here).
- Adaptors may also **contain some proline-rich stretches (that can bind other proteins that contain SH3 domains)**, and they also often **contain critical tyrosine residues that may be phosphorylated by tyrosine kinases**.
- The amino acid residues that are close to a tyrosine moiety that is phosphorylated determine which specific SH2 domains may bind that site.
    - For instance, an adaptor with a YxxM motif (where Y represents tyrosine, M represents methionine, and x refers to any amino acid) will bind an SH2 domain in the lipid kinase phosphatidylinositol 3-kinase (PI3-kinase).
- The same adaptor protein may recruit a tyrosine kinase with a specific SH3 domain to a proline-rich stretch, and tyrosine phosphorylation of the adaptor may thus result in a tyrosine kinase and PI3-kinase being perched next to each other, resulting in the phosphorylation and activation of PI3-kinase.
- Signal transduction can therefore be visualized as a kind of social networking phenomenon. An initial signal (tyrosine phosphorylation, for instance) results in proteins being brought close to one another at designated hubs (adaptors), resulting in the activation of specific enzymes that eventually influence the nuclear localization or activity of specific downstream transcription factors or induce other cellular events, such as actin polymerization.

THE IMMUNE RECEPTOR FAMILY

Immune receptors are a unique family of receptor complexes typically made up of integral membrane proteins of the immunoglobulin (Ig) superfamily that are involved in ligand recognition, associated with other transmembrane signaling proteins that have unique tyrosine-containing motifs in their cytoplasmic tails. Whereas the signaling components are generally separate proteins from those involved in ligand recognition, in a few members of the family, the receptor consists of a single chain in which the extracellular domain is involved in ligand recognition and the cytoplasmic tail contains tyrosine residues that contribute to signaling.

- The signaling proteins of the immune receptor family are often positioned close to non-receptor tyrosine kinases of the Src family.
- The latter also possess N-terminal lipid anchors that tether them to the inner leaflet of the plasma membrane.
- The cytoplasmic tyrosine containing motifs on the signaling proteins of the immune receptor family are generally one of two different types.
    - **ITAMs** (immunoreceptor tyrosine-based activating motifs) are found on receptors involved in cell activation and have the sequence **YxxL / me (x)** $_{6-8}$**YxxL / I,** where Y represents a tyrosine residue, L represents leucine, I represents isoleucine, and x refers to any amino acid.
        - ITAM motifs can be phosphorylated on both tyrosine residues that are present in this motif by Src family kinases when immune receptors are activated.
        - Tyrosinephosphorylated ITAMs recruit a distinct tyrosine kinase of the Syk/ZAP-70 family, which contains tandem SH2 domains that each bind to one of the two phosphorylated YxxL/I motifs of the ITAM.

- Binding of Syk (or ZAP-70) to a phospho-ITAM results in a conformational change in this kinase and its activation.
- The activated Syk or ZAP-70 kinase then drives immune cell activation.

- Some immune receptors inhibit cellular responses, and signaling chains in these receptors may contain a slightly different tyrosine containing motif that is called an **ITIM** (immunoreceptor tyrosine-based inhibitory motif), which has the consensus sequence V/L/IxYxxL, where V refers to valine.
    - Phosphorylated ITIMs recruit tyrosine or inositol lipid phosphatases, enzymes that remove phosphate residues from phosphotyrosine moieties or from certain lipid phosphates and thus counteract ITAM-based immune receptor activation.

Members of the immune receptor family include antigen receptors on both B cells and T cells, the IgE receptor on mast cells, and activating and inhibitory Fc receptors on innate immune cells and B lymphocytes (Fig. 7-5).

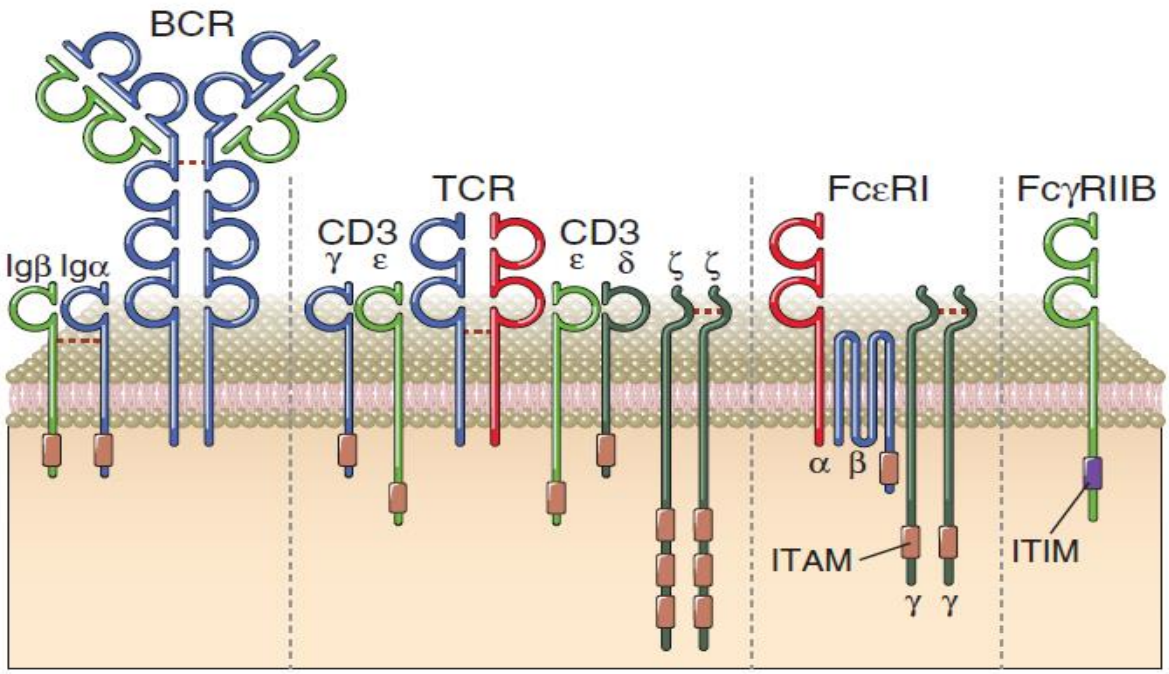


FIGURE 7–5 selected members of the immune receptor family. Four selected members of the immune receptor family are depicted. Typically, immune receptors that activate immune cells have separate chains for recognition and associated chains that contain cytosolic ITAMs. Examples shown here include the B cell receptor (BCR), the T cell receptor (TCR), and the high-affinity receptor for IgE (FcεRI). Inhibitory receptors in the immune system typically have ITIM motifs on the cytosolic portion of the same chain that uses its extracellular domain for ligand recognition. The inhibitory receptor shown, FcγRIIB, is found on B cells and myeloid cells.

ITAMs are found in the cytoplasmic tails of several immune receptor complexes that are involved in signal transduction, including the ζ chain and CD3 proteins of the T cell receptor (TCR) complex, Igα and Igβ proteins associated with membrane Ig molecules (the antigen receptors) of B cells, and components of several Fc receptors and of the NKG2D activating receptor on natural killer (NK) cells.

ITIM-containing inhibitory receptors include CD22, FcγRIIB, and several inhibitory NK cell receptors.

General Features of Antigen Receptor Signaling

Signaling downstream of the T and B cell antigen receptors is characterized by a similar sequence of events, consisting of the following.

- Receptor ligation typically involves the clustering of receptors by multivalent ligands, resulting in activation of an associated Src family kinase.
- Receptor ligation may also result in the unfolding of the cytoplasmic tail of a polypeptide chain that is part of the receptor.
- The unfolding event (or conformational change) may allow previously hidden tyrosine residues of a cytosolic ITAM motif to become available for phosphorylation by a Src family kinase.
- The activated Src family kinase phosphorylates available tyrosines in the ITAMs of signaling proteins that are part of the receptor complex.
- The two phosphorylated tyrosines in a single ITAM are recognized by a Syk family tyrosine kinase that has tandem SH2 domains that each recognize an ITAM phosphotyrosine.
- Recruitment of the Syk family kinase to the phosphorylated ITAM results in the activation of this tyrosine kinase and the subsequent tyrosine phosphorylation of adaptor proteins and enzymes that activate distinct signaling pathways downstream of the immune receptor.

This sequence of events is described in more detail in the context of T cell and B cell receptor signaling later.

Alterations in the strength of TCR and B cell receptor (BCR) signaling influence the fates of lymphocytes during their development and activation.

In other words, the presence of different numbers of activated signaling molecules induced by antigen-ligated receptors is interpreted differently by lymphocytes.

- For instance, during maturation of T cells in the thymus, weak antigen receptor signals are required for positive selection, the process that preserves useful cells by matching coreceptors to the appropriate MHC molecules, and gradations of signal strength may determine positive selection of developing T cells into the CD4 or CD8 lineage.
- In contrast, strong antigen receptor signals during maturation may contribute to lymphocyte death by apoptosis.

The strength of TCR and BCR signaling may also differentially influence the type of immune response that is generated by a given antigen.

Antigen receptor signaling is fine-tuned and modulated by three mechanisms that are unique to this class of receptors.

- **Progressive ITAM use.** One of the ways in which different quantities of signal output might be generated by antigen receptors is the phosphorylation of different numbers of ITAM tyrosines after receptor engagement. The TCR complex has six signaling chains and ten ITAMs, and increasing numbers of ITAMs may be phosphorylated as the affinity of different ligands for the TCR increases. The number of ITAMs phosphorylated may therefore provide a cytosolic interpretation of the affinity of the antigen that binds to the TCR, and antigen affinity can thus influence the nature of the cellular response at different stages of differentiation and activation. The BCR has only two ITAMs, but because this number increases when the receptor is cross-linked by multivalent antigens, the degree of cross-linking by antigens may determine the number of ITAMs that might be used and thus generate different responses to antigens of differing affinity and valency.
- **Increased cellular activation by coreceptors.** A **coreceptor** is a transmembrane signaling protein on a lymphocyte that can facilitate antigen receptor activation by simultaneously binding to the same antigen complex

that is recognized by the antigen receptor. The coreceptor brings with it signaling enzymes linked to its cytoplasmic tail and can thereby facilitate ITAM phosphorylation and activation of the antigen receptor when antigen draws it into the vicinity of the antigen receptor. Coreceptors on T cells are the CD4 and CD8 proteins that demarcate the two functionally distinct subsets. Complement receptor type 2 (CR2/CD21) is the coreceptor on B cells.

- **Modulation of signaling by inhibitory receptors.** Key **inhibitory receptors** in T cells include CTLA-4 and PD-1, whereas important inhibitory signals in B cells are delivered through receptors such as CD22 and FcγRIIB, among others. The roles of these inhibitors are mentioned later in this chapter.

In addition, antigen receptor signals may, in some circumstances, cooperate with signals from receptors, known as **costimulatory receptors** that add yet another level of control to the process of lymphocyte activation. Costimulatory receptors provide "second signals" for lymphocytes (antigen recognition provides the first signal) and ensure that immune responses are optimally triggered by infectious pathogens and substances that mimic microbes. Unlike coreceptors, costimulatory receptors do not recognize components of the same ligands as do antigen receptors; signal outputs downstream of costimulatory receptors are integrated with the signals derived from the antigen receptor, and these signals cooperate to fully activate lymphocytes. The prototypic costimulatory receptor is CD28 on T cells, which is activated by the costimulatory molecules B7-1 and B7-2 (CD80 and CD86), ligands induced on antigen presenting cells (APCs) as a result of their exposure to microbes.

The T cell Receptor Complex and T Cell Signaling

- The TCR was discovered in the early 1980s, around the same time that the structure of major histocompatibility complex (MHC) molecules associated with peptides, the ligands for T cells, was being defined.
- A number of separate approaches were used to molecularly identify the TCR.
    - One approach depended on the identification of genes that were expressed specifically in T cells and that also could be shown to have undergone a gene rearrangement event specifically in these cells (a characteristic feature of antigen receptor genes).
        - The first gene thus identified was homologous to Ig genes and proved to be a chain of the heterodimeric γδ TCR.
    - In another approach, clonal populations of T cells were created and monoclonal antibodies were generated against different T cell clones. Monoclonal antibodies that each recognized only a specific T cell clone were identified. These clonotype-specific antibodies identified a chain of the TCR. In yet another study, one chain of the TCR was identified serendipitously, when sequencing of a T cell–specific library of cDNAs unexpectedly revealed a novel gene with homology to immunoglobulins. We now know that the TCR is similar to antibodies, but there are important differences between these two types of antigen receptors (Table 7-1).

The Structure of the T cell Receptor for Antigen

The antigen receptor of MHC-restricted CD4+ helper T cells and CD8+ cytotoxic T lymphocytes (CTLs) is a heterodimer consisting of two transmembrane polypeptide chains, designated TCR α and β, covalently linked to each other by a disulfide bridge between extracellular cysteine residues (Fig. 7-6).

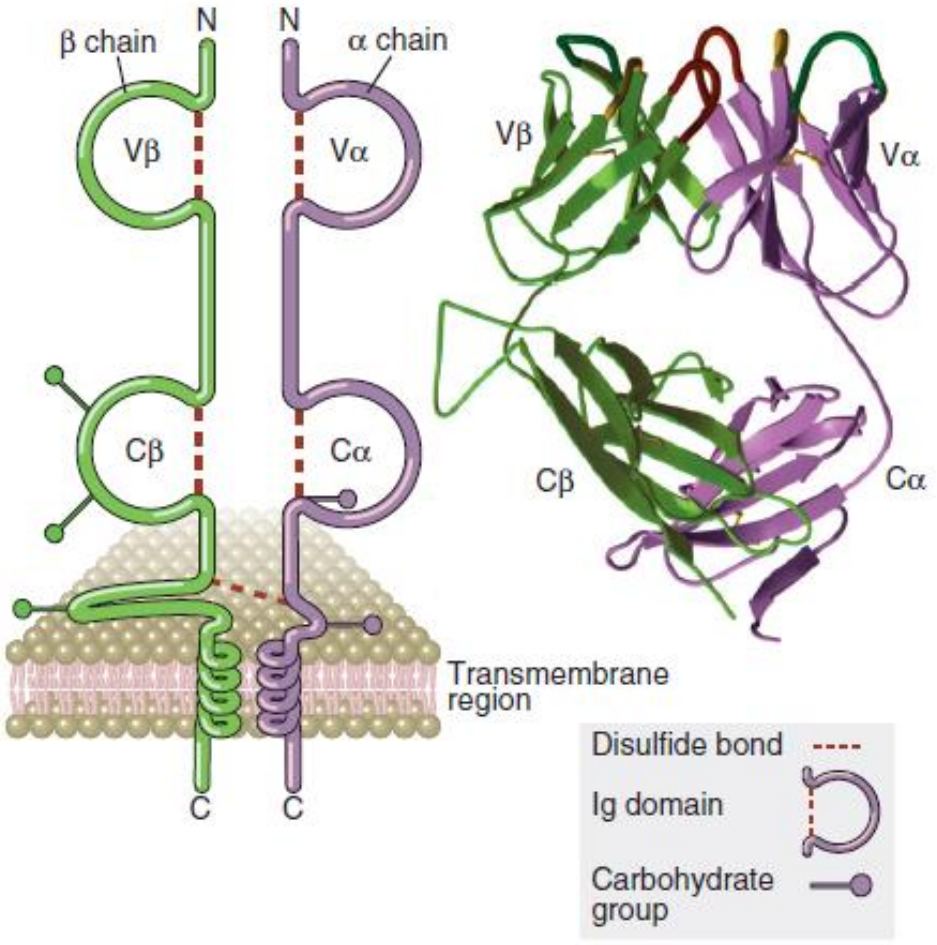


FIGURE 7–6 Structure of the T cell receptor. The schematic diagram of the αβ TCR *(left)* shows the domains of a typical TCR specific for a peptide-MHC complex. The antigen-binding portion of the TCR is formed by the Vβ and Vα domains. The ribbon diagram *(right)* shows the structure of the extracellular portion of a TCR as revealed by x-ray crystallography. The hypervariable segment loops that form the peptide-MHC binding site are at the top. *(Modified from Bjorkman PJ. MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. Cell 89:167-170, 1997. Copyright Cell Press.)*

- These T cells are called αβ T cells. A less common type of TCR, found on γδ T cells, is composed of TCR γ and δ chains.
- Each TCR α and β chain consists of one Ig-like N-terminal variable (V) domain, one Ig-like constant (C) domain, a hydrophobic transmembrane region, and a short cytoplasmic region.
- Thus, the extracellular portion of the TCR αβ heterodimer is structurally similar to the antigen-binding fragment (Fab) of an Ig molecule, which is made up of the V and C regions of a light chain and the V region and one C region of a heavy chain.
- The V regions of the TCR α and β chains contain short stretches of amino acids where the variability between different TCRs is concentrated, and these form the hypervariable or complementarity-determining regions (CDRs).
- Three CDRs in the α chain and three similar regions in the β chain together form the part of the TCR that specifically recognizes peptide-MHC complexes (Fig. 7-7).
- The β chain V domain contains a fourth hypervariable region that does not appear to participate in antigen recognition but is the binding site for microbial products called superantigens.
- Each TCR chain, like Ig heavy and light chains, is encoded by multiple gene segments that undergo somatic rearrangements during the maturation of the T lymphocytes.
- The C regions of both α and β chains continue into short hinge regions, which contain cysteine residues that contribute to a disulfide bond linking the two chains.
- The hinge is followed by hydrophobic transmembrane portions, an unusual feature of which is the presence of positively charged amino acid residues,

including a lysine residue (in the α chain) or a lysine and an arginine residue (in the β chain).

- These residues interact with negatively charged residues present in the transmembrane portions of other polypeptides (those of the CD3 complex and ζ) that are part of the TCR complex.
- Both TCR α and β chains have carboxyl-terminal cytoplasmic tails that are 5 to 12 amino acids long. Like membrane Ig on B cells (see later), these cytoplasmic regions are too small to transduce signals, and specific molecules physically associated with the TCR serve the signal-transducing functions of this antigen receptor complex.

The CD3 and ζ proteins are noncovalently associated with the TCR αβ heterodimer, and when the TCR recognizes antigen, these associated proteins transduce the signals that lead to T cell activation.

The components of the TCR complex are illustrated in Figures 7-8 and 7-9.

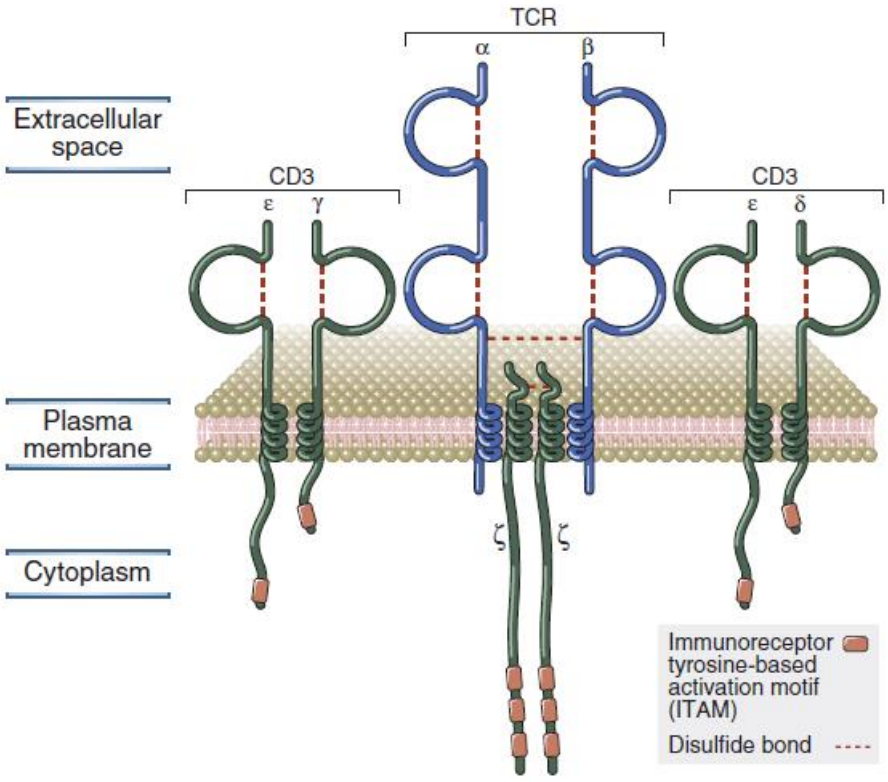


FIGURE 7-8 Components of the TCR complex. The TCR complex of MHC-restricted T cells consists of the αβ TCR noncovalently linked to the CD3 and ζ proteins. The association of these proteins with one another is mediated by charged residues in their transmembrane regions, which are not shown.

- The CD3 proteins and the ζ chain are identical in all T cells regardless of specificity, which is consistent with their role in signaling and not in antigen recognition.
- The CD3 proteins are required not only for signaling in T cells but for surface expression of the functionally complete receptor complex on T cells.
- The CD3 γ, δ, and ε proteins are homologous to each other. The N-terminal extracellular regions of the γ, δ, and ε chains each contains a single Ig-like domain, and therefore these three proteins are members of the Ig superfamily.
- The transmembrane segments of all three CD3 chains contain a negatively charged aspartic acid residue that binds to positively charged residues in the transmembrane domains of the TCR α and β chains.
- Each TCR complex contains one TCR αβ heterodimer associated with one CD3 γε heterodimer, one CD3 δε heterodimer, and one disulfide-linked ζζ homodimer.
- The cytoplasmic domains of the CD3 γ, δ, and ε proteins range from 44 to 81 amino acid residues in length, and each of these domains contains one ITAM.
- The ζ chain has a short extracellular region of nine amino acids, a transmembrane region containing a negatively charged aspartic acid residue (similar to the CD3 chains), and a long cytoplasmic region (113 amino acids) that contains three ITAMs. It is normally expressed as a homodimer.

The ζ chain is also associated with signaling receptors on lymphocytes other than T cells, such as the Fcγ receptor (FcγRIII) of NK cells.

Signal Initiation by the T cell Receptor

Ligation of the TCR by MHC-peptide ligands results in the clustering of coreceptors with the antigen receptor and phosphorylation of ITAM tyrosine residues.

- Phosphorylation of ITAM tyrosines initiates signal transduction and the activation of downstream tyrosine kinases, which in turn phosphorylate tyrosine residues on other adaptor proteins.
- The subsequent steps in signal transduction are generated by the specific recruitment of key enzymes that each initiate distinct downstream signaling pathways.
- It is thought that the TCR, like other immune receptors, is activated when multiple receptor molecules are brought together by binding to adjacent antigenic epitopes.
- However, cross-linking of the TCR poses a challenge because the induction of receptor clustering would require a high density of identical MHC-peptide complexes on APCs, and APCs generally express very few peptide-MHC complexes, perhaps as few as 100 per cell, that may be recognized by a given TCR (see Chapter 6).

How, then, is the signal from the TCR initiated? It is known that antigen recognition by the TCR induces ITAM phosphorylation by active Src family kinases, but the actual mechanism of signal initiation remains to be conclusively determined. There is growing evidence that ITAMs in the TCR complex are "folded" and unavailable before the TCR recognizes antigen. Recognition of MHC-peptide complexes may induce a conformational change in the TCR, making the ITAMs associated with the linked CD3 or ζ chains available for tyrosine phosphorylation by Src family kinases. Alternatively, the activity of Src family kinases may be enhanced after receptor

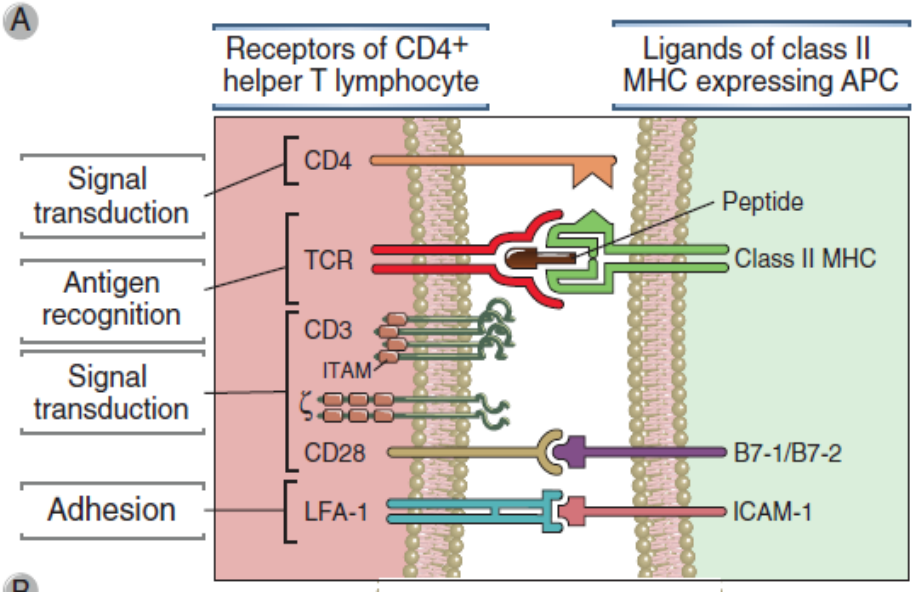
ligation (Fig. 7-10). The CD4 and CD8 coreceptors (described next) greatly facilitate the activation process by bringing Lck (which is loosely associated with the tail of the coreceptor proteins) close to the CD3 and ζ ITAMs (see Fig. 7-10). Eventually, a relatively stable interface is formed between the T cell and the APC, and this interface is known as the immunologic synapse (discussed later).

Figure 7-10 are given in following section.

The Role of the CD4 and CD8 Coreceptors in T cell Activation

CD4 and CD8 are T cell coreceptors that bind to nonpolymorphic regions of MHC molecules and facilitate signaling by the TCR complex during T cell activation (see Fig.7-9).

A



B

T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen presenting cells
CD8	Signal transduction	Class I MHC	Antigen presenting cells, CTL target cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen presenting cells
CTLA-4	Signal transduction (negative regulation)	B7-1/B7-2	Antigen presenting cells
LFA-1	Adhesion	ICAM-1	Antigen presenting cells, endothelium
VLA-4	Adhesion	VCAM-1	Endothelium

FIGURE 7–9 Ligand-receptor pairs involved in T cell activation. **A,** The major surface molecules of CD4+ T cells involved in the activation of these cells (the receptors) and the molecules on APCs (the ligands) recognized by the receptors are shown. CD8+ T cells use most of the same molecules, except that the TCR recognizes peptide–class I MHC complexes, and the coreceptor is CD8, which recognizes class I MHC. Immunoreceptor tyrosine-based activation motifs (ITAMs) are the regions of signaling proteins that are phosphorylated on tyrosine residues and become docking sites for other signaling molecules. CD3 is composed of three polypeptide chains, named γ, δ, and ε, arranged in two pairs (γε and δε); we show CD3 as three protein chains. **B,** The important properties of the major "accessory" molecules of T cells, so called because they participate in responses to antigens but are not the receptors for antigen, are summarized. CTLA-4 (CD152) is a receptor for B7 molecules that delivers inhibitory signals; its role in shutting off T cell responses is described in Chapter 9. VLA molecules are integrins involved in leukocyte binding to endothelium (see Chapter 3). APC, antigen-presenting cell; ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function-associated antigen 1; MHC, major histocompatibility complex; TCR, T cell receptor; VLA, very late antigen.

These proteins are called **coreceptors** because they bind to MHC molecules and thus recognize a part of the same ligand (peptide-MHC complexes) that interacts with the TCR. Mature αβ T cells express either CD4 or CD8, but not both. CD8 and CD4 interact with class I and class II MHC molecules, respectively, and are responsible for the class I or class II MHC restriction of these subsets of T cells (see Fig. 7-9).

CD4 and CD8 are transmembrane glycoprotein members of the Ig superfamily (Fig. 7-11). CD4 is expressed as a monomer on the surface of peripheral T cells and thymocytes and is also present on mononuclear phagocytes and some dendritic cells. It is the receptor on T cells for the envelope protein of the human immunodeficiency virus. CD4 has four extracellular Ig-like domains, a hydrophobic transmembrane region, and a highly basic cytoplasmic tail 38 amino acids long. The two N-terminal Ig-like domains of the CD4 protein bind to the nonpolymorphic β2 domain of the class II MHC molecule.

Most CD8 molecules exist as disulfide-linked heterodimers composed of two related chains called CD8α and CD8β (see Fig. 7-11). Both the α chain and the β chain have a single extracellular Ig domain, a hydrophobic transmembrane region, and a highly

basic cytoplasmic tail about 25 amino acids long. The Ig domain of CD8 binds to the nonpolymorphic α3 domain of class I MHC molecules. Some T cells express CD8 αα homodimers, but this different form appears to function like the more common CD8 αβ heterodimers. These homodimers are also present on a subset of murine dendritic cells (see Chapter 6).

The cytoplasmic tails of both CD4 and CD8 bind the Src family kinase Lck. The ability of these coreceptors to bind to MHC molecules helps these proteins to be drawn adjacent to the TCR that contacts the same MHC-peptide complex on the APC. As a result, on the cytosolic face of the membrane, Lck is drawn very close to the ITAMs in CD3 and ζ proteins and phosphorylates the ITAMs, thus facilitating the subsequent recruitment and activation of the kinase ZAP-70.

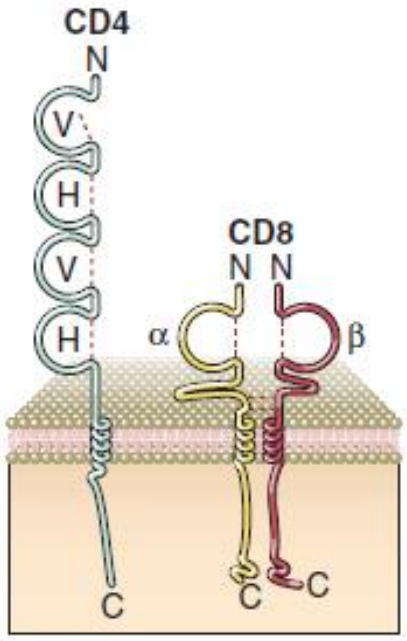


FIGURE 7–11 A schematic view of the structure of the CD4 and CD8 coreceptors. The CD4 protein is an integral membrane monomer consisting of four extracellular Ig domains, a transmembrane domain, and a cytoplasmic tail. The CD8 protein is either a disulfide-linked αβ integral membrane heterodimer or a disulfide linked αα homodimer (not shown). Each chain has a single extracellular Ig domain. The cytoplasmic portions of both CD4 and CD8 can associate with Lck (not shown).

Activation of Tyrosine Kinases and a Lipid Kinase during T cell Activation

Phosphorylation of residues in proteins and lipids plays a central role in the transduction of signals from the TCR complex and coreceptors.

Within seconds of TCR ligation, many of the tyrosine residues within the ITAMs of the CD3 and ζchains become phosphorylated (see Fig.7-10).

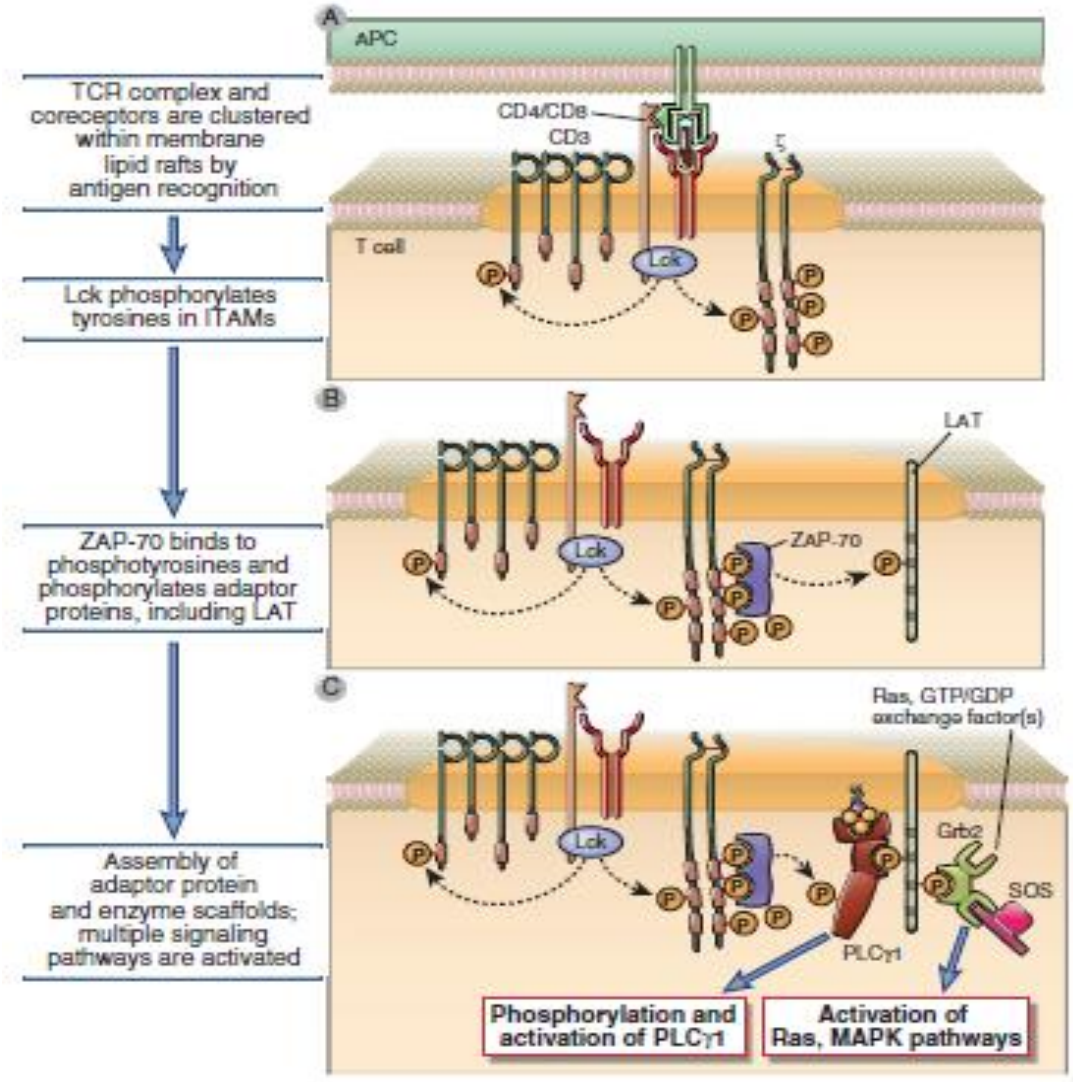


FIGURE 7–10 Early tyrosine phosphorylation events in T cell activation. On antigen recognition, there is clustering of TCR complexes with coreceptors (CD4, in this case). CD4-associated Lck becomes active and phosphorylates tyrosines in the ITAMs of CD3 and ζ chains **(A)**. ZAP-70 binds to the phosphotyrosines of the ζ chains and is itself phosphorylated and activated. (The illustration shows one ZAP-70 molecule binding to two phosphotyrosines of one ITAM in the ζ chain, but it is likely that initiation of a T cell response requires the assembly of multiple ZAP-70 molecules on each ζ chain.) Active ZAP-70 then phosphorylates tyrosines on various adaptor molecules, such as LAT **(B)**. The adaptors become docking sites for cellular enzymes such as PLCγ1 and exchange factors that activate Ras and other small G proteins upstream of MAP kinases **(C)**, and these enzymes activate various cellular responses.

In addition to coreceptor-associated Lck, another Src family kinase that is found in physical association with the TCR complex is CD3-associated Fyn, and it may play a role similar to that of Lck. Knockout mice lacking Lck show some defects in T cell development, and double knockout mice lacking both Lck and Fyn show even more severe defects. The tyrosine-phosphorylated ITAMs in the ζ chain become "docking sites" for the Syk family tyrosine kinase called **ZAP-70** (ζ - associated protein of 70 kD). ZAP-70 contains two SH2 domains that can bind to ITAM phosphotyrosines. Each ITAM has two tyrosine residues, and both of these must be phosphorylated to provide a docking site for one ZAP-70 molecule. The bound ZAP-70 becomes a substrate for the adjacent Lck, which phosphorylates specific tyrosine residues of ZAP-70. As a result, ZAP-70 acquires its own tyrosine kinase activity and is then able to phosphorylate a number of other cytoplasmic signaling molecules. A critical threshold of ZAP-70 activity may be needed before downstream signaling events will proceed, and this threshold is achieved by the recruitment of multiple ZAP-70 molecules to the phosphorylated ITAMs on the ζ chains and on CD3 tails.

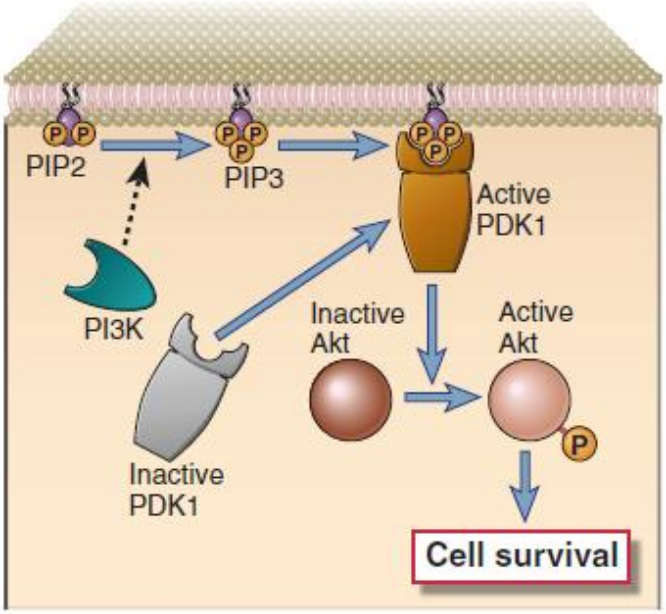


FIGURE 7–12 Role of PI3-kinase in T cell responses. Membrane PIP3, generated by PI3-kinase (PI3K), activates PDK1, which phosphorylates and activates the Akt kinase. This enzyme phosphorylates downstream targets that are involved in cell survival.

Another signaling pathway in T cells involves the activation of PI3-kinase, which phosphorylates a specific membrane-associated inositol lipid (Fig. 7-12).
This enzyme is recruited to the TCR complex and associated adaptor proteins and generates phosphatidylinositol trisphosphate (PIP3) from membrane phosphatidylinositol bisphosphate (PIP2) on the inner leaflet of the plasma membrane. Certain signaling proteins in the cytosol have specialized PH domains that have an affinity for PIP3, and as a result, PH domain–containing proteins can bind to the inside of the cell membrane only when PIP3 is generated.

Examples of PH domain–containing proteins include kinases such as Itk in T cells and Btk in B cells. Another important PIP3-dependent kinase is PDK1, which is required for the phosphorylation and activation of an important downstream kinase called Akt. Activated Akt phosphorylates crucial targets and contributes to cell survival in a number of ways. Phosphorylation by Akt leads to the inactivation of two proapoptotic members of the Bcl-2 family, BAD and BAX. Akt also inactivates a Forkhead family transcription factor that induces the expression of Fas ligand, and this kinase also targets caspase-9 for degradation.
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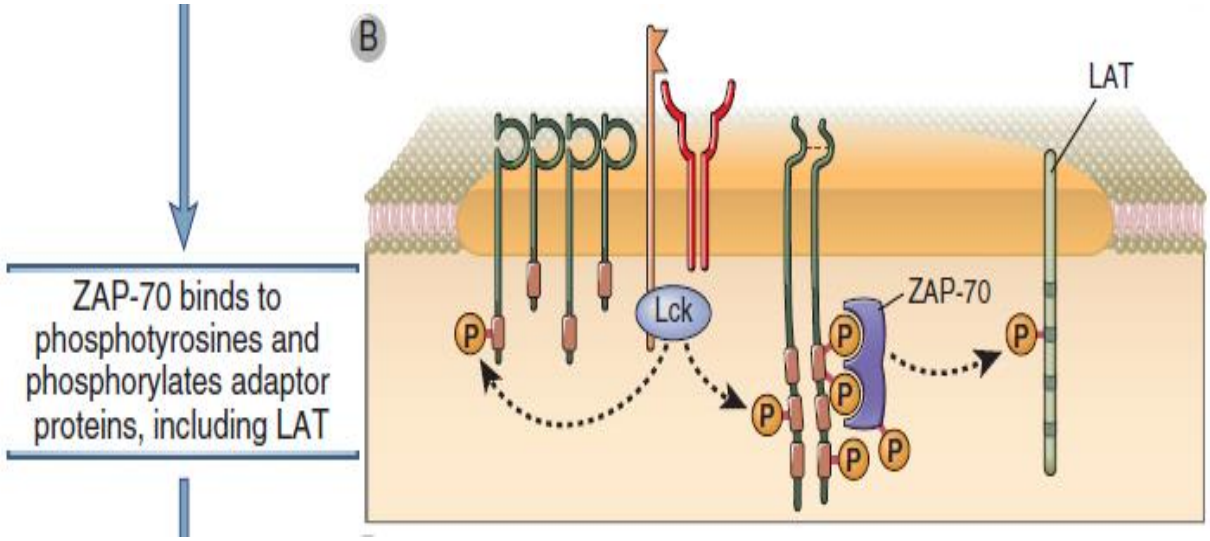
Recruitment and Modification of Adaptor Proteins

Activated ZAP-70 phosphorylates several adaptor proteins that are able to bind signaling molecules (see Fig. 7-10).

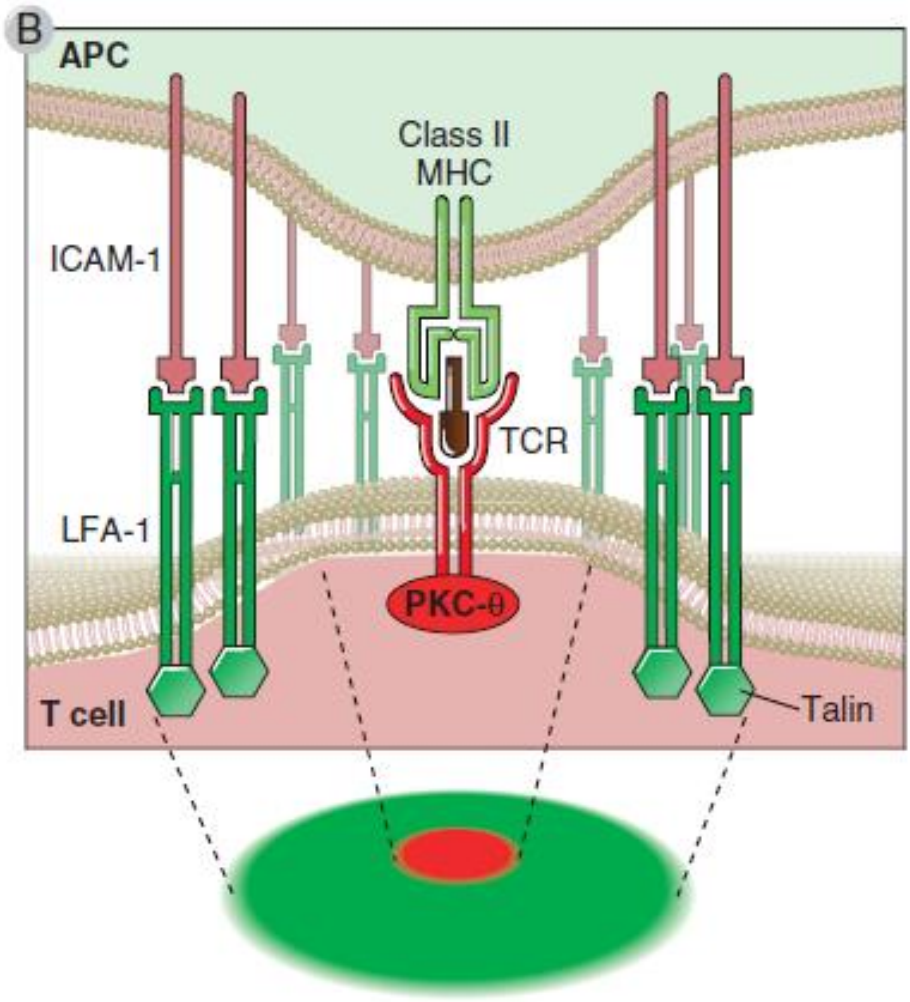
- A key early event in T cell activation is the ZAP - 70–mediated tyrosine phosphorylation of adaptor proteins such as SLP-76 and LAT.
- Phosphorylated LAT directly binds PLCγ1, a key enzyme in T cell activation (discussed later), and coordinates the recruitment of several other adaptor proteins, including SLP-76, GADS, and Grb-2, to the cluster of TCR and TCR-associated proteins, sometimes referred to as the signalosome.
- Thus, LAT serves to bring a variety of downstream components of TCR signaling pathways close to their upstream activators.

Because the function of many of these adaptors depends on their tyrosine phosphorylation by active ZAP-70, only antigen recognition (the physiologic stimulus for ZAP-70 activation) triggers the signal transduction pathways that lead to functional T cell responses.



Formation of the Immunologic Synapse

When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact (Fig. 7-13).



- This region of physical contact between the T cell and the APC forms a bull'seye– like structure that is called an **immunologic synapse** or a supramolecular activation cluster (SMAC).
- The T cell molecules that are rapidly mobilized to the center of the synapse include the TCR complex (the TCR, CD3, and ζ chains), CD4 or CD8 coreceptors, receptors for costimulators (such as CD28), enzymes such as

PKC-θ, and adaptor proteins that associate with the cytoplasmic tails of the transmembrane receptors.

- At this portion of the synapse, called the c-SMAC (for central supramolecular activation cluster), the distance between the T cell plasma membrane and that of the APC is about 15 nm.
- Integrins remain at the periphery of the synapse, where they function to stabilize the binding of the T cell to the APC, forming the peripheral portion of the SMAC called the p-SMAC.
- In this outer part of the synapse, the two membranes are about 40 nm apart.
- Many signaling molecules found in synapses are initially localized to regions of the plasma membrane that have a lipid content different from the rest of the cell membrane and are called **lipid rafts** or glycolipid-enriched microdomains.
- TCR and costimulatory receptor signaling is initiated in these rafts, and signaling initiates cytoskeletal rearrangements that allow rafts to coalesce and form the immunologic synapse.

Immunologic synapses may serve a number of functions during and after T cell activation.

- The synapse forms a stable contact between an antigen specific T cell and an APC displaying that antigen and becomes the site for assembly of the signaling machinery of the T cell, including the TCR complex, coreceptors, costimulatory receptors, and adaptors.

  Although TCR signal transduction is clearly initiated before the formation of the synapse and is required for synapse formation, the immunologic synapse itself may provide a unique interface for TCR triggering.

T cell activation needs to overcome the problems of a generally low affinity of TCRs for peptide-MHC ligands and the presence of few MHC molecules displaying any one peptide on an APC.

The synapse represents a site at which repeated engagement of TCRs may be sustained by this small number of peptide-MHC complexes on the APC, thus facilitating prolonged and effective T cell signaling.

- The synapse may ensure the specific delivery of secretory granule contents and cytokines from a T cell to APCs or targets that are in contact with the T cell.

  Vectorial delivery of secretory granules containing perforin and granzymes from CTLs to target cells has been shown to occur at the synapse.

  Similarly, CD40L-CD40 interactions are facilitated by the accumulation of these molecules on the T cell and APC interfaces of the immunologic synapse.

  Some cytokines are also secreted in a directed manner into the synaptic cleft, from where they are preferentially delivered to the cell that is displaying antigen to the T lymphocyte.
- The synapse may also be an important site for the turnover of signaling molecules, primarily by monoubiquitination and delivery to late endosomes and lysosomes.

  This degradation of signaling proteins may contribute to the termination of T cell activation and is discussed later.

MAP Kinase Signaling Pathways in T Lymphocytes

Small guanine nucleotide–binding proteins (G proteins) activated by antigen recognition stimulate at least three different mitogen-activated protein (MAP) kinases, which in turn activate distinct transcription factors.

G proteins are involved in diverse activation responses in different cell types. Two major members of this family activated downstream of the TCR are Ras and Rac. Each activates a different component or set of transcription factors, and together they mediate many cellular responses of T cells.

- **The Ras pathway is activated in T cells after TCR ligation, leading to the activation of the extracellular receptor-activated kinase (ERK), a prominent member of the MAP kinase family, and eventually to the activation of downstream transcription factors.**
    - Ras is loosely attached to the plasma membrane through covalently attached lipids. In its inactive form, the guanine nucleotide–binding site of Ras is occupied by guanosine diphosphate (GDP).
    - When the bound GDP is replaced by guanosine triphosphate (GTP), Ras undergoes a conformational change and can then recruit or activate various cellular enzymes, the most important of which is c-Raf.
    - Activation of Ras by GDP/GTP exchange is seen in response to the engagement of many types of receptors in many cell populations, including the TCR complex in T cells.
    - Mutated Ras proteins that are constitutively active (i.e., they constantly assume the GTP-bound conformation) are associated with neoplastic transformation of many cell types.
    - Nonmutated Ras proteins are active GTPases that convert the GTP bound to Ras into GDP, thus returning Ras to its normal, inactive state.

- The mechanism of Ras activation in T cells involves the adaptor proteins LAT and Grb-2 (Fig. 7-14).

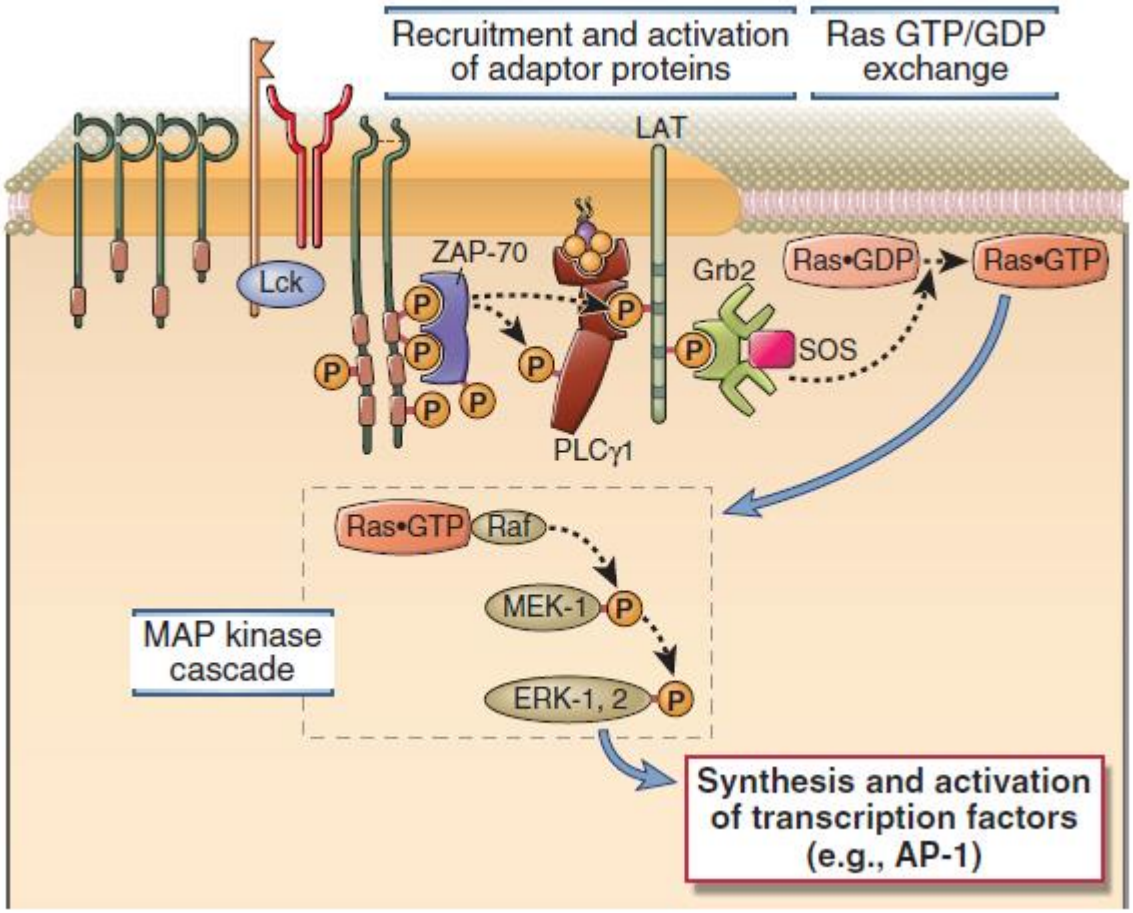


FIGURE 7–14 The Ras-MAP kinase pathway in T cell activation. ZAP-70 that is activated by antigen recognition phosphorylates membrane-associated adaptor proteins (such as LAT), which then bind another adaptor, Grb-2, that provides a docking site for the GTP/GDP exchange factor SOS. SOS converts Ras · GDP to Ras · GTP. Ras · GTP activates a cascade of enzymes, which culminates in the activation of the MAP kinase ERK. A parallel Rac-dependent pathway generates another active MAP kinase, JNK (not shown).

- When LAT is phosphorylated by ZAP-70 at the site of TCR clustering, it serves as the docking site for the SH2 domain of Grb-2.
- Once attached to LAT, Grb-2 recruits the Ras GTP/GDP exchange factor called SOS (so named because it is the mammalian homologue

of a *Drosophila* protein called son of sevenless) to the plasma membrane.

- SOS catalyzes GTP for GDP exchange on Ras. This generates the GTP-bound form of Ras (written as Ras·GTP), which then activates a "MAP kinase" cascade of three kinases, the first two of which phosphorylate and activate the next kinase in the cascade.
- The last kinase in the cascade initiated by Ras is a MAP kinase called ERK.
- Ras·GTP activates a kinase called c-Raf, which then activates a dual specificity kinase that phosphorylates ERK on closely spaced threonine and tyrosine residues.
- This dual specificity kinase is an example of a MAP kinase kinase (a kinase that activates a MAP kinase).
- The activated ERK MAP kinase translocates to the nucleus and phosphorylates a protein called Elk, and phosphorylated Elk stimulates transcription of c-Fos, a component of the activation protein 1 (AP-1) transcription factor.

- In parallel with the activation of Ras through recruitment of Grb-2 and SOS, the adaptors phosphorylated by TCR-associated kinases also recruit and activate a GTP/GDP exchange protein called Vav that acts on another small guanine nucleotide–binding protein called **Rac** (see Fig. 7-14). The Rac·GTP that is generated initiates a parallel MAP kinase cascade, resulting in the activation of a distinct MAP kinase called c-Jun N-terminal kinase (JNK). JNK is sometimes called stress-activated protein (SAP) kinase because in many cells, it is activated by various forms of noxious stimuli such as ultraviolet light, osmotic stress, or proinflammatory cytokines such as tumor

necrosis factor (TNF) and IL-1. Activated JNK then phosphorylates c-Jun, the second component of the AP-1 transcription factor.

A third member of the MAP kinase family, in addition to ERK and JNK, is p38, and it too is activated by Rac·GTP and in turn activates various transcription factors. Rac·GTP also induces cytoskeletal reorganization and may play a role in the clustering of TCR complexes, coreceptors, and other signaling molecules into the synapse. The activities of ERK and JNK are eventually shut off by the action of dual-specificity protein tyrosine/threonine phosphatases. These phosphatases are induced or activated by ERK and JNK themselves, providing a negative feedback mechanism to terminate T cell activation.

Calcium- and PKC-Mediated Signaling Pathways in T Lymphocytes

TCR signaling leads to the activation of the γ1 isoform of the enzyme phospholipase C (PLCγ1), and the products of PLCγ1-mediated hydrolysis of membrane lipids activate enzymes that induce specific transcription factors in T cells (Fig. 7-15).

- PLCγ1 is a cytosolic enzyme specific for inositol phospholipids that is recruited to the plasma membrane by tyrosine-phosphorylated LAT within minutes of ligand binding to the TCR.
- Here, the enzyme is phosphorylated by ZAP-70 and by other kinases, such as the Tec family kinase called Itk.
- Phosphorylated PLCγ1 catalyzes the hydrolysis of a plasma membrane phospholipid called PIP2, generating two breakdown products, the soluble sugar triphosphate, inositol 1,4,5-trisphosphate (IP3), and membrane-bound diacylglycerol (DAG).
- **IP3 and DAG then activate two distinct downstream signaling pathways in T cells.**

Pathway I: IP3 produces a rapid increase in cytosolic free calcium within minutes after T cell activation.

- IP3 diffuses through the cytosol to the endoplasmic reticulum, where it binds to its receptor, a ligand-gated calcium channel, and stimulates release of membrane-sequestered calcium stores.
- The released calcium causes a rapid rise (during a few minutes) in the cytosolic free calcium ion concentration, from a resting level of about 100 nM to a peak of 600 to 1000 nM.
- The depletion of endoplasmic reticulum calcium is sensed by an endoplasmic reticulum membrane protein called STIM1, which activates a “storeoperated”

plasma membrane ion channel called a **CRAC** (calcium release–activated calcium) channel.

- The result is an influx of extracellular calcium that sustains cytosolic levels at about 300 to 400 nM for more than an hour.
- A key component of the CRAC channel is a protein called **Orai**, which was discovered as a gene that is defective in a rare human immunodeficiency disease.
- Cytosolic free calcium acts as a signaling molecule by binding to a ubiquitous calcium-dependent regulatory protein called calmodulin.
- Calcium-calmodulin complexes activate several enzymes, including a protein serine/threonine phosphatase called **calcineurin** that is important for transcription factor activation, as discussed later.

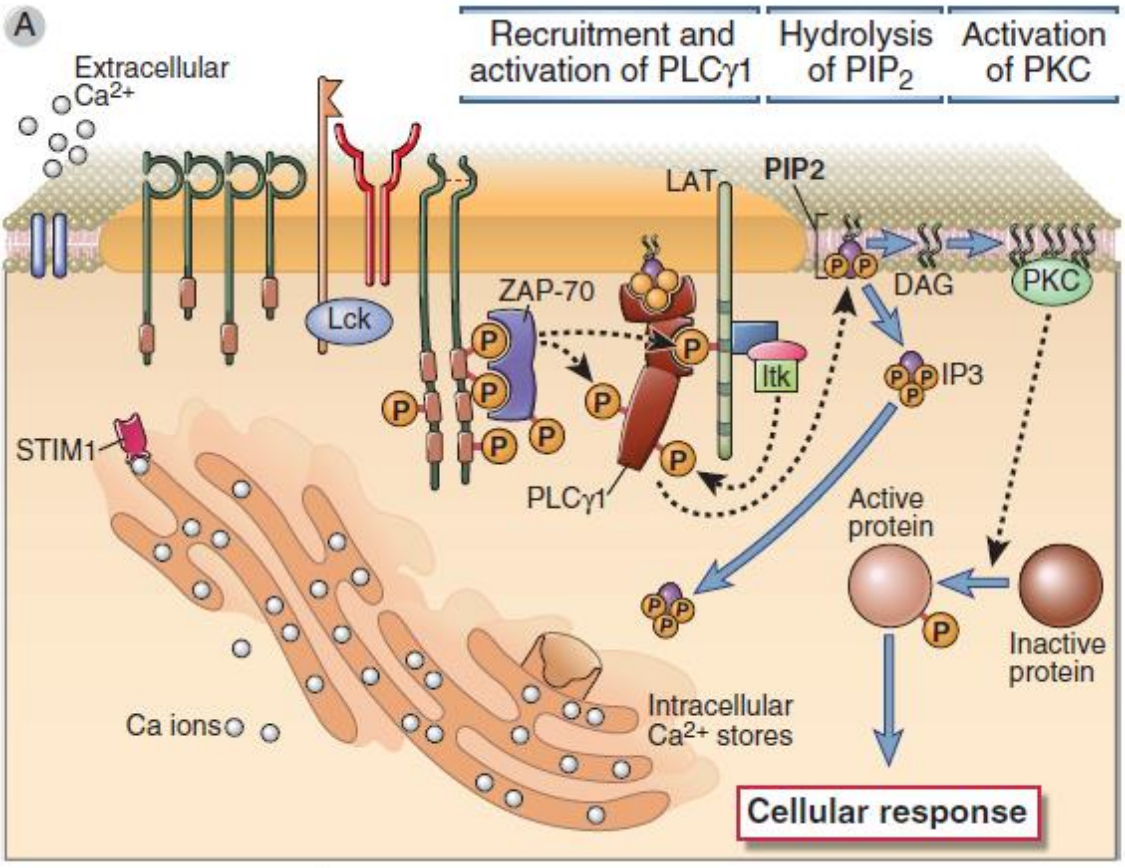
Pathway II: Diacylglycerol (DAG), the second breakdown product of PIP2, is a membrane-bound lipid that activates the enzyme protein kinase C (PKC).

- There are several isoforms of PKC that participate in the generation of active transcription factors, discussed later.
- The combination of elevated free cytosolic calcium and DAG activates certain isoforms of membrane-associated PKC by inducing a conformational change that makes the catalytic site of the kinase accessible to its substrates.
- Numerous downstream proteins are phosphorylated by PKC. The PKC-θ isoform localizes to the immunologic synapse and is involved in the activation and nuclear translocation of the nuclear factor κB (NF-κB) transcription factor.
- Pathways of NF-κB activation are discussed later in this chapter.

So far, we have described several signal transduction pathways initiated by ligand binding to the TCR that result in the activation of different types of enzymes:

- Small G protein–MAP kinase pathways leading to activation of kinases such as ERK and JNK;
- A PLCγ1-calcium–dependent pathway leading to activation of the phosphatase calcineurin; and
- A DAG-dependent pathway leading to activation of PKC.
- Each of these pathways contributes to the expression of genes encoding proteins needed for T cell clonal expansion, differentiation, and effector functions.

In the following section, we describe the mechanisms by which these different signaling pathways stimulate the transcription of various genes in T cells.



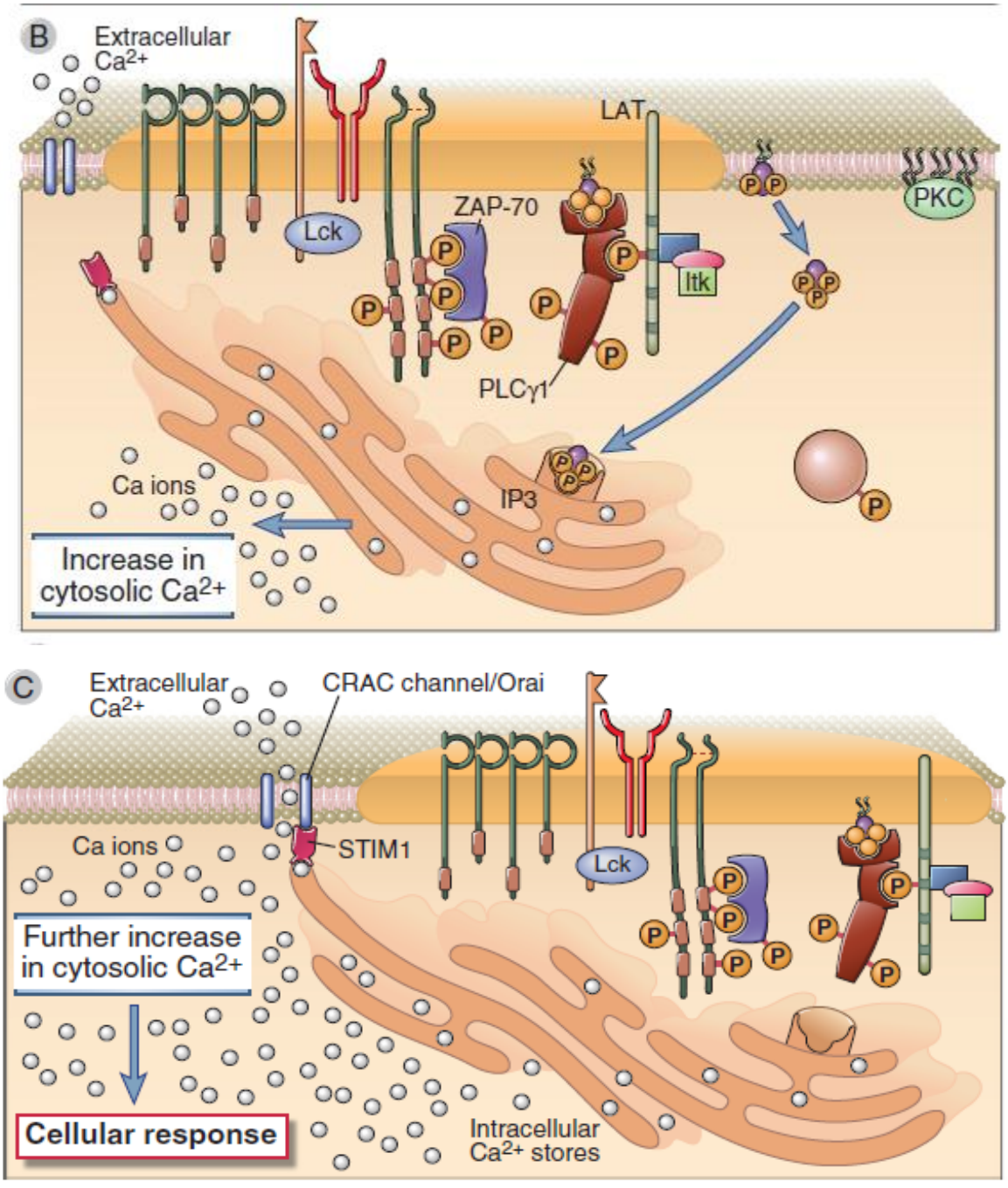


FIGURE 7–15 T cell signaling downstream of PLCγ1. **A,** The LAT adaptor protein that is phosphorylated on T cell activation binds the cytosolic enzyme PLCγ1, which is phosphorylated by ZAP-70 and other kinases, such as Itk, and activated. Active PLCγ1 hydrolyzes membrane PIP2 to generate IP3, which stimulates an increase in cytosolic calcium, and DAG, which activates the enzyme PKC. **B,** Depletion of endoplasmic reticulum calcium is sensed by STIM1. **C,** STIM1 which induces the opening of the CRAC channel that facilitates entry of extracellular calcium into the cytosol. Orai is a component of the CRAC channel. Increased cytosolic calcium and PKC then activate various transcription factors, leading to cellular responses.

Activation of Transcription Factors That Regulate T cell Gene Expression

The enzymes generated by TCR signaling activate transcription factors that bind to regulatory regions of numerous genes in T cells and thereby enhance transcription of these genes (Fig. 7-16).

- Much of our understanding of the transcriptional regulation of genes in T cells is based on analyses of cytokine gene expression.
- The transcriptional regulation of most cytokine genes in T cells is controlled by the binding of transcription factors to nucleotide sequences in the promoter and enhancer regions of these genes.
- For instance, the IL-2 promoter, located 5′ of the coding exons of this gene, contains a segment of approximately 300 base pairs in which are located binding sites for several different transcription factors.
- All these sites must be occupied by transcription factors for maximal transcription of the IL-2 gene.
- Different transcription factors are activated by different cytoplasmic signal transduction pathways, and the requirement for multiple transcription factors accounts for the need to activate many signaling pathways after antigen recognition.
- It is likely that the same principles are true for many genes in T cells, including genes encoding cytokine receptors and effector molecules, although different genes may be responsive to different combinations of transcription factors.
- Three transcription factors that are activated in T cells by antigen recognition and appear to be critical for most T cell responses are nuclear factor of activated T cells (NFAT), AP-1, and NF-κB.

- **NFAT** is a transcription factor required for the expression of IL-2, IL-4, TNF, and other cytokine genes.
  - NFAT is present in an inactive, serine-phosphorylated form in the cytoplasm of resting T lymphocytes.
  - It is activated by the calcium-calmodulin–dependent phosphatase calcineurin. Calcineurin dephosphorylates cytoplasmic NFAT, thereby uncovering a nuclear localization signal that permits NFAT to translocate into the nucleus.
  - Once it is in the nucleus, NFAT binds to the regulatory regions of IL-2, IL-4, and other cytokine genes, usually in association with other transcription factors, such as AP-1.
  - The mechanism of activation of NFAT was discovered indirectly by studies of the mechanism of action of the immunosuppressive drug cyclosporine.
  - This drug and the functionally similar compound, FK506, are natural products of fungi and are widely used therapeutic agents to treat allograft rejection.
  - They function largely by blocking T cell cytokine gene transcription. Cyclosporine binds to a cytosolic protein called cyclophilin, and FK506 binds to a protein called FK506-binding protein (FKBP).
  - Cyclophilin and FKBP are also called immunophilins. Cyclosporine-cyclophilin complexes and FK506-FKBP complexes bind to and inhibit calcineurin and thereby block translocation of NFAT into the nucleus.

- **AP-1** is a transcription factor found in many cell types; it is specifically activated in T lymphocytes by TCR mediated signals.
    - AP-1 is actually the name for a family of DNA-binding factors composed of dimers of two proteins that bind to one another through a shared structural motif called a leucine zipper.
    - The best characterized AP-1 factor is composed of the proteins Fos and Jun.
    - TCR-induced signals lead to the appearance of active AP-1 in the nucleus of T cells.
    - Activation of AP-1 typically involves synthesis of the Fos protein and phosphorylation of preexisting Jun protein.
    - Transcription and synthesis of Fos can be enhanced by the ERK pathway, as described before, and also by PKC.
    - JNK phosphorylates c-Jun, and AP-1 complexes containing the phosphorylated form of Jun have increased transcription-enhancing activity.
    - AP-1 appears to physically associate with other transcription factors in the nucleus, including NFAT, and works best in combination with NFAT.
    - Thus, AP-1 activation represents a convergence point of several TCR-initiated signaling pathways.
- **NF-κB** is a transcription factor that is activated in response to TCR signals and is essential for cytokine synthesis.
    - NF-κB proteins are homodimers or heterodimers of proteins that are homologous to the product of a cellular proto-oncogene called c-*rel* and are important in the transcription of many genes in diverse cell types, particularly in innate immune cells.

    - In resting T cells, NF-κB is present in the cytoplasm in a complex with other proteins called inhibitors of κB (IκBs), which make a nuclear localization signal on NF-κB inaccessible, thus preventing the entry of this factor into the nucleus.
    - TCR signals lead to serine phosphorylation of IκBα and then its ubiquitination and proteasomal degradation.
    - The enzymes responsible for phosphorylation of IκB are called IκB kinases, and these are discussed toward the end of this chapter.
    - Once released from IκB, NF-κB is able to migrate into the nucleus and bind to and regulate the promoters of target genes.

- The links between different signaling proteins, activation of transcription factors, and functional responses of T cells are often difficult to establish because there are complex and incompletely understood interactions between signaling pathways.
- Also, for the sake of simplicity, we often discuss signaling in the context of linear pathways, but it is likely that this does not reflect the more complex and interconnected reality.
- Finally, we have focused on selected pathways to illustrate how antigen recognition may lead to biochemical alterations, but it is clear that many other signaling molecules are also involved in antigen-induced lymphocyte activation.

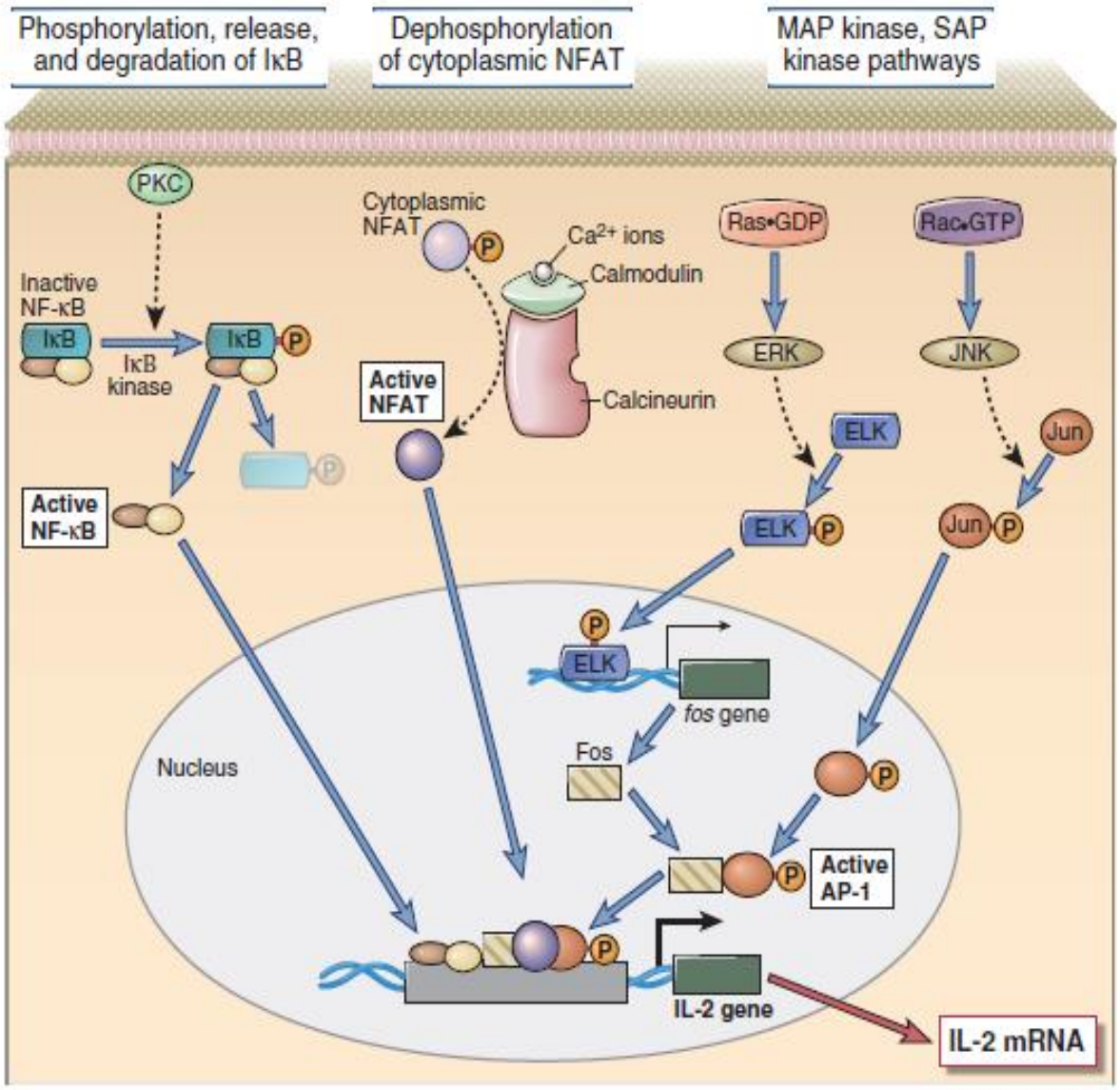


FIGURE 7–16 Activation of transcription factors in T cells. Multiple signaling pathways converge in antigen-stimulated T cells to generate transcription factors that stimulate expression of various genes (in this case, the IL-2 gene). The calcium-calmodulin pathway activates NFAT, and the Ras and Rac pathways generate the two components of AP-1. Less is known about the link between TCR signals and NF-κB activation. (NF-κB is shown as a complex of two subunits, which in T cells are typically the p50 and p65 proteins, named for their molecular sizes in kilodaltons.) PKC is important in T cell activation, and the PKC-θ isoform is particularly important in activating NF-κB. These transcription factors function coordinately to regulate gene expression. Note also that the various signaling pathways are shown as activating unique transcription factors, but there may be considerable overlap, and each pathway may play a role in the activation of multiple transcription factors.

Modulation of T cell signaling by Protein Tyrosine Phosphatases

Tyrosine phosphatases remove phosphate moieties from tyrosine residues on proteins and generally inhibit TCR signaling.

- Two tyrosine phosphatases that serve an **important inhibitory role** in lymphocytes and other hematopoietic cells are called **SHP-1 and SHP-2 (for SH2 domain–containing phosphatases 1 and 2)**.
- Inhibitory phosphatases are **typically recruited by inhibitory receptors** that are induced after a lymphocyte has been activated by tyrosine kinases.
- These phosphatases **inhibit signal transduction** by removing phosphates from tyrosine residues in key signaling molecules and thus **functionally antagonize tyrosine kinases**.
- Another inhibitory phosphatase that **does not act on phosphoproteins but rather is specific for an inositol phospholipid** is called **SHIP (SH2 domain–containing inositol phosphatase).**
- **Like SHP-1 and SHP-2, SHIP binds to phosphorylated ITIM sequences on specific inhibitory receptors. SHIP removes a phosphate group from PIP3, a phospholipid in the inner leaflet of the plasma membrane, and thus antagonizes PI3-kinase signaling in lymphocytes.**
- **Although most phosphatases attenuate lymphocyte signaling, one tyrosine phosphatase, CD45, facilitates lymphocyte activation.**
- The **CD45 protein is a receptor tyrosine phosphatase** expressed in all hematopoietic cells. It is an integral membrane protein whose cytoplasmic tail contains tandem protein tyrosine phosphatase domains.
- **CD45 dephosphorylates inhibitory tyrosine residues in the Src family kinases Lck and Fyn and thus contributes to the generation of active kinases.**

Costimulatory Receptors of T Cells

Costimulatory signals are delivered by receptors that recognize ligands that are induced on APCs by microbes and cooperate with TCR signals to augment signaling and activate T cells.

The two-signal hypothesis for T cell activation. TCR signaling aided by coreceptors drives the T cell's response to foreign structures. In immunologic jargon, this response by the TCR to MHC and peptide on an APC is referred to as signal 1. T cells are fully activated only when a foreign peptide is recognized in the context of the activation of the innate immune system by a pathogen or some other cause of inflammation. Costimulatory ligands represent the danger signals (or signal 2) induced on antigen-presenting cells by microbes. "Foreignness" must combine with "danger" for optimal T cell activation to occur.

The CD28 Family of Costimulatory Receptors

The best defined costimulators for T lymphocytes are a pair of related proteins, called B7-1 (CD80) and B7-2 (CD86), which are expressed on activated dendritic cells, macrophages, and B lymphocytes.

The CD28 molecule on T cells is the principal costimulatory receptor for delivery of second signals for T cell activation. The biologic roles of the B7 and CD28 proteins are considered in more detail in upcoming topics.

Another important activating member of the CD28 family is a receptor called ICOS (inducible costimulator), which plays an important role in T follicular helper cell development and will be discussed in Chapters 9 and 11.

- **The CD2/SLAM Family of Costimulatory Receptors**

  Although the best studied and most prominent family of costimulatory receptors on T cells is the CD28 family, other proteins also contribute to

optimal T cell activation and differentiation. One important family of proteins that plays a role in the activation of T cells and NK cells is a group of proteins structurally related to a receptor called **CD2** (Fig. 7-17).

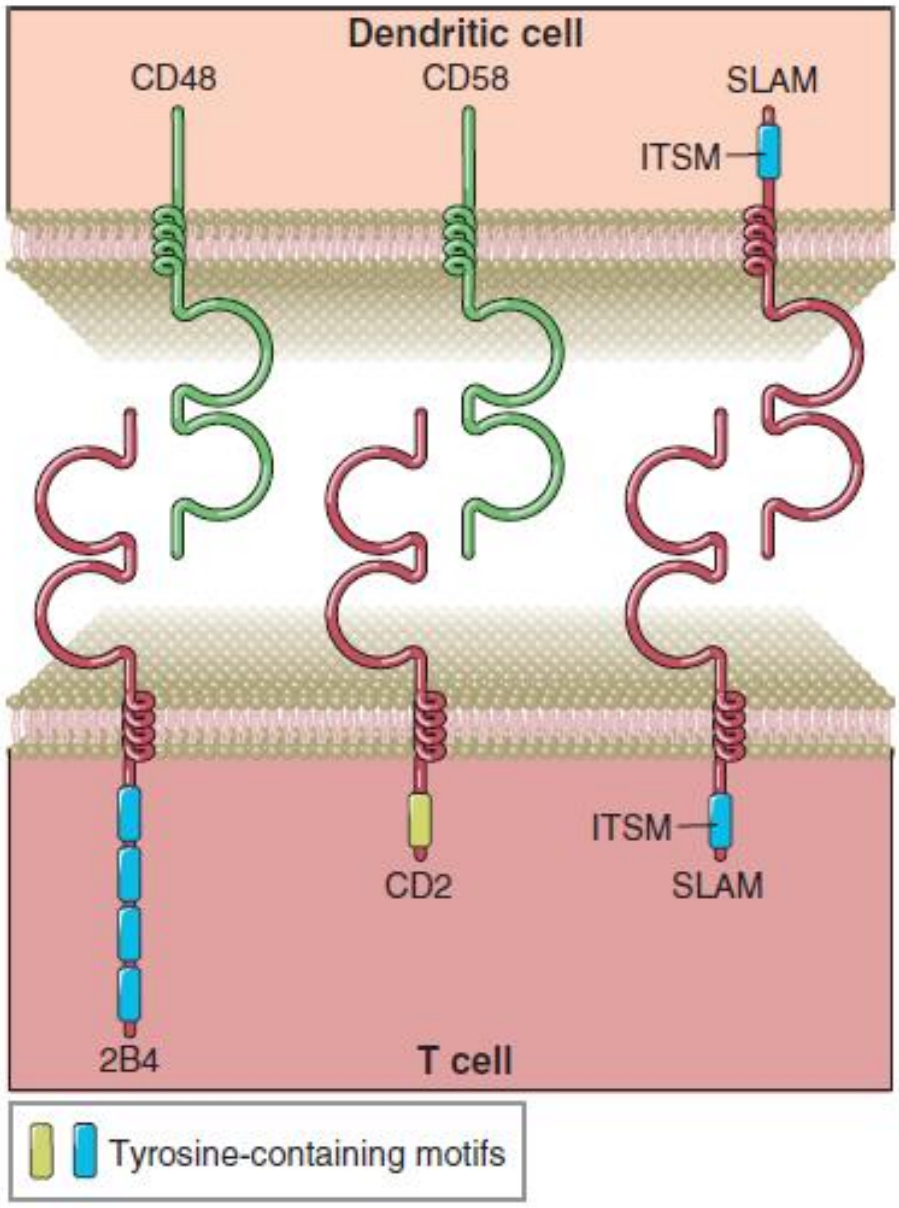


FIGURE 7–17 selected costimulatory receptors of the CD2 family and their ligands. 2B4, CD2, and SLAM contain two extracellular Ig-like domains, and their cytoplasmic tails also contain tyrosine-containing motifs. The tyrosine-based motif in the tail regions of SLAM and SLAM family members such as 2B4 is called an ITSM and binds to SAP or SAP-like proteins (not shown).

CD2 is a glycoprotein present on more than 90% of mature T cells, on 50% to 70% of thymocytes, and on NK cells. The molecule contains two extracellular Ig domains, a hydrophobic transmembrane region, and a long (116 amino acid residues) cytoplasmic tail. The principal ligand for CD2 in humans is a molecule called leukocyte function-associated antigen 3 (LFA-3, or CD58), also a member of the CD2 family. LFA-3 is expressed on a wide variety of hematopoietic and nonhematopoietic cells, either as an integral membrane protein or as a phosphatidylinositol-anchored membrane molecule. In mice, the principal ligand for CD2 is CD48, which is also a member of the CD2 family and is distinct from but structurally similar to LFA-3.

CD2 functions both as an intercellular adhesion molecule and as a signal transducer. Some anti-CD2 antibodies increase cytokine secretion by and proliferation of human T cells cultured with anti-TCR/CD3 antibodies, indicating that CD2 signals can enhance TCR-triggered T cell responses. Some anti-CD2 antibodies block conjugate formation between T cells and other LFA-3–expressing cells, indicating that CD2 binding to LFA-3 also promotes cell-cell adhesion. Such antibodies inhibit both CTL activity and antigen-dependent helper T cell responses.

Knockout mice lacking both CD28 and CD2 have more profound defects in T cell responses than do mice lacking either molecule alone. This indicates that CD28 and CD2 may compensate for each other, an example of the redundancy of costimulatory receptors of T cells. On the basis of such findings, anti-CD2 antibodies are currently being tested for their efficacy in psoriasis.

A distinct subgroup of the CD2 family of proteins is known as the **SLAM** (signaling lymphocytic activation molecule) family. SLAM, like all members of the CD2 family, is an integral membrane protein that contains two extracellular Ig domains and a relatively long cytoplasmic tail. The cytoplasmic tail of SLAM, but not of CD2, contains a specific tyrosine-based motif, TxYxxV/I (where T is a threonine

residue, Y is a tyrosine residue, V is a valine, I is an isoleucine, and x is any amino acid), known as an immunoreceptor tyrosine-based switch motif (ITSM) that is distinct from the ITAM and ITIM motifs found in other activating and inhibitory receptors. It is called a switch motif because in some receptors, this motif can orchestrate a "switch" from the binding of a tyrosine phosphatase, SHP-2, in the absence of an adaptor to the binding of other enzymes in the presence of an adaptor called SAP (SLAM-associated protein), thus potentially mediating a change from an inhibitory to an activating function.

The extracellular Ig domains of SLAM are involved in homophilic interactions. SLAM on a T cell can interact with SLAM on a dendritic cell and, as a result, the cytoplasmic tail of SLAM may deliver signals to T cells. The ITSM motif binds to SAP, and the latter forms a bridge between SLAM and Fyn (a Src family kinase that is also physically linked to CD3 proteins in T cells). SLAM and other members of the SLAM family function as costimulatory receptors in T cells, NK cells, and some B cells. As we shall discuss in Chapter 20, mutations in the *SH2D1A* gene encoding SAP are the cause of a disease called the X-linked lymphoproliferative syndrome (XLP).

An important member of the SLAM family in NK cells, CD8+ T cells, and γδ T cells is called **2B4** (see Fig. 7-17). 2B4 recognizes a known ligand for CD2 called CD48. Like SLAM, the cytoplasmic tail of 2B4 contains ITSM motifs, binds to the SAP adaptor protein, and signals by recruiting Fyn. Defective 2B4 signaling may contribute in a major way to the immune deficit in patients with the X-linked lymphoproliferative syndrome.

Suggestive Questions based on Lecture 1.

1. What do you mean by the term Signal Transduction? Write a short note on Cytosolic Phase and Nuclear Phase.
2. Write about the possible outcomes of Signal Transduction.
3. Justify the statement "The initiation of signaling from a cell surface receptor may require ligand induced clustering of the receptor, known as receptor cross-linking, or may involve a conformational alteration of the receptor that is induced by its association with ligand."
4. Write a short note on Protein Kinases and its types.
5. Justify the statement "For every type of phosphorylation event, there is a specific **phosphatase**, an enzyme that can remove a phosphate residue and thus modulate signaling."
6. Write a short note on various categories of cellular receptors based on the signaling mechanisms they use and the intracellular biochemical pathways they activate.
7. Comment on the statement "Signaling molecules are often composed of distinct modules, each with a specific binding or catalytic function."
8. Write short notes on Modular Signaling Proteins and Adaptors.
9. Write a short note on various adapters that participate in lymphocyte activation.
10. Why Signal transduction are compared as a kind of social networking phenomenon.
11. Write a short note on ITAMs and ITIMs.
12. Write a short notes on general features of Antigen Receptor Signaling.
13. Comment on the statement "Alterations in the strength of TCR and B cell receptor (BCR) signaling influence the fates of lymphocytes during their development and activation."

14. Explain in brief the steps involved in fine tuning of Antigen receptor signaling that are modulated by three mechanisms that are unique to the class of receptors like ITAMs, Coreceptors, and Associated Receptors.
15. Comment on the role of costimulatory receptors that controls the process of lymphocyte activation.
16. Diagrammatically explain the antigen receptor of MHC-restricted CD4+ helper T cells and CD8+ cytotoxic T lymphocytes (CTLs).
17. Diagrammatically explain the components of TCR complex involved in signal transduction process.
18. Comment on the statement "Ligation of the TCR by MHC-peptide ligands results in the clustering of coreceptors with the antigen receptor and phosphorylation of ITAM tyrosine residues."
19. Write short note on The Role of the CD4 and CD8 Coreceptors in T cell Activation.
20. Justify the statement "CD4 and CD8 are T cell coreceptors that bind to nonpolymorphic regions of MHC molecules and facilitate signaling by the TCR complex during T cell activation".
21. Comment on the statement "Phosphorylation of residues in proteins and lipids plays a central role in the transduction of signals from the TCR complex and coreceptors."
22. Write a short note on role of PI3-Kinase in T cell responses.
23. Briefly explain the process of recruitment and modification of Adapter Proteins.
24. Comment on the statement that "Activated ZAP-70 phosphorylates several adaptor proteins that are able to bind signaling molecules."
25. Write a short note on the concept of formation of Immunologic Synapse.

26. Discuss the statement “When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact”.
27. Point out and describe in brief various functions of Immunological Synapse.
28. Explain the role of MAP-Kinase Pathways in relation to T – Lymphocytes.
29. Explain the role of Calcium- and PKC-Mediated Signaling Pathways in T Lymphocytes.
30. Explain in brief the two distinct downstream signaling pathways activated by IP3 and DAG in T cells.
31. Write short notes on the role of NFAT, AP-1, and NF-κB. (Transcription factors) in regulation of T cell gene expression.
32. Explain how T cell signaling is modulated by Protein Tyrosine Phosphatases.