Cell Mediated Immunity: B-Cell Signal Transduction

ZCT – 210 [Topic No: 4] Lecture No: 2
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THE B LYMPHOCYTE ANTIGEN RECEPTOR COMPLEX

- The B lymphocyte antigen receptor is a transmembrane form of an antibody molecule associated with two signaling chains.

[Here we will focus on some salient features of the membrane forms of Ig and their associated proteins and discuss how they deliver signals to B cells. Because the signaling pathways are similar to those in T cells, we will summarize these without much detail. However, there are both similarities and significant differences between B and T cell antigen receptors (see Table 7-1).]

TABLE 7–1 Properties of Lymphocyte Antigen Receptors: T Cell Receptor and Immunoglobulins

	T Cell Receptor (TCR)	Immunoglobulin (Ig)
Components	α and β chains	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain: one V domain, three or four C domains Light chain: one V domain and one C domain
Number of CDRs	Three in each chain for antigen binding	Three in each chain
Associated signaling molecules	CD3 and ζ	Igα and Igβ
Affinity for antigen (K_d)	10^{-5}-10^{-7} M	10^{-7}-10^{-11} M (secreted Ig)
Changes after cellular activation		
Production of secreted form	No	Yes
Isotope switching	No	Yes
Somatic mutations	No	Yes

Structure of the B cell Receptor for Antigen

- Membrane IgM and IgD, the antigen receptors of naïve B cells, have short cytoplasmic tails consisting of only three amino acids (lysine, valine, and lysine).
- These tails are too small to transduce signals generated after the recognition of antigen.

- Ig-mediated signals are transduced by two other molecules, called Igα and Igβ that are disulfide linked to one another and are expressed in B cells noncovalently associated with membrane Ig (Fig. 7-18).
- These proteins each contain an ITAM motif in their cytoplasmic tails, are required for the transport of membrane Ig molecules to the cell surface, and together with membrane Ig form the **B cell receptor (BCR) complex**.

B cell receptor complexes in class-switched B cells, including memory B cells, contain membrane immunoglobulins that may be of the IgG, IgA, or IgE classes.

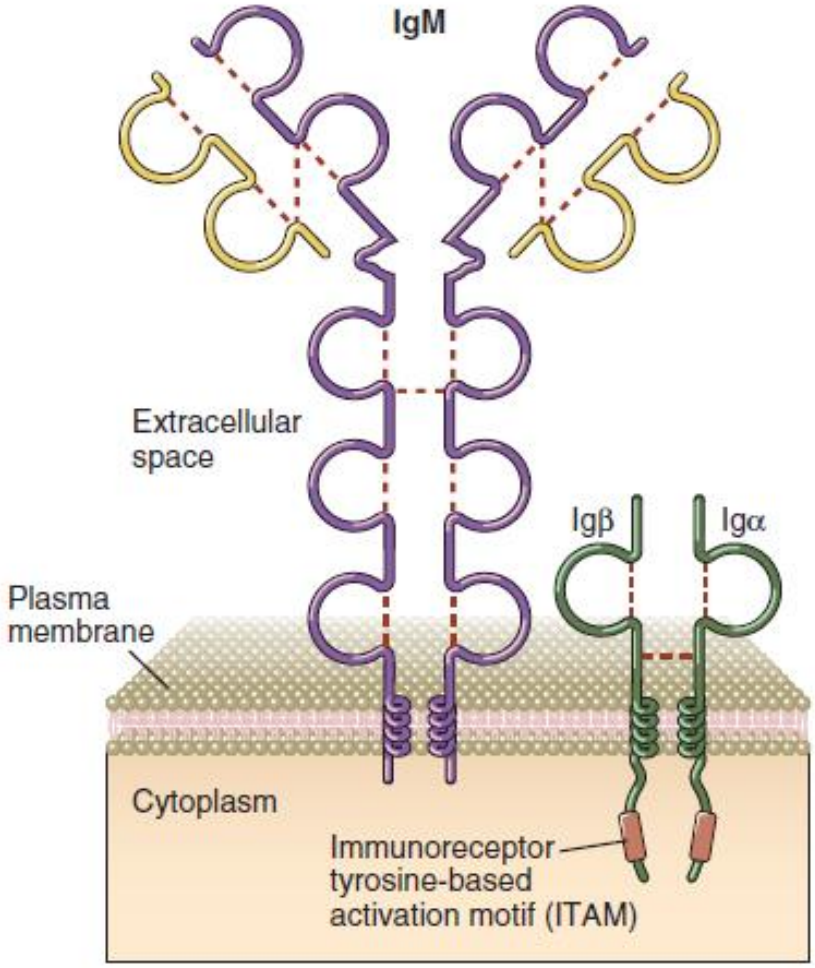


FIGURE 7–18 B cell antigen receptor complex. Membrane IgM (and IgD) on the surface of mature B cells is associated with the invariant Igβ and Igα molecules, which contain ITAMs in their cytoplasmic tails that mediate signaling functions. Note the similarity to the TCR complex.

Signal Initiation by the B cell Receptor

Signal initiation by antigens occurs by cross-linking of the BCR and is facilitated by the coreceptor for the BCR.

- It is thought that cross-linking of membrane Ig by multivalent antigens brings Src family kinases together and, by promoting their physical interaction, fully activates these enzymes, enabling them then to phosphorylate the tyrosine residues on the ITAMs of Igα and Igβ.
- It is also possible that as in T cells, antigen binding facilitates a conformational change in BCR-associated ITAMs, making them accessible to already active Src family kinases that modify ITAM tyrosines, but there is at present no firm evidence to support such a model.
- The phosphorylation of ITAM tyrosine residues triggers all subsequent signaling events downstream of the BCR (Fig. 7-19).
- Cross-linked Ig receptors enter lipid rafts, where many adaptor proteins and signaling molecules are concentrated.
- Igα and Igβ are loosely connected to Src family tyrosine kinases such as Lyn, Fyn, and Blk, and these enzymes are also linked by lipid anchors to the inside of the plasma membrane.
- The phosphorylation of the tyrosine residues in the ITAMs of Igα and Igβ provides a docking site for the tandem SH2 domains of the Syk tyrosine kinase.
- Syk is activated when it associates with phosphorylated tyrosines of ITAMs and may itself be phosphorylated on specific tyrosine residues by BCR-associated Src family kinases, leading to further activation.

- If the antigen is monovalent and incapable of cross-linking multiple Ig molecules, some signaling may nevertheless occur, but additional activation by helper T cells may be necessary to fully activate B cells.

Role of the CR2/CD21 Complement Receptor as a Coreceptor for B Cells

The activation of B cells is enhanced by signals that are provided by complement proteins and the CD21 coreceptor complex, which link innate immunity to the adaptive humoral immune response (Fig. 7-20).

- The complement system consists of a collection of plasma proteins that are activated either by binding to antigen-complexed antibody molecules (the classical pathway) or by binding directly to some microbial surfaces and polysaccharides in the absence of antibodies (the alternative and lectin pathways).
- Thus, polysaccharides and other microbial components may activate the complement system directly, during innate immune responses.
- Proteins and other antigens that do not activate complement directly may be bound by preexisting antibodies or by antibodies produced early in the response, and these antigen-antibody complexes activate complement by the classical pathway.
- Recall that complement activation results in the proteolytic cleavage of complement proteins.
- The key component of the system is a protein called C3, and its cleavage results in the production of a molecule called C3b that binds covalently to the microbe or antigen-antibody complex.
- C3b is further degraded into a fragment called C3d, which remains bound to the microbial surface or on the antigen-antibody complex.
- B lymphocytes express a receptor for C3d that is called the type 2 complement receptor (CR2, or CD21).

- The complex of C3d and antigen or C3d and antigen antibody complex binds to B cells, with the membrane Ig recognizing antigen and CR2 recognizing the bound C3d (see Fig. 7-20).
- CR2 is expressed on mature B cells as a complex with two other membrane proteins, CD19 and CD81 (also called TAPA-1).
- The CR2-CD19-CD81 complex is often called the B cell coreceptor complex because CR2 binds to antigens through attached C3d at the same time that membrane Ig binds directly to the antigen.
- Binding of C3d to the B cell complement receptor brings CD19 in proximity to BCR-associated kinases, and the cytoplasmic tail of CD19 rapidly becomes tyrosine phosphorylated.
- Phosphorylation of the tail of CD19 results in the efficient recruitment of Lyn, a Src family kinase that can amplify BCR signaling by greatly enhancing the phosphorylation of ITAM tyrosines in Igα and Igβ.
- Phosphorylated CD19 also activates other signaling pathways, notably one dependent on the enzyme PI3-kinase, which in turn further augment signaling initiated by antigen binding to membrane Ig.
- PI3-kinase is required for the activation of Btk and PLCγ2 because these enzymes must bind to PIP3 on the inner leaflet of the plasma membrane to be fully activated, in a manner analogous to that shown in Figure 7-12.
- The net result of coreceptor activation is that the response of the antigen-stimulated B cell is greatly enhanced.

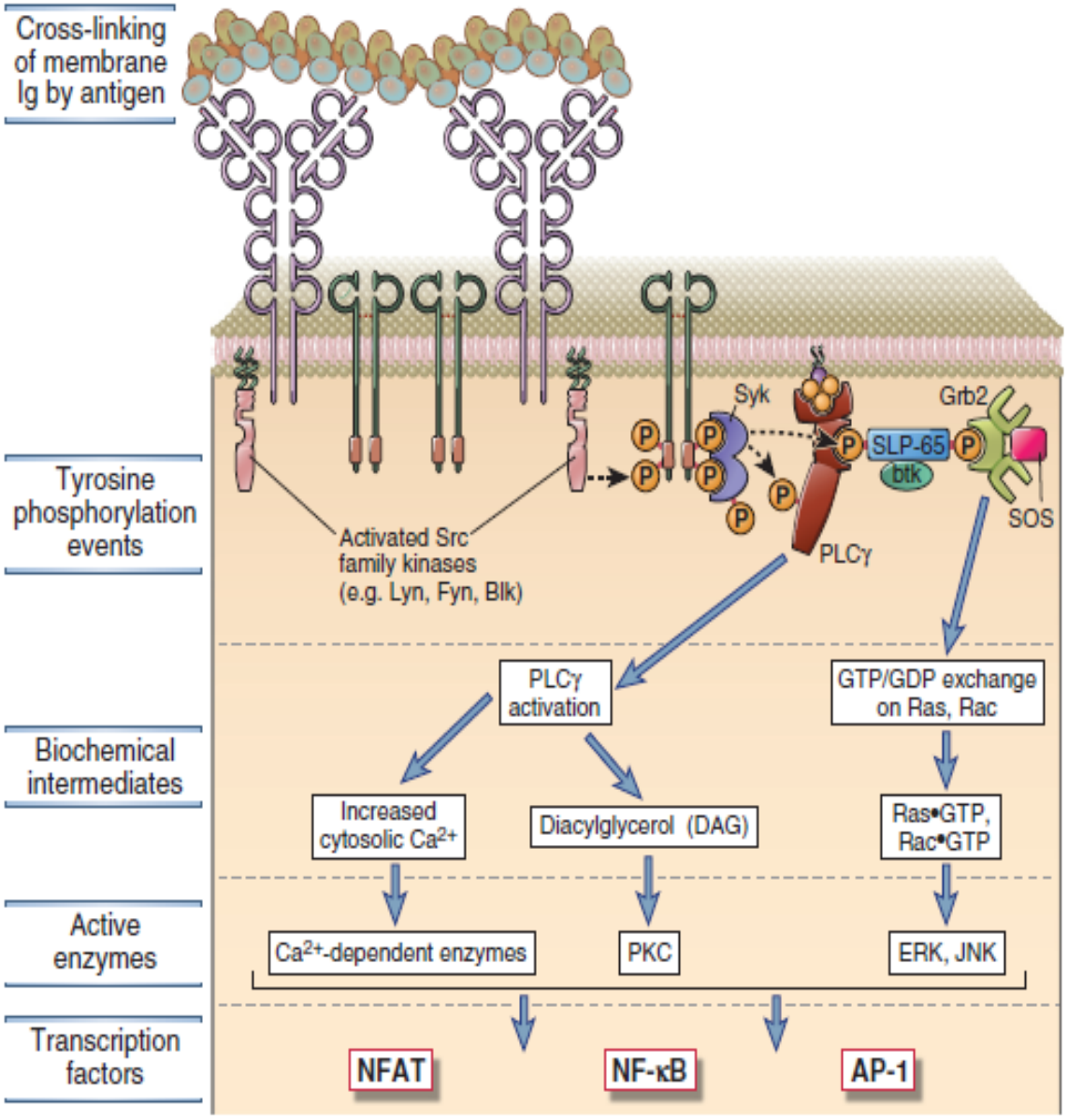


FIGURE 7–19 Signal transduction by the BCR complex. Antigen-induced cross-linking of membrane Ig on B cells leads to clustering and activation of Src family tyrosine kinases and tyrosine phosphorylation of the ITAMs in the cytoplasmic tails of the Igα and Igβ molecules. This leads to docking of Syk and subsequent tyrosine phosphorylation events as depicted. Several signaling cascades follow these events, as shown, leading to the activation of several transcription factors. These signal transduction pathways are similar to those described in T cells.

Signaling Pathways Downstream of the B cell Receptor

After antigen binding to the BCR, Syk and other tyrosine kinases activate numerous downstream signaling pathways that are regulated by adaptor proteins (see Fig. 7-19).

- The cross-linking of the BCR or the activation of the BCR by a coreceptor-dependent mechanism results in ITAM phosphorylation and recruitment of Syk to the ITAM, followed by the activation of this dual SH2 domain–containing kinase.
- Activated Syk phosphorylates critical tyrosine residues on adaptor proteins such as SLP-65 (SH2-binding leukocyte phosphoprotein of 65 kD, also called BLNK, B cell linker protein).
- This facilitates the recruitment to these adaptor proteins of other SH2 domain – and phosphotyrosine-binding (PTB) domain–containing enzymes, including guanine nucleotide exchange proteins that can separately activate Ras and Rac, PLCγ2, and the Btk tyrosine kinase, among others.
- Recruitment facilitates the activation of these downstream effectors, each generally contributing to the activation of a distinct signaling pathway.

The Ras–MAP kinase pathway is activated in antigen stimulated B cells.

- The GTP/GDP exchange factor SOS is recruited to BLNK through the binding of the Grb-2 adaptor protein; Ras is then converted by this exchange factor from an inactive GDP-bound form to an active GTP-bound form.
- Activated Ras contributes to the activation of the ERK MAP kinase pathway discussed earlier in the context of T cell signaling.
- In a parallel fashion, the activation of the Rac small GTP protein may contribute to the activation of the JNK MAP kinase pathway.

- **A specific phosphatidylinositol-specific phospholipase C (PLC) is activated in response to BCR signaling, and this in turn facilitates the activation of downstream signaling pathways.**
  - In B cells, the dominant isoform of PLC is the γ2 isoform, whereas T cells express the related γ1 isoform of the enzyme.
  - PLCγ2 becomes active when it binds to BLNK and is phosphorylated by Syk and Btk.
  - As described in the context of TCR signaling, active PLC breaks down membrane PIP2 to yield soluble IP3 and leaves DAG in the plasma membrane.
  - IP3 mobilizes calcium from intracellular stores, leading to a rapid elevation of cytoplasmic calcium, which is subsequently augmented by an influx of calcium from the extracellular milieu.
  - In the presence of calcium, DAG activates some isoforms of protein kinase C (mainly PKC-β in B cells), which phosphorylate downstream proteins on serine/threonine residues.
- **PKC-β activation downstream of the BCR contributes to the activation of NF-κB in antigen-stimulated B cells.**
  - This process is similar to that in T cells triggered by PKC-θ, the PKC isoform present in T cells, and the pathway of NF-κB activation downstream of PKCs is described later in this chapter.

These signaling cascades ultimately lead to the activation of a number of transcription factors that induce the expression of genes whose products are required for functional responses of B cells.

- Some of the transcription factors that are activated by antigen receptor–mediated signal transduction in B cells are Fos (downstream of Ras and ERK

activation), JunB (downstream of Rac and JNK activation), and NF-κB (downstream of Btk, PLCγ2, andPKC-β activation).

[These were discussed earlier when we described T cell signaling pathways. These and other transcription factors, many not mentioned here, are involved in stimulating proliferation and differentiation of B cells.]

As in T cells, our knowledge of antigen-induced signaling pathways in B cells and their links with subsequent functional responses is incomplete. We have described some of these pathways to illustrate the main features, but others may play important roles in B cell activation. The same signaling pathways are used by membrane IgM and IgD on naive B cells and by IgG, IgA, and IgE on B cells that have undergone isotype switching because all these membrane isotypes associate with Igα and Igβ.

THE ATTENUATION OF IMMUNE RECEPTOR SIGNALING

- Activation of lymphocytes has to be tightly controlled to limit immune responses against microbes for avoidance of "collateral damage" to host tissues.
- In addition, the immune system needs mechanisms that will prevent reactions against self-antigens.
- Attenuation of signaling is essential to prevent uncontrolled inflammation and lymphoproliferation.

Here we discuss the biochemical mechanisms that serve to limit and terminate lymphocyte activation.

Inhibitory signaling in lymphocytes is mediated primarily by inhibitory receptors and also by enzymes known as E3 ubiquitin ligases that mark certain signaling molecules for degradation.

- Inhibitory receptors typically recruit and activate phosphatases that counter signaling events induced by antigen receptors (Fig. 7-21).
- The functional responses of all cells are regulated by a balance between stimulatory and inhibitory signals, and we will first describe, from a broad mechanistic standpoint, how inhibitory receptors may function in NK cells, T cells, and B cells.
- We will then describe how ubiquitin E3 ligases may attenuate signaling in lymphocytes. The biologic relevance of signal attenuation through inhibitory receptors in NK cells, T cells, and B cells is addressed in Chapters 4, 9, and 11, respectively.

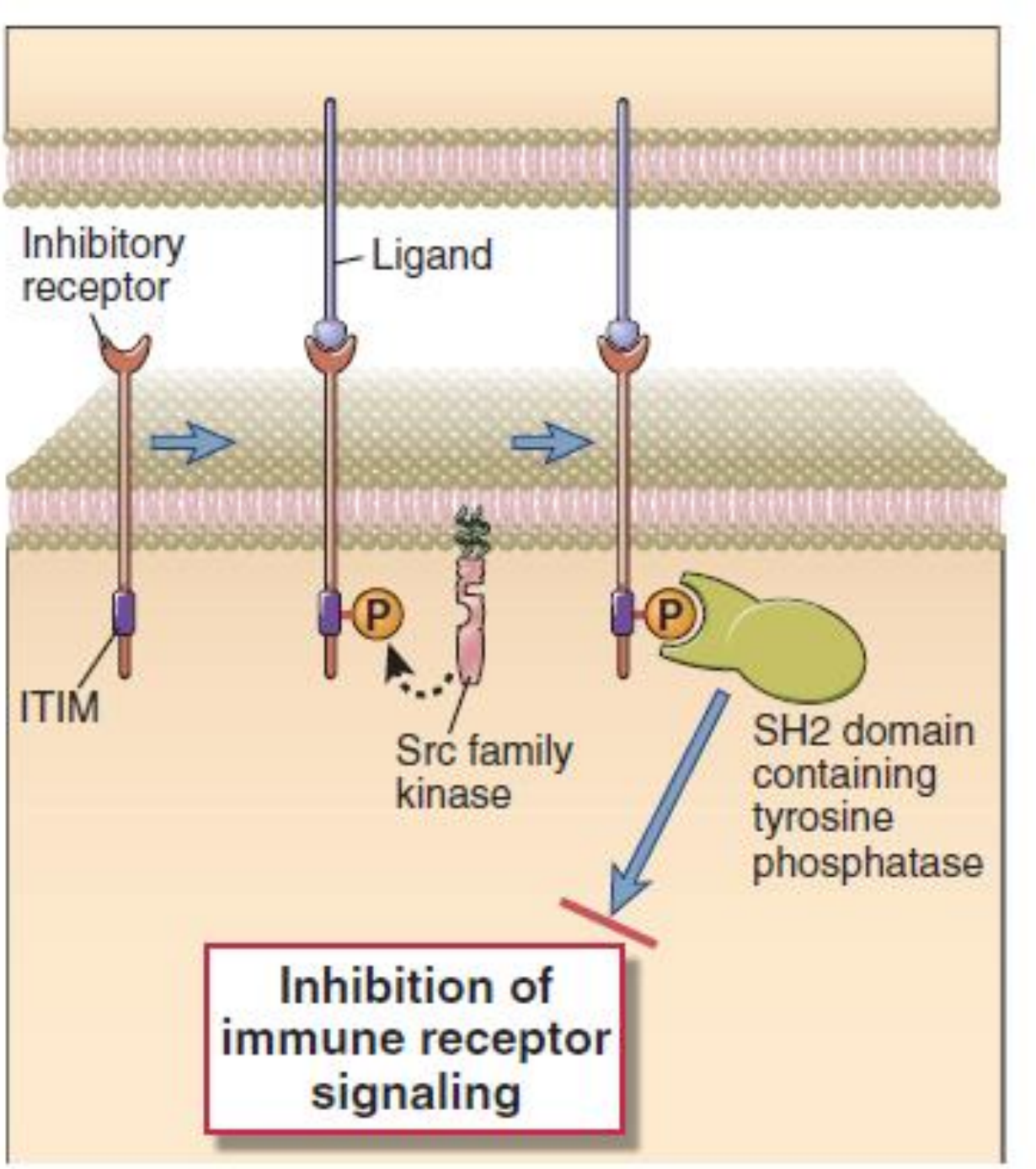


FIGURE 7–21 Inhibitory signaling in lymphocytes. A schematic depiction is provided of an inhibitory receptor with an extracellular ligand-binding domain and a cytosolic ITIM motif. Ligand binding results in phosphorylation of the ITIM tyrosine by a Src family kinase, followed by recruitment of an SH2 domain–containing tyrosine phosphatase that can attenuate immune receptor signaling.

Inhibitory Receptors in NK Cells, B Cells, and T Cells

- Most but not all inhibitory receptors in the immune system contain cytosolically oriented ITIM motifs that can recruit SH2 domain–containing phosphatases and thus attenuate signaling in a broadly similar manner (see Fig 7-21).
- Inhibitory receptors play key roles in NK cells, T cells, and B cells as well as in other cells of innate immunity.
- In human NK cells, the key inhibitory receptors can broadly be divided into three groups: KIRs or killer Ig-like receptors (see Chapter 4); ILT (Ig-like transcript) family proteins that are closely related to KIRs; and C-type lectins, the major one being a heterodimer consisting of the NKG2A C-type lectin and CD94.
- These inhibitory receptors are not restricted to NK cells and may also be present on some activated T cells.
- KIRs contain extracellular Ig domains that can recognize class I HLA molecules, and a subset of these receptors contains cytosolic ITIM motifs.
- ILT-2, part of an evolutionarily older family of inhibitory receptors than KIRs, also has extracellular Ig domains that bind HLA class I and cytosolic ITIM motifs.
- The CD94/NKG2A dimer binds to an atypical class I MHC molecule called HLA-E, and the NKG2A chain of this dimer contains cytosolic ITIM motifs.
- Tyrosine residues on the ITIMs of these and other inhibitory receptors can be phosphorylated by Src family kinases linked to lymphocyte activation and, as described earlier, recruit SH2 domain–containing tyrosine phosphatases such as SHP-1 and SHP-2 and an SH2 domain– containing inositol phosphatase called SHIP.

- SHP-1 and SHP-2 attenuate tyrosine kinase–initiated signaling from activating receptors in NK cells as well as from the BCR and TCR in B and T cells, respectively.
- SHIP removes phosphate moieties from PIP3 and thus inhibits PI3- kinase activity in lymphocytes, NK cells, and innate immune cells.
- The prototypical inhibitory receptor of the CD28 family, **CTLA-4** (also called CD152), has the ability to inhibit T cell responses induced on activated T cells and has a higher affinity than CD28 for B7 proteins.
- CTLA-4 is involved in the maintenance of unresponsiveness (tolerance) to self-antigens and is discussed in this context in Chapter 14.
- Another inhibitory receptor of the same family is called **PD-1** (programmed death 1), and this is also discussed in Chapter 14.
- CTLA-4 contains a tyrosine-containing motif in its tail that may be inhibitory; PD-1 contains cytosolic ITIM and ITSM motifs, and its cytosolic tail is critical for the initiation of inhibitory signals.
- The key inhibitory receptors in B cells include FcγRIIB and CD22/Siglec-2.
- FcγRIIB, an important attenuator of signaling in activated B cells as well as in dendritic cells and macrophages, can bind IgG-containing immune complexes through extracellular Ig domains.
- It primarily recruits SHIP and antagonizes PI3-kinase signaling.
- This receptor dampens B cell activation in the latter part of a humoral immune response and will be discussed in more detail in Chapter 11.

E3 Ubiquitin Ligases and the Degradation of Signaling Proteins

- One of the major ways of degrading cytosolic and nuclear proteins involves the covalent attachment of ubiquitin residues to these proteins.
- Although ubiquitination of proteins is frequently linked to the degradation of these proteins in proteasomes, proteins can be ubiquitinated in a number of ways, each form of ubiquitination serving a very different function.
- In the context of signal transduction, different types of ubiquitination mediate signal attenuation on the one hand and signal generation on the other.
- Ubiquitination was briefly discussed in Chapter 6 in the context of class I MHC–based antigen processing and presentation.
- Ubiquitin is a 76–amino acid protein that is activated in an ATP-dependent fashion by an E1 enzyme, then "carried" by an E2 enzyme, and transferred to lysine residues on specific substrates that are recognized by specific E3 ubiquitin ligases.
- In many cases, after the C terminus of a ubiquitin moiety is covalently linked to a lysine residue on a target protein, the C-terminal ends of subsequent ubiquitin moieties may be covalently attached to lysine residues on the preceding ubiquitin to generate a polyubiquitin chain.
- The geometric shape of the polyubiquitin chain is very different, depending on which specific lysine residue on the preceding ubiquitin molecule in the chain is the site for covalent binding of the next ubiquitin molecule, and the shape of the ubiquitin chain has important functional consequences.
- If lysine in position 48 of the first ubiquitin moiety forms an isopeptide bond with the C terminus of the next ubiquitin and so on, a lysine-48 type of ubiquitin chain will be generated that can be recognized by the proteasomal cap, and the protein will be targeted for degradation in the proteasome.

- Some E3 ligases generate a different type of polyubiquitin chain called a lysine-63 type of chain, which does not target proteins for degradation but instead generates a structure for latching the marked proteins onto other specific proteins; this is important in NF-κB signaling, as discussed later.
- For some functions, in particular targeting membrane proteins to lysosomes rather than to proteasomes, only a single ubiquitin moiety may need to be attached to a protein target.
- Several E3 ligases are found in T cells; some of them are involved in signal activation and others in signal attenuation.
- The prototype of E3 ligases involved in terminating T cell responses is Cbl-b, but several others serve similar functions.
- Recruitment of Cbl-b to the TCR complex and associated adaptor proteins leads to the monoubiquitination, endocytosis, and lysosomal degradation of the TCR complex, and this may be a mechanism for the attenuation of TCR signaling (Fig. 7-22).
- CD28 signals block the inhibitory activity of Cbl-b, and this is one mechanism by which costimulation augments TCR signals.
- In knockout mice lacking Cbl-b, the T cells respond to antigen even without CD28-mediated costimulation and produce abnormally high amounts of IL-2.
- These mice develop autoimmunity as a result of the enhanced activation of their T cells.

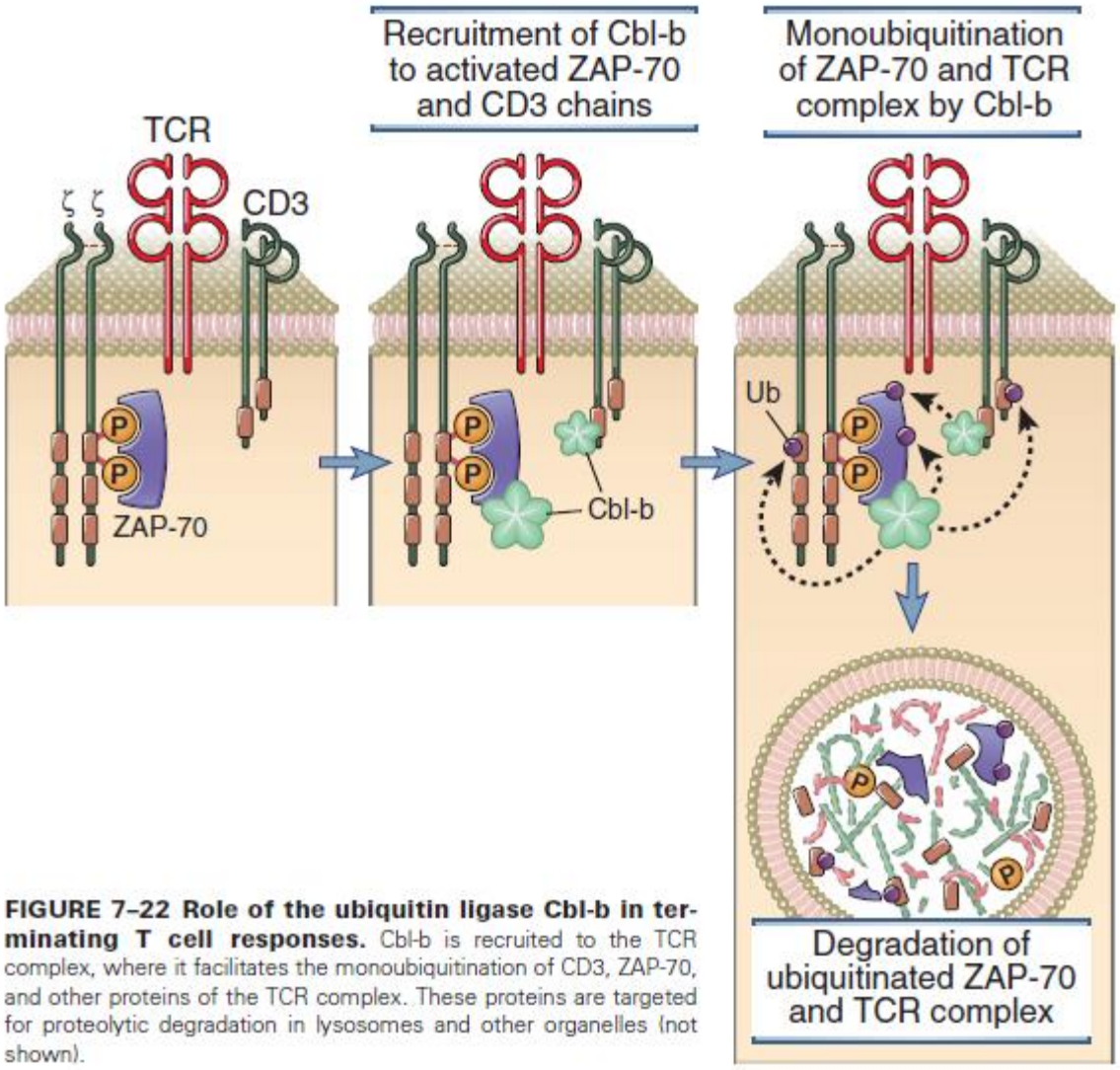


FIGURE 7–22 Role of the ubiquitin ligase Cbl-b in terminating T cell responses. Cbl-b is recruited to the TCR complex, where it facilitates the monoubiquitination of CD3, ZAP-70, and other proteins of the TCR complex. These proteins are targeted for proteolytic degradation in lysosomes and other organelles (not shown).

CYTOKINE RECEPTORS AND SIGNALING

- Cytokines, the secreted "**messenger molecules**" of the immune system.

Here we will describe receptors for cytokines and their mechanisms of signaling.

- All cytokine receptors consist of one or more transmembrane proteins whose extracellular portions are responsible for cytokine binding and whose cytoplasmic portions are responsible for initiation of intracellular signaling pathways.
- For most cytokine receptors, these signaling pathways are typically activated by ligand induced receptor clustering, bringing together the cytoplasmic portions of two or more receptor molecules, and thus inducing the activity of unique non-receptor tyrosine kinases.
- In the case of the TNF receptor family of cytokine receptors, preformed receptor trimers apparently undergo a conformational change after contacting their cognate trimeric ligands.

Classes of Cytokine Receptors

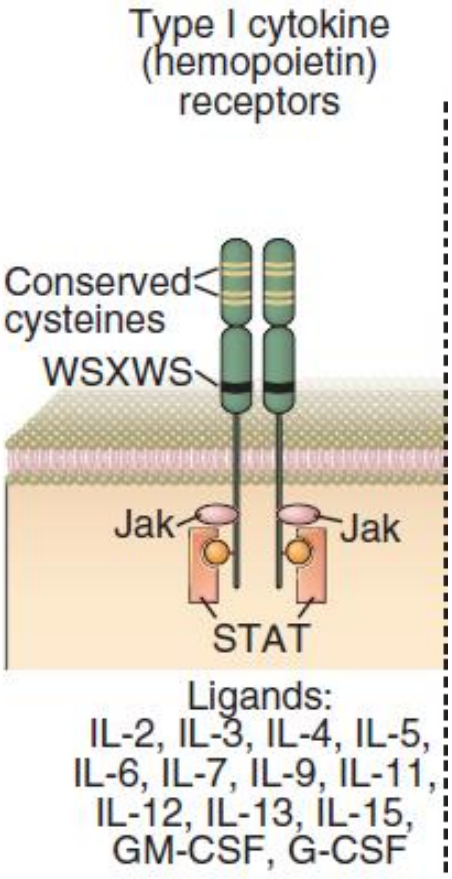
- The most widely used classification of cytokine receptors is based on structural homologies of the extracellular Cytokine-binding domains and shared intracellular signaling mechanisms (Fig. 7-23).
- Signaling through type I and type II cytokine receptors occurs by a similar mechanism, known as JAK-STAT signaling, that is described in more detail later. Cytokine receptors of the TNF receptor family activate a number of pathways, a prominent one being the NF-κB pathway, which will also be considered in detail later.
- Signaling through the IL-1R and the TLR families uses a common cytoplasmic domain, and a major component downstream is ubiquitin E3 ligase–dependent activation of the NF-κB pathway. Chemokines, which are

chemotactic cytokines, activate a large subfamily of receptors and have been discussed in Chapter 3 (Abbas-Immunology).

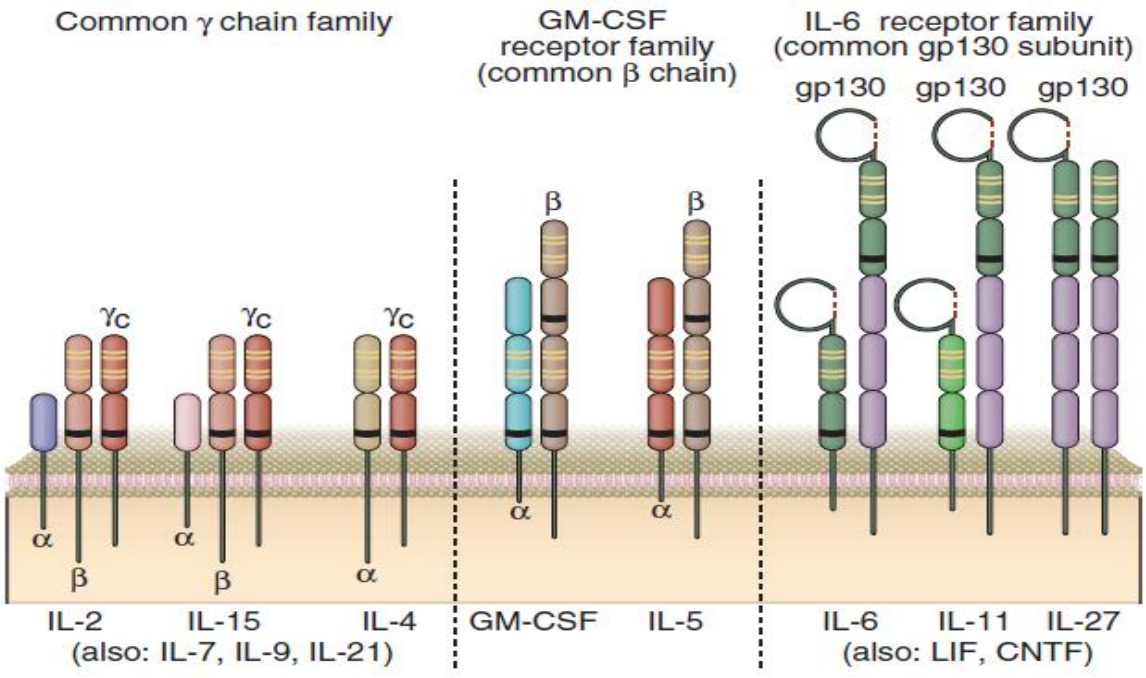
- Chemokine receptors are seven-transmembrane GPCRs described in the early part of this chapter and are not elaborated on here.

Type I Cytokine Receptors (Hematopoietin Receptor Family)

- Type I cytokine receptors are dimers or trimers that typically consist of unique ligand-binding chains and one or more signal-transducing chains, which are often shared by receptors for different cytokines.
- These chains contain one or two domains with a conserved pair of cysteine residues and a membrane proximal peptide stretch containing a tryptophan-serine-X-tryptophan-serine (WSXWS) motif, where X is any amino acid (Fig. 7-23A).

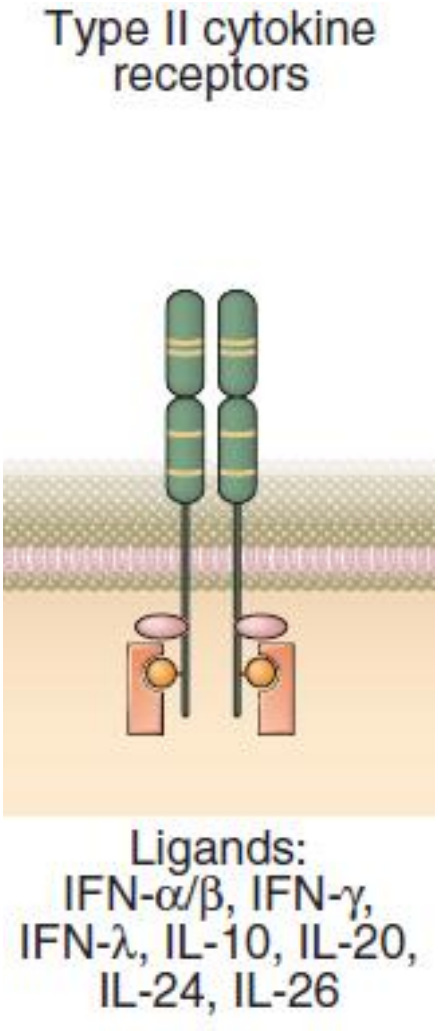


- The conserved sequences of the receptors form structures that bind cytokines that have four α-helical bundles and are referred to as type I cytokines, but the specificity for individual cytokines is determined by amino acid residues that vary from one receptor to another.
- This receptor family can be divided into subgroups based on structural homologies or the use of shared signaling polypeptides (Fig. 7-23B).
    - One group contains a signaling component called the common γ chain (CD132); in this group are the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21.
    - A distinct subgroup of type I receptors includes receptors that share a common β chain (CD131) subunit.
    - This subgroup includes the receptors for IL-3, IL-5, and GM-CSF.
    - Another subgroup of receptors uses the gp130 signaling component, and this includes the receptors for IL-6, IL-11, and IL-27.
    - All the type I cytokine receptors engage JAK-STAT signaling pathways.



Type II Cytokine Receptors (Interferon Receptor Family)

- The type II receptors are similar to type I receptors by virtue of possessing two extracellular domains with conserved cysteines, but type II receptors do not contain the WSXWS motif.
- These receptors consist of one ligand binding polypeptide chain and one signal-transducing chain.
- All the type II cytokine receptors, like the type I receptors, engage JAK-STAT signaling pathways.
- This family includes receptors for type I and type II interferons and for IL-10, IL-20, and IL-26.



TNF Receptor Family

- These receptors are part of a large family of preformed trimers (some of which are not considered cytokine receptors) with conserved cysteine-rich extracellular domains and shared intracellular signaling mechanisms that typically stimulate gene expression but in some cases induce apoptosis.
- Some important receptors of this family, most of which will be discussed in other chapters in their biologic contexts, include the TNF receptors TNFRI and TNFRII, the CD40 protein, Fas, the lymphotoxin receptor, and the BAFF receptor family.
- The ligands for these receptors also form trimers. Some of these ligands are membrane bound, whereas others are soluble.
- Binding of the ligands to the preformed trimeric receptors typically induces a conformational change and recruits adaptor proteins to the receptor complex.
- These adaptors in turn recruit enzymes that include both E3 ubiquitin ligases, which mediate nondegradatory polyubiquitination, and protein kinases, which initiate downstream signaling.
- In the case of the TNF receptor illustrated in Figure 7-24, the receptor recruits the adaptor protein TRADD (TNF receptor–associated death domain), and TRADD in turn can recruit proteins called TRAFs (TNF receptor associated factors), which possess a unique type of E3 ligase activity that will be discussed in the section on NF-κB signaling.
- The type I TNF receptor (there are two different receptors for TNF) and Fas (CD95) can also recruit adaptors that induce the activation of caspase-8, and these receptors, in certain cells, can thereby induce apoptosis.

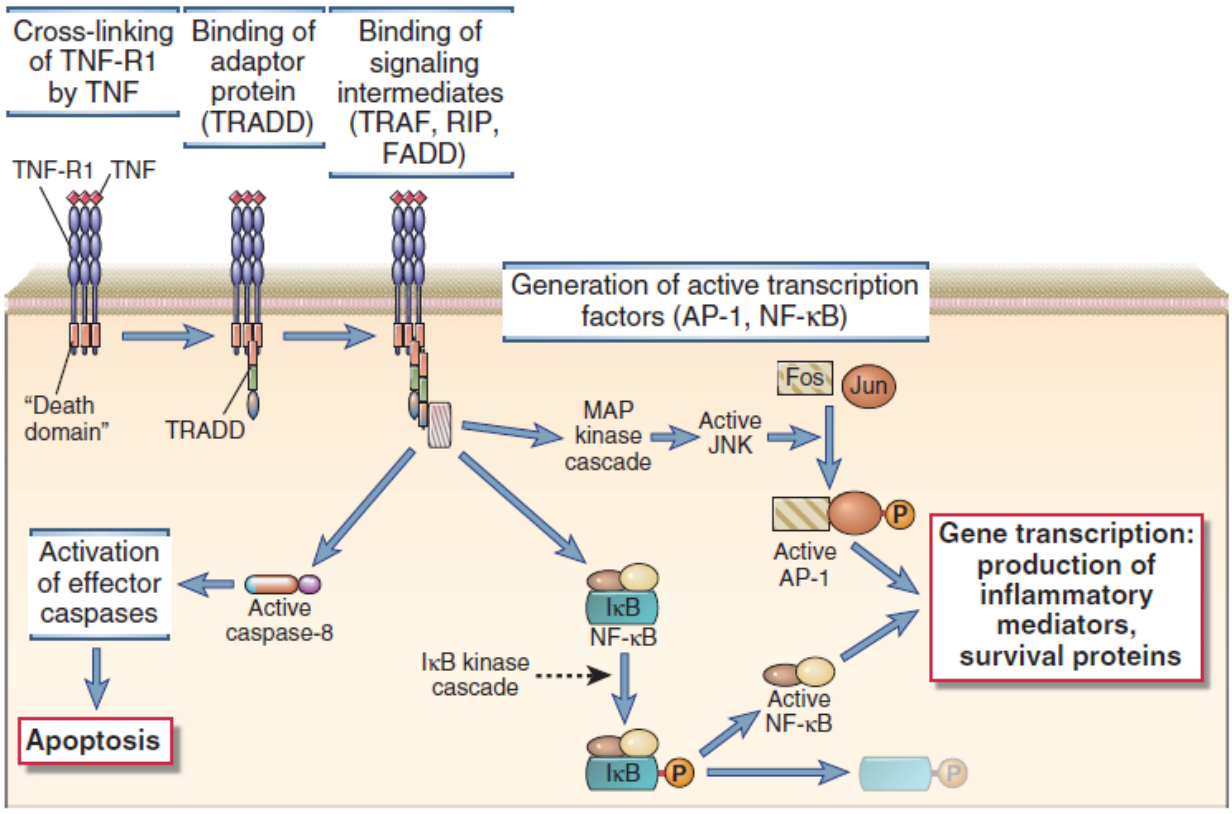
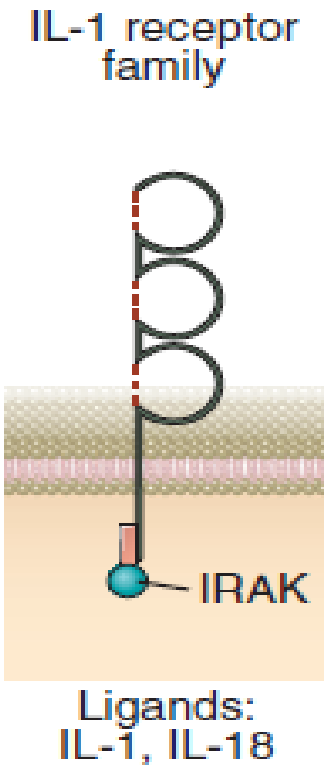


FIGURE 7–24 Signaling through the TNF receptor can result in NF-κB and MAP kinase activation or in the induction of apoptotic death. Ligation of the type I TNF receptor results in the recruitment of an adaptor protein called TRADD, which in turn can activate TRAF molecules (E3 ubiquitin ligase) and the RIP1 kinase. Downstream consequences include the activation of the NF-κB pathway and the JNK MAP kinase pathway or the induction of apoptotic death.

IL-1/TLR Family

- The receptors of this family share a conserved cytosolic sequence, called the Toll-like/IL-1 receptor (TIR) domain, and engage similar signal transduction pathways that induce new gene transcription.
- Briefly, engagement of the IL-1R or of TLRs results in receptor dimerization and the recruitment of one or more of four known TIR domain–containing adaptors to the TIR domain of the cytoplasmic tail of the receptor.
- The adaptors link TLRs to different members of the IRAK (IL-1R– ***associated*** kinase) family.

- IRAKs can in turn link adaptors to TRAF6, an E3 ubiquitin ligase required for NF-κB activation. Other pathways activated by TLR signaling include MAP kinase activation and the phosphorylation of IRF3 and IRF7, transcriptional inducers of type I interferons.



- In a general sense, the MAL/MyD88 adaptor pair links TLRs to the early induction of NF-κB signaling and to MAP kinase activation, whereas the TRAM/TRIF adaptor pair leads to the delayed activation of NF-κB and the activation of IRF3.
- TLR4, for instance, activates MAL/Myd88 signaling initially from the cell surface and TRAM/TRIF signaling subsequently after receptor endocytosis. The mechanisms connecting IL-1R/TLR signaling and NF-κB activation are discussed below.

JAK-STAT Signaling

Cytokine receptors of the type I and type II receptor families engage signal transduction pathways that involve non-receptor tyrosine kinases called Janus kinases or JAKs and transcription factors called signal transducers and activators of transcription (STATs).

- The discovery of the JAK-STAT pathways came from biochemical and genetic analyses of interferon signaling.
- There are four known Janus kinases (JAK1-3 and TYK2) and seven STATs (STAT1-4, 5a, 5b, and 6).
- The sequence of events in the JAK-STAT signaling pathways is now well defined (Fig. 7-25).

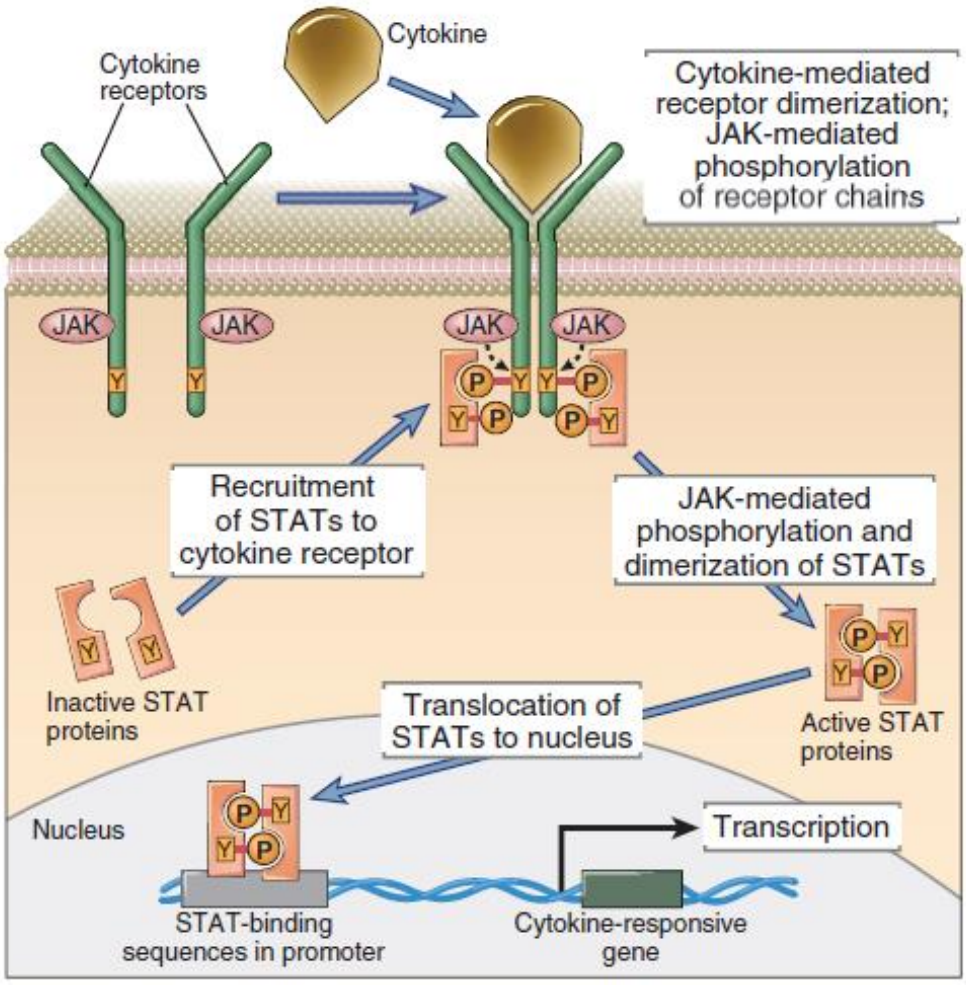


FIGURE 7–25 Type I and type II cytokines induce JAK-STAT signaling. Ligation of receptors for type I and type II cytokines results in the activation of an associated JAK tyrosine kinase, the phosphorylation of the receptor tail, and the recruitment of an SH2 domain–containing activator of transcription (STAT) to the receptor. The recruited STAT is activated by JAK phosphorylation, dimerizes, enters the nucleus, and turns on the expression of cytokine target genes.

- Inactive JAK enzymes are noncovalently attached to the cytoplasmic domains of type I and type II cytokine receptors.
- When two receptor molecules are brought together by binding of a cytokine molecule, the receptor-associated JAKs are activated and phosphorylate tyrosine residues in the cytoplasmic portions of the clustered receptors.
- Some of these phosphotyrosine moieties of the receptors are then recognized and bind to Src homology 2 (SH2) domains of monomeric cytosolic STAT proteins.
- The STAT proteins are thus brought close to JAKs and are phosphorylated by the receptor-associated kinases.
- The SH2 domain of one STAT monomer is able to bind to a phosphotyrosine residue on an adjacent STAT protein.
- The STAT dimers that are generated migrate to the nucleus, where they bind to specific DNA sequences in the promoter regions of cytokine-responsive genes and activate gene transcription.
- An intriguing question is how the specificity of responses to many different cytokines is achieved, given the limited numbers of JAKs and STATs used by the various cytokine receptors.
- The likely answer is that unique amino acid sequences in the different cytokine receptors provide the scaffolding for specifically binding, and thereby activating, different combinations of JAKs and STATs.

- The SH2 domains of different STAT proteins selectively bind to phosphotyrosines and flanking residues of different cytokine receptors.
- This is largely responsible for the activation of particular STATs by various cytokine receptors and therefore for the specificity of cytokine signaling.
- Several type I and type II cytokine receptors are heterodimers of two different polypeptide chains, each of which binds a different JAK.
- Furthermore, two different STATs may heterodimerize on phosphorylation.
- Therefore, there is a significant amount of combinatorial diversity in the signaling that can be generated from a limited number of JAK and STAT proteins.
- In addition, cytokines activate signaling pathways and transcription factors other than STATs.
- For instance, the IL-2 receptor β chain activates Ras-dependent MAP kinase pathways that may be involved in gene transcription and growth stimulation.
- Other cytokine receptors may similarly activate other signaling pathways in concert with the JAK-STAT pathways to elicit biologic responses to the cytokines.
- Several mechanisms of negative regulation of JAKSTAT pathways have been identified.
- Proteins called suppressors of cytokine signaling (SOCS) can be identified by the presence of an SH2 domain and a conserved 40– amino acid C-terminal region called a SOCS box.
- SOCS proteins serve as adaptors for multisubunit E3 ligase activity.
- They can bind to activated STATs and JAKs, and the tightly associated E3 ligases ubiquitinate the JAKs and STATs, thus targeting them for proteasomal degradation.

- SOCS protein levels can be regulated by TLR ligands, by cytokines themselves, and by other stimuli. In this way, SOCS serve as negative feedback regulators of the cytokine-mediated activation of cells.
- Other inhibitors of JAK-STAT signaling include tyrosine phosphatases, such as SHP-1 and SHP-2, which can dephosphorylate and therefore deactivate JAK molecules.
- Another family of inhibitory proteins, called protein inhibitors of activated STAT (PIAS), were originally defined as negative regulators of STATs.
- PIAS proteins bind phosphorylated STATs and prevent their interaction with DNA.
- It is now known that PIAS proteins also interact with and block the function of other transcription factors associated with cytokine signaling, including NF-κB and SMADs (transcription factors downstream of members of the TGF-β receptor family).

Pathways of NF-κB Activation

NF-κB is a transcription factor that plays a central role in inflammation, lymphocyte activation, cell survival, and the formation of secondary lymphoid organs.

- It is also an important player in lymphocyte development and in the pathogenesis of many cancers, including malignant neoplasms derived from activated lymphocytes.
- NF-κB is activated by many cytokine and TLR stimuli and by antigen recognition and is discussed here as the prototype of a transcription factor with fundamental roles in innate and adaptive immunity.
- There are five NF-κB proteins.
- The domain that is common to all NF-κB proteins is a DNA-binding domain called a **Rel homology domain**.
- For a transcription factor to be active, it must both bind DNA and contain an activation domain that can facilitate transcriptional initiation.
- Three NF-κB proteins have both Rel homology domains and activation domains. These are p65/RelA, RelB, and c-Rel.
- **NF-κB1/p50 and NF-κB2/p52** proteins contain a DNA-binding Rel homology domain but lack activation domains.
- NF-κB1 typically forms active heterodimers with p65/RelA or with c-Rel, and these heterodimers are typically considered **"canonical"** (according to recognized rules or scientific laws) NF-κB heterodimers (Fig. 7-26).
- Canonical NF-κB heterodimers reside in the cytosol bound to an inhibitor of NF-κB called IκBα.
- Canonical NF-κB heterodimers are activated by a number of signaling receptors that drive inflammation or lymphocyte activation.

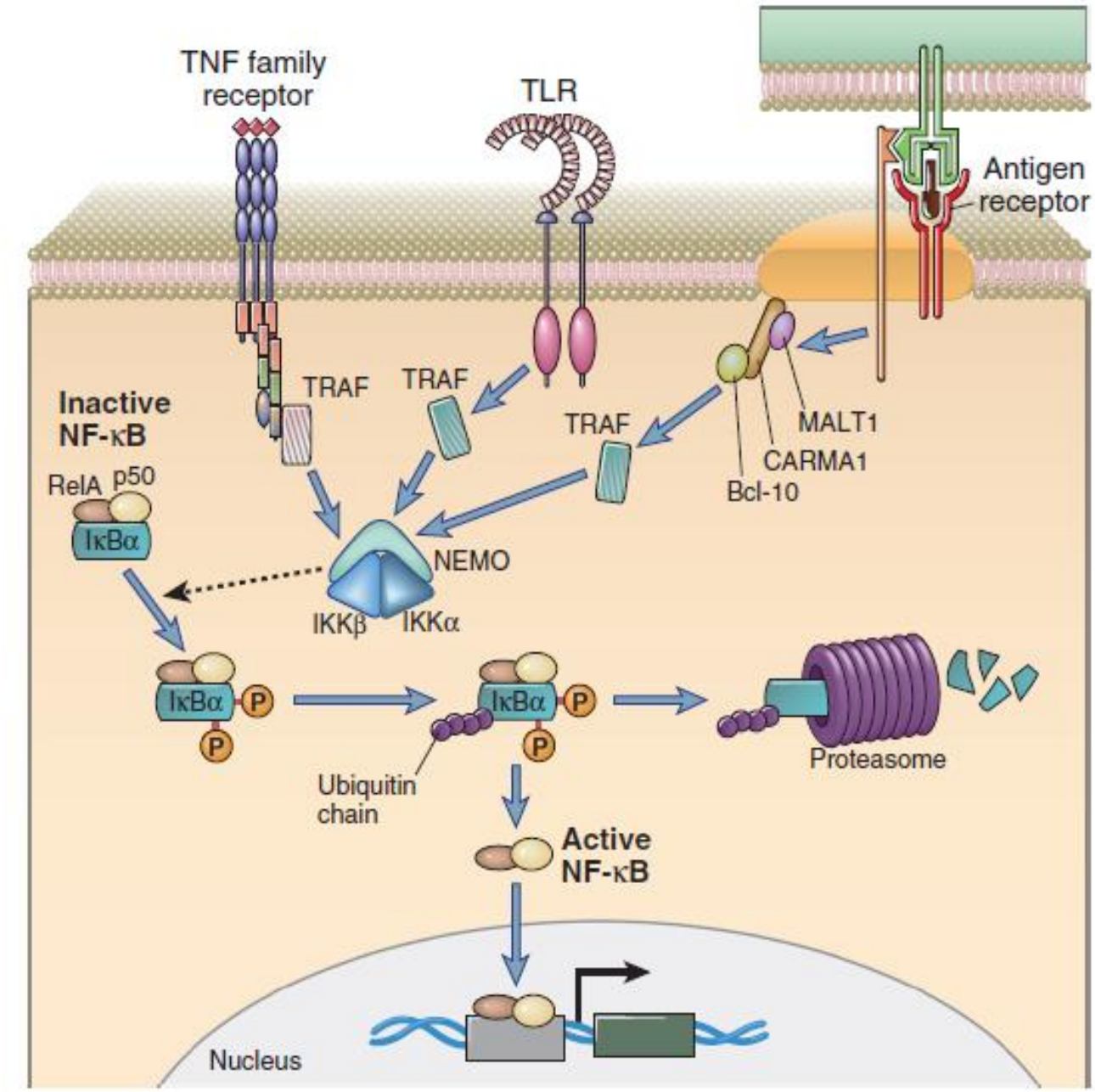


FIGURE 7–26 The canonical NF-**κ**B pathway. Antigen receptors activate specific PKCs that activate the CARMA1/Bcl-10/MALT1 complex, which in turn contributes to the induction of a TRAF E3 ligase that can polyubiquitinate NEMO/IKKγ, a component of the IκB kinase (IKK) complex, forming lysine-63–linked ubiquitin chains. This leads to the phosphorylation and activation of IKKβ by an upstream kinase. IKKβ phosphorylates the inhibitor of NF-κB (IκBα) and targets it for lysine-48 polyubiquitination and proteasomal degradation. Degradation of IκBα leads to the entry of active NF-κB into the nucleus. TLRs, members of the IL-1R family, and many members of the TNF receptor family activate TRAF family members that can activate this pathway.

As we have noted earlier,

- TLRs, the BCR, the TCR, and many cytokine receptors of the TNF and IL-1R family activate NF-κB.
- This NF-κB pathway induces the tagging and degradation of IκBα, allowing the unfettered heterodimeric NF-κB transcription factor to migrate into the nucleus.
- Most receptors that activate NF-κB do so by inducing this pathway.
- Two very different types of polyubiquitination events are required for canonical NF-κB activation.
- There are a few common steps in the canonical pathway that apply to all upstream signal inputs.
    - Upstream signaling leads to the activation of a unique type of ubiquitin E3 ligase that can add a lysine-63 type of ubiquitin chain to a protein called NEMO or IKKγ that is a noncatalytic subunit of a trimeric enzyme complex called the IκB kinase (IKK) complex.
        - This complex contains two other subunits called **IKKα and IKKβ**, both of which have the potential to be catalytically active serine/threonine kinases.
        - Ubiquitination of NEMO allows IKKβ to be activated by an upstream kinase.
    - Active IKKβ phosphorylates the inhibitory protein bound to NF-κB, IκBα, on two specific serine residues and thus tags this protein for lysine - 48 ubiquitination.
    - Polyubiquitinated IκBα is targeted for degradation in the proteasome, and the canonical NF-κB heterodimer is then free to enter the nucleus (see Fig. 7-26).

- We have discussed earlier how TCR and BCR signaling contributes to the activation of PKC-θ and PKC-β, respectively.
- These PKCs can phosphorylate a protein called CARMA1 that forms a complex with two proteins called Bcl-10 and MALT1.
- The CARMA1/MALT1/Bcl-10 complex can contribute to the activation of a lysine-63 type of ubiquitin E3 ligase called TRAF6.
- Active TRAF6 can activate TAK1 and also add a lysine-63 ubiquitin chain to NEMO, thus facilitating the activation of IKKβ.
- TLRs and the IL-1R also activate TRAF6 to initiate IKK activation.
- Many members of the TNF receptor family, including the TNF receptor and CD40, can activate canonical NF-κB signaling through the activation of other TRAF proteins such as TRAF2, TRAF3, and TRAF5.
- Heterodimers of NF-κB2 and RelB make up a "noncanonical" form of NF-κB, and these heterodimers are activated by a separate signaling pathway that is particularly important for lymphoid organ biogenesis and the survival of naive B lymphocytes.
- The two key receptors that induce the non-canonical or alternative NF-κB pathway, the LTβR (lymphotoxin β receptor) and the BAFFR (BAFF receptor), activate an IKK-like complex that contains IKKα homodimers.
- This leads to ubiquitination and degradation of a part of the NF-κB2–RelB dimer and release of the active protein.

Suggestive Questions:

1. Justify the statement that "The B lymphocyte antigen receptor is a transmembrane form of an antibody molecule associated with two signaling chains."
2. Compare between the properties of T – cell Receptors and Immunoglobulins.
3. Diagrammatically describe the structure of the B-Cell Receptor for antigen.
4. Write a short note on B – cell Receptor Complex.
5. Comment on the statement "signal initiation by antigens occurs by cross - linking of the BCR".
6. Comment on the role of CR2/CD21 complement receptor as a coreceptor for B – cells.
7. Justify the statement "The activation of B cells is enhanced by signals that are provided by complement proteins and the CD21 coreceptor complex".
8. Comment on the statement "The Ras–MAP kinase pathway is activated in antigen stimulated B cells."
9. Justify the statement "A specific phosphatidylinositol-specific phospholipase C (PLC) is activated in response to BCR signaling, and this in turn facilitates the activation of downstream signaling pathways."
10. Write short note on Inhibitory Receptors in NK Cells, B Cells, and T Cells
11. Justify the connection between E3 Ubiquitin Ligases and the Degradation of Signaling Proteins.
12. Explain the various classes of cytokine receptors with diagram.
13. Write short notes on:
    - Type I Cytokine Receptors (Hematopoietin Receptor Family)
    - Type II Cytokine Receptors (Interferon Receptor Family)
    - TNF Receptor Family

- IL-1/TLR Family
- G – Protein Coupled Receptor (7 transmembrane)

14. Explain systematically the JAK-STAT Pathway with diagram.
15. Justify the role of JAK-STAT Pathway in Cytokine Gene Expression.
16. Comment on the statement "NF-κB is a transcription factor that plays a central role in inflammation, lymphocyte activation, cell survival, and the formation of secondary lymphoid organs."
17. Write short note on The canonical NF-**κ**B pathway