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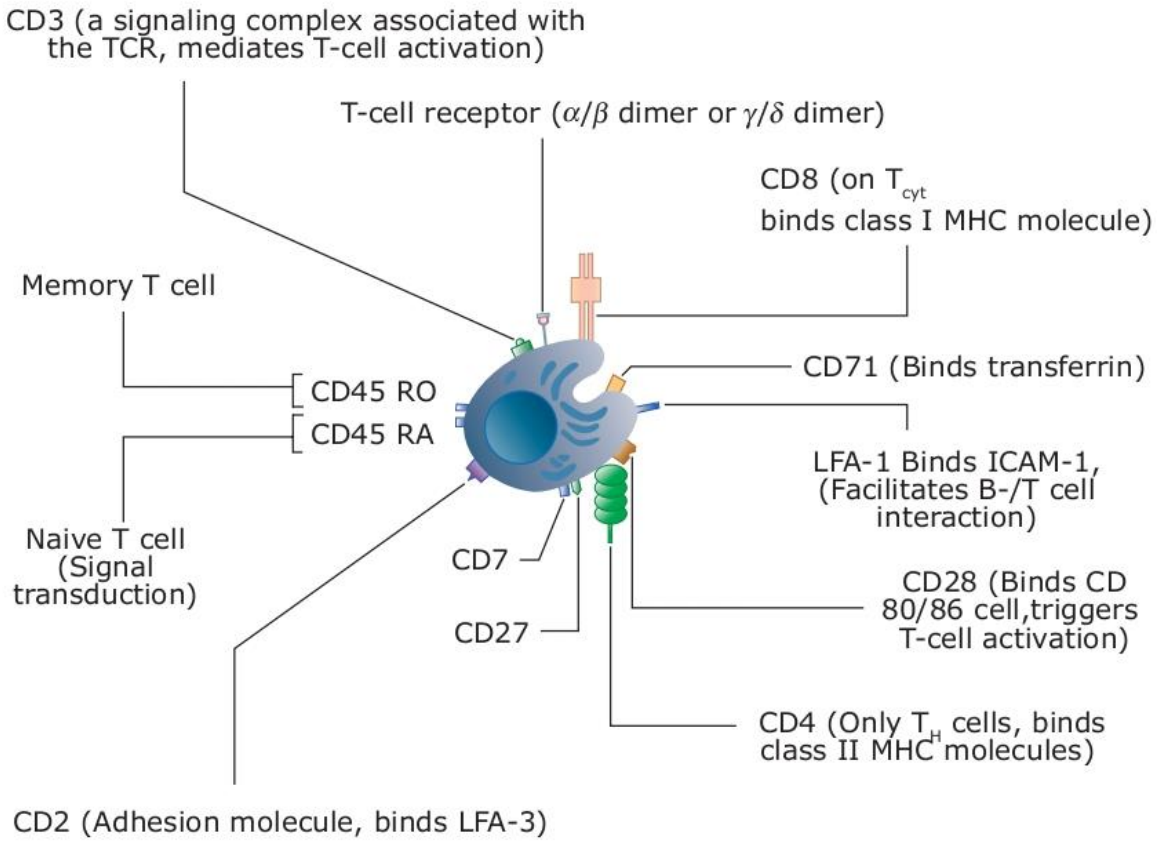
T CELL- IMPORTANT RECEPTORS



1. TCR-CD3 COMPLEX

1. The Tcell Receptor is a Heterodimer with variable and constant regions

1. There are two types of T-cell receptors, both of which are heterodimers (dimers made up of two different polypeptides).
   a. The majority of recirculating **T cells bear αβ heterodimers,** which bind to ligands made up of an antigenic peptide presented in a molecular groove on the surface of a type I or type II MHC molecule.
   b. A second subset of T cells instead expresses a heterodimeric T-cell receptor composed of a different pair of protein chains, **termed γ and δ.** T cells bearing γδ receptors have particular localization patterns (often in mucosal tissues) and some γδ T cells recognize different types of antigens from those bound by αβ T cells. Although some γδ T cells recognize conventional MHC-presented peptide antigens, other γδ T cells bind lipid or glycolipid moieties presented by noncanonical MHC molecules.
2. TCR proteins are members of the immunoglobulin superfamily of proteins and therefore the domain structures of αβ and γδ TCR heterodimers are strikingly similar to those of the immunoglobulins.
3. The **α** chain has a molecular weight of **40–50 kDa,** and the **β chain's** is **40–45 kDa**. Like the antibody light chains, the TCR chains have **two immunoglobulin-like domains**, each of which contains an intrachain **disulfide bond** spanning **60 to 75 amino acids.**
4. The **amino-terminal (variable) domain** in both chains exhibits marked sequence variation, but the sequences of the remainder of each chain are conserved (constant).
5. Each of the TCR variable domains has three hypervariable regions, which appear to be equivalent to the **complementarity-determining regions (CDRs)** in immunoglobulin light and heavy chains.A fourth hypervariable region on the **TCRβ** chain does not appear to contact antigen, and its functional significance is therefore uncertain.
6. At the C-terminal end of the constant domain, each TCR chain contains a short connecting sequence, in whicha cysteine residue forms a disulfide link with the other chain of the heterodimer.
7. C-terminal to this disulfide is a transmembrane region of 21 or 22 amino acids, which anchors each chain in the plasma membrane. The ***transmembrane domains contain positively charged amino acid residues*** that ***promote interaction with corresponding negatively charged residues on the chains of the signal transducing CD3 complex.***
8. Finally, TCR chain contains only a very short cytoplasmic tail at the carboxyl-terminal end.

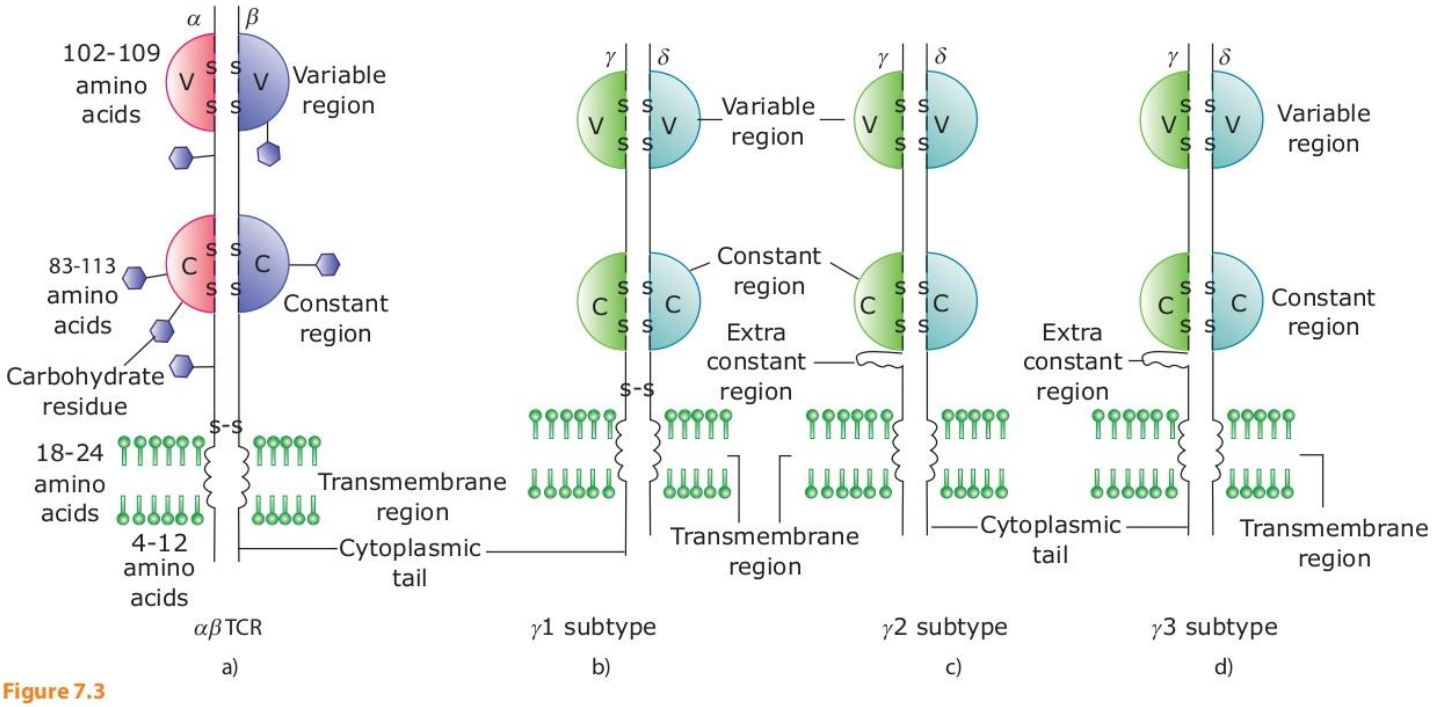
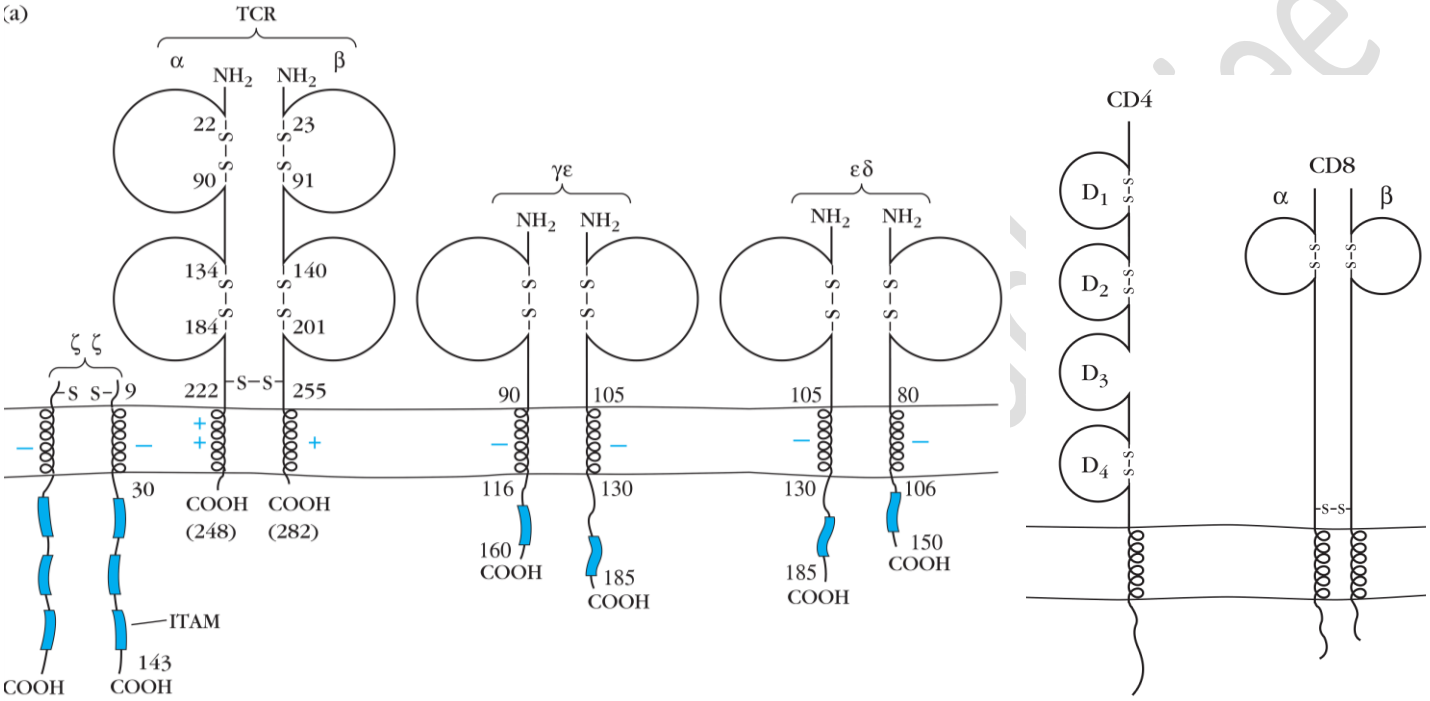


Figure 7.3
(a) Schematic diagram of TCR of αβ cell; (b), (c) and (d) Schematic diagram of TCRs of the three subtypes of γδ cells—γ1, γ2 and γ3 respectively.

2. THE T-CELL SIGNAL TRANSDUCTION COMPLEX INCLUDES CD3

1. Signaling through the TCR depends on a complex of proteins referred to collectively as CD3. The CD3 complex is made up of three dimers: **a γε (delta epsilon) pair, a γε (gamma epsilon) pair, and a third pair that is made up either of two CD3ζ(zeta) molecules or a ζη (zeta, eta) heterodimer**.
2. The cytoplasmic tails of the CD3 molecules are studded with **ITAM sequences that serve as docking sites for adapter proteins following activation induced tyrosine phosphorylation.**
3. Each of the CD3 dimers contains negatively charged amino acids in its transmembrane domain that form ionic bonds with the positively charged residues on the intramembrane regions of the T-cell receptor.



3. THE T CELL CO-RECEPTOS CD4 AND CD8 ALSO BIND THE MHC COMPLEX

1. The T-cell receptor is noncovalently associated with a number of accessory molecules on the cell surface. However, the only two such molecules that also recognize the MHC-peptide antigen are **CD4** and **CD8**.
2. **$CD4^+$T cells**
   a. **They** recognize peptides that are combined with class II MHC molecules, and function primarily as helper or regulatory T cells.
   b. CD4 is a **55 kDa** monomeric membrane glycoprotein that contains **four extracellular immunoglobulin-like domains (D1–D4)**, a **hydrophobic transmembrane region**, and a **long cytoplasmic tail** containing three serine residues that can be phosphorylated
3. **$CD8^+$ T cells**
   a. They recognize antigen that is expressed on the surface of class I MHC molecules, and function mainly as cytotoxic T cells.
   b. CD8 takes the form of a ***disulfide-linked αβ heterodimer or homodimer.***
   c. Both the α and β chains of CD8 are small glycoproteins of approximately **30 to 38 kDa.** Each chain consists of a single, extracellular, immunoglobulin-like domain, a stalk region, **a hydrophobic transmembrane region**, and a **cytoplasmic tail containing 25 to 27 residues**, several of which can be phosphorylated.

Signaling through the antigen receptor, even when combined with that through CD4 or CD8, is insufficient to activate a T cell that has had no prior contact with antigen (a naïve T cell). A naïve T cell needs to be simultaneously signalled through the TCR and its co-receptor, CD28, in order to be activated.

The TCR and CD28 molecules on a naïve T cell must co-engage the MHC-presented peptide and the CD28 ligand, CD80 (or CD86), respectively, on the antigen presenting cell for full activation to occur.

4.T CELL SIGNALLING AND ACTIVATION

When the antigens from the pathogens are internalized through PRRs by APC, they are processed as peptides and become associated with MHC class-II molecules.

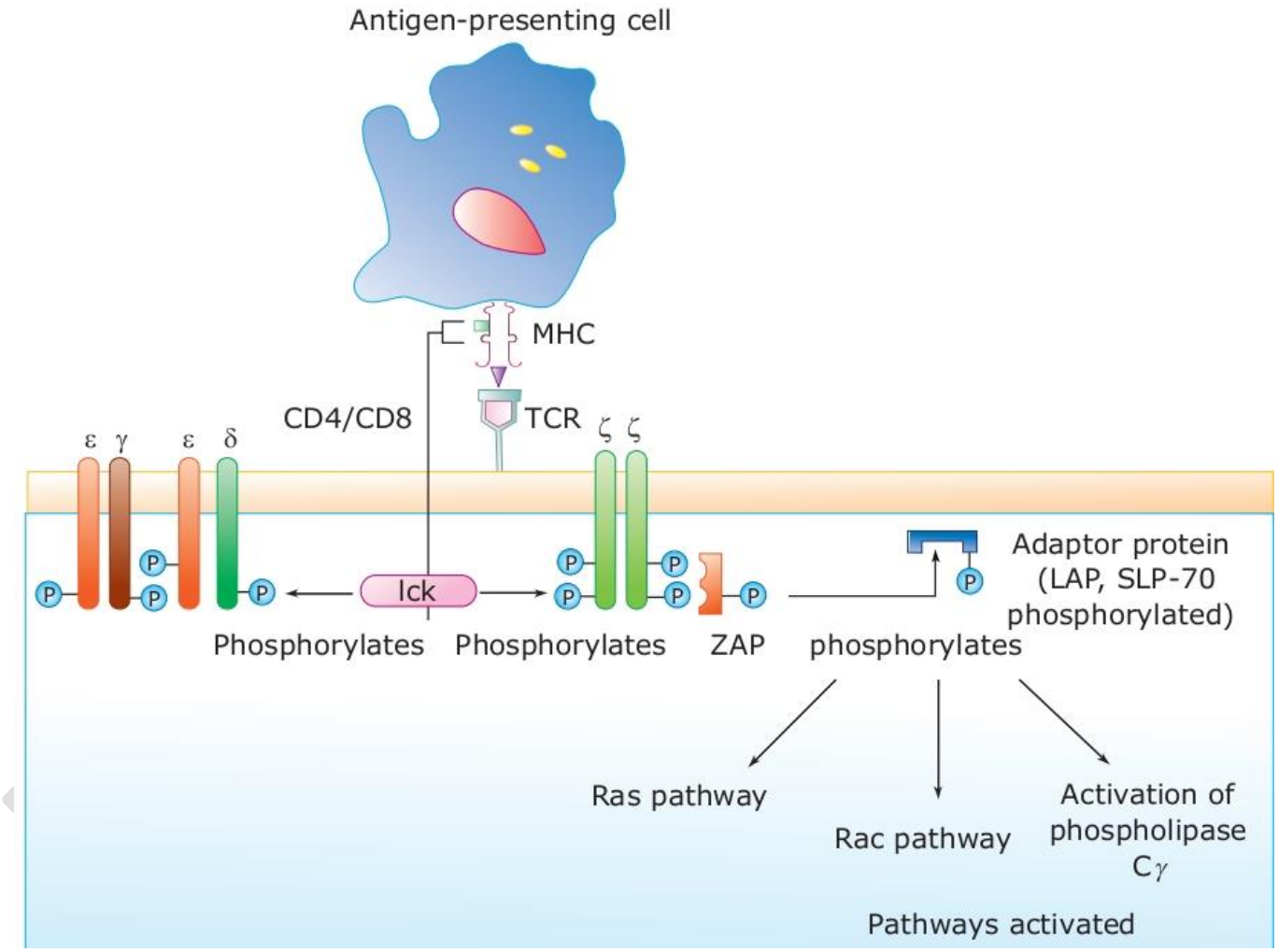
This interaction enhances the **signal I** in T_H cells. A subsequent antigen non-specific co-stimulatory **signal II** is provided by the interaction between CD28 and B7 of APC. **Signal III** is the polarizing signal that is mediated by various soluble or membrane-bound factors, such as IL-12 and CC-chemokine ligand 2 (CCL2), that promote the development of T_H1 or T_H2 cells, respectively.

T_H cell gene activation: Activation of T cells after antigen recognition results in the expression of three sets of genes:

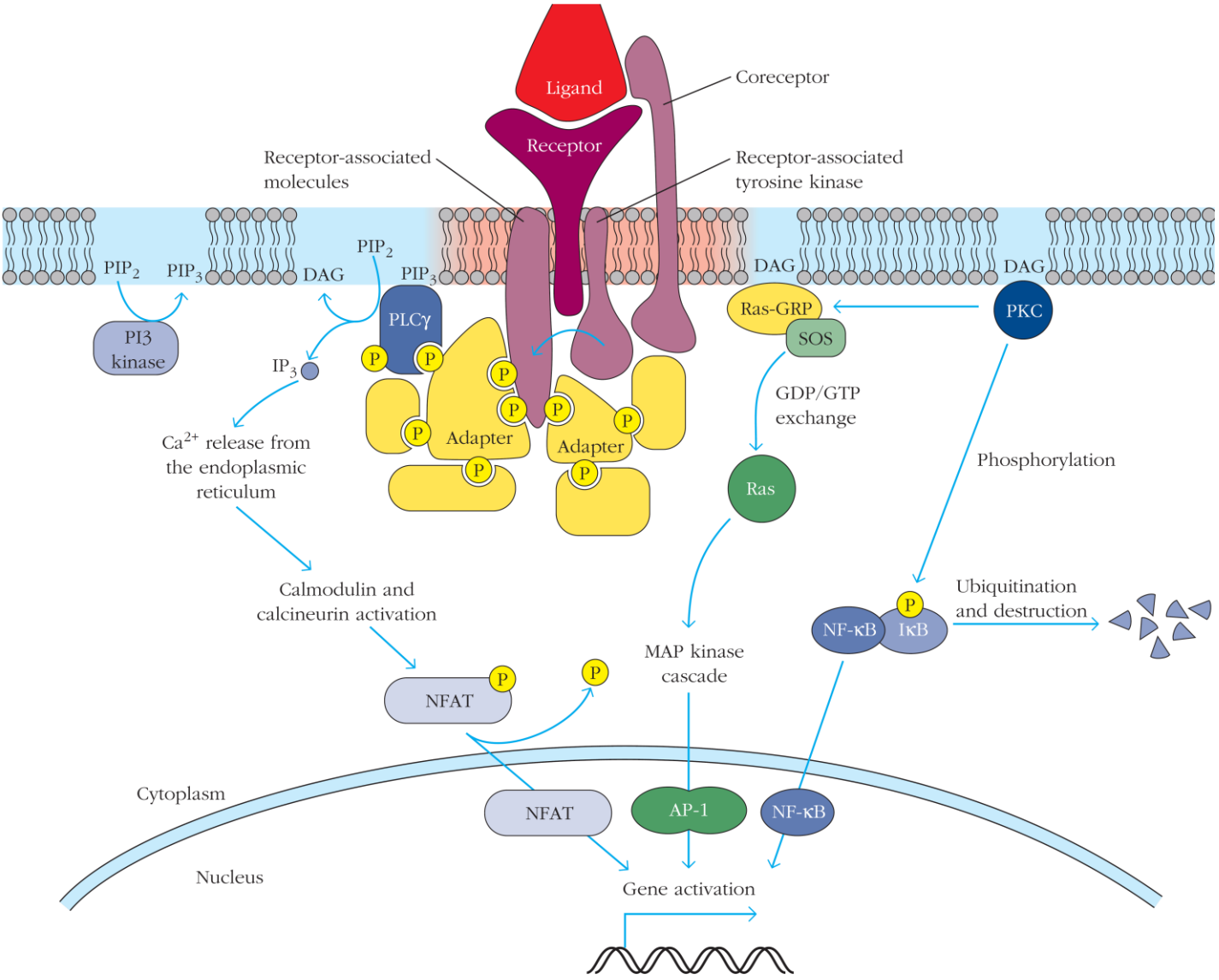
Immediate genes, expressed within ***half an hour of antigen recognition***, encoding number of transcription factors, like **c-Fos, c-Myc, c-Jun. NF-AT and NF-kB.**

Early genes expressed ***within 2 hours*** encode cytokines like **IL-2, IL-2R, IL-3 and IL-6**.

Late genes expressed ***more than 2 days*** encode **various adhesion molecules**.



Concepts in lymphocyte signaling



Ligand binding to receptors on a cell induces a variety of downstream effects, many of which culminate in transcription factor activation. Here we illustrate a few of the pathways that are addressed in this chapter. Binding of receptor to ligand induces clustering of receptors and signaling molecules into regions of the membrane referred to as lipid rafts (red). Receptor binding of ligand may be accompanied by binding of associated co-receptors to their own ligands, and causes the activation of receptor-associated tyrosine kinases, which phosphorylate receptor-associated proteins. Binding of downstream adapter molecules to the phosphate groups on adapter proteins creates a scaffold at the membrane that then enables activation of a variety of enzymes including phospholipase Cγ (PLCγ), PI3 kinase, and additional tyrosine kinases. PLCγ cleaves phosphatidyl inositol bisphosphate (PIP_2) to yield inositol trisphosphate (IP_3), which interacts with receptors on endoplasmic reticulum vesicles to cause the release of calcium ions. These in turn activate calcineurin, which dephosphorylates the transcription factor NFAT, allowing it to enter the nucleus. Diacylglycerol (DAG), remaining in the membrane after PLCγ cleavage of PIP_2, binds and activates protein kinase C (PKC), which phosphorylates and activates enzymes leading to the destruction of the inhibitor of the transcription factor NF-κB. With the release of the inhibitor, NF-κB enters the nucleus and activates a series of genes important to the immune system. Binding of the adapter protein Ras-GRP to the signaling complex allows for the binding and activation of the Guanine nucleotide Exchange Factor (GEF) Son of Sevenless (SOS), which in turn initiates the phosphorylation cascade of the MAP kinase pathways. This leads to the entry of a third set of transcription factors into the nucleus, and activation of the transcription factor AP-1. (Many details that are explained in the text have been omitted from this figure for clarity.)

LCK IS THE FIRST TYROSINE KINASE ACTIVATED IN T CELL SIGNALING

1. When the T-cell receptor interacts with its cell-bound antigen, receptors, co-receptors, and signaling molecules cluster into the cholesterol-rich lipid rafts of the plasma membrane .
2. The ***Src-family tyrosine kinase Lck*** is normally found associated with CD4 and CD8, and the association between Lck and CD4 is particularly close. Antigen-induced clustering of the receptor–co-receptor complex brings Lck into the vicinity of the membrane-associated tyrosine phosphatase, ***CD45***, which removes the inhibitory phosphate group on Lck.
3. Reciprocal phosphorylation by nearby Lck molecules at their activating tyrosine sites then induces Lck to phosphorylate ***CD3 ITAM residues.***
4. Once the CD3 ITAMs are phosphorylated, a second tyrosine kinase, **ZAP-70,** docks at the phosphorylated tyrosine

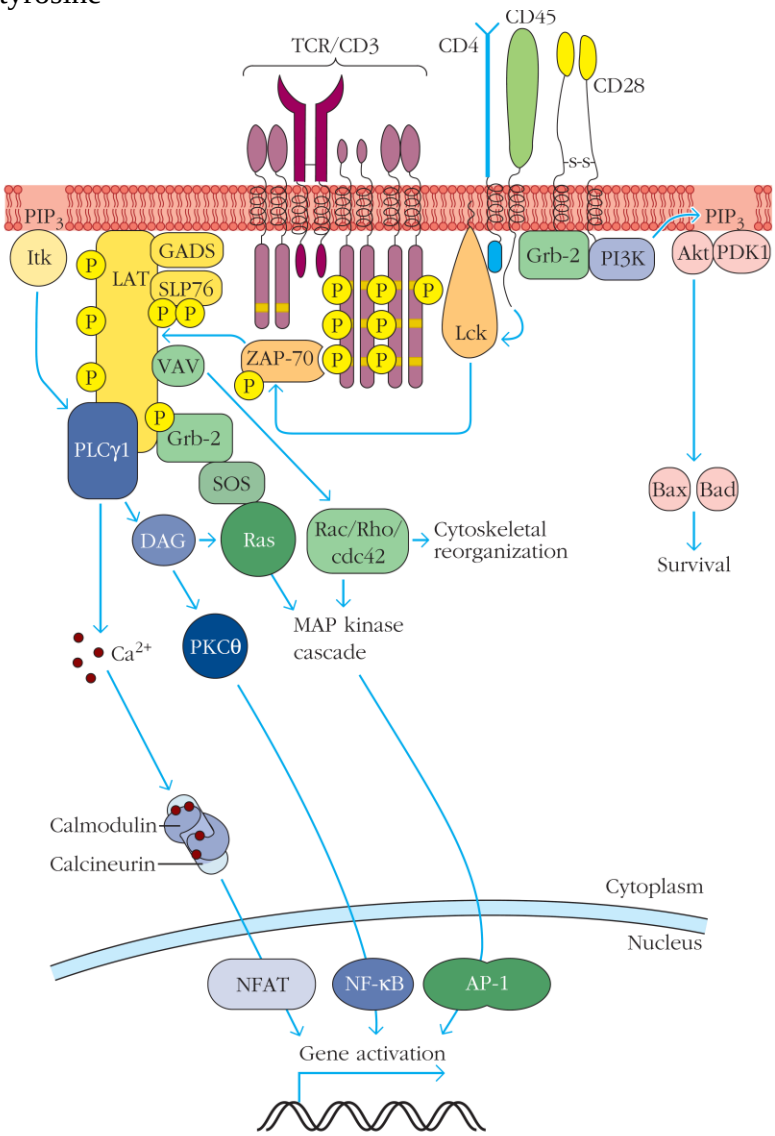


FIGURE 3-32 Signal transduction pathways emanating from the TCR. T cell antigen binding activates the src-family kinase Lck, which phosphorylates the kinase ZAP-70. ZAP-70 in turn phosphorylates the adapter molecules LAT, SLP76, and GADS which form a scaffold enabling the phosphorylation and activation of PLCγ1 and PKCθ with the consequent effects on transcription factor activation described in the text. The GEF proteins Vav and SOS are also activated on binding to LAT, leading to activation of the Ras/MAP kinase transcription factor pathway and the Rac/Rho/cdc42 pathway, leading to changes in cell shape and motility. PI3 kinase, translocated to the cytoplasmic side of CD28, forms PIP_3, inducing localization of the enzymes PDK1 and Akt to the membrane. This leads to further NF-κB activation and increased cell survival as described.

T CELLS USE DOWNSTREAM SIGNALING STRATEGIES SIMILAR TO THOSE OF B CELLS

1. In T cells, one of the earliest adapter molecules to be incorporated into the signaling complex is **LAT (*L*inker protein of *A*ctivated *T* cells)**, a transmembrane protein associated with lipid raft s in the plasma membrane.
2. Following TCR ligation, ***LAT is phosphorylated*** on multiple residues by ***ZAP-70***, and these phosphorylated residues now provide docking sites for several important enzymes bearing SH2 domains, including **PLCγ1** ***Phosphorylated LAT*** also binds to the ***adapter protein GADS***, which is constitutively associated with the ***adapter SLP-76***. This combination of adapter proteins is critical to T-cell receptor signaling, providing the structural framework for most downstream signaling events.

3. **PLCγ1** , ***localized to the plasma membrane*** by binding to LAT, is further activated by tyrosine phosphorylation, mediated by the **kinase Itk** (which belongs to a family of kinases referred to as Tec kinases).
4. PLCγ1 breaks down PIP_2, releasing **IP3**, which induces the release of calcium and the **activation of NFAT** via calcineurin activation. The **DAG** created by PIP_2 hydrolysis ***binds***, in T cells, to a specialized form of PKC called PKC θ (theta). The signaling cascade culminates in the ***degradation of the inhibitors of NF-κB*** and the translocation of the active transcription factor into the nucleus.
5. **Phosphorylated LAT** also associates with the SH2 domain of **Grb2**, the now-familiar adapter molecule that brings in components of the Ras pathway to the signaling complex.
6. **Grb2** binds constitutively to **SOS**, the GEF that facilitates activation of the Ras pathway. In T cells, the **Ras pathway** is important both to the activation of the **transcription factor AP-1**, which functions to signal cytokine secretion, and to the passage of the signals that reorganize the actin cytoskeleton.

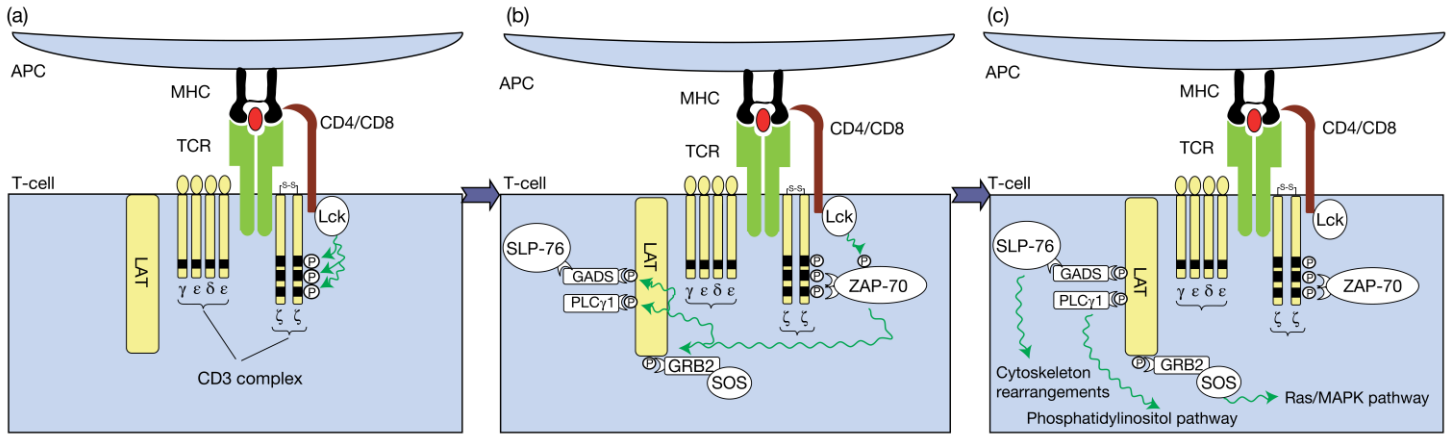


Figure 7.8 Signaling events downstream of T-cell receptor (TCR) engagement. (a) Engagement of the TCR with the correct peptide–MHC combination leads to CD4/CD8 recruitment to the TCR complex through interactions with MHC on the antigen-presenting cell (APC) (note that, for simplicity, co-stimulation between B7 and CD28 is not depicted). Because CD4 and CD8 are constitutively associated with the Lck kinase, this brings Lck into close proximity to the ITAMs within the CD3 co-receptor complex. Lck then phosphorylates CD3ζ on multiple sites, that creates binding sites for recruitment of the ZAP-70 kinase. (b) ZAP-70 recruitment to the CD3 co-receptor complex leads to its phosphorylation and activation by Lck. Active ZAP-70 then propagates TCR signals through phosphorylation of LAT at several sites. Phosphorylated LAT serves as a platform for recruitment of multiple signaling complexes, as depicted. (c) Molecules recruited to LAT instigate three main signaling cascades, as depicted, which cooperatively achieve T-cell activation. See main text for further details.

5.T CELL ACTIVATION AND TWO SIGNAL HYPOTHESIS

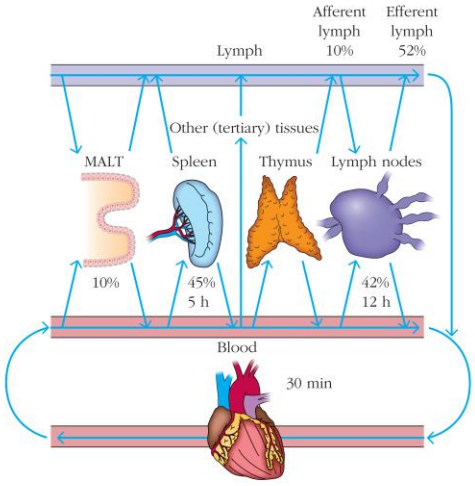
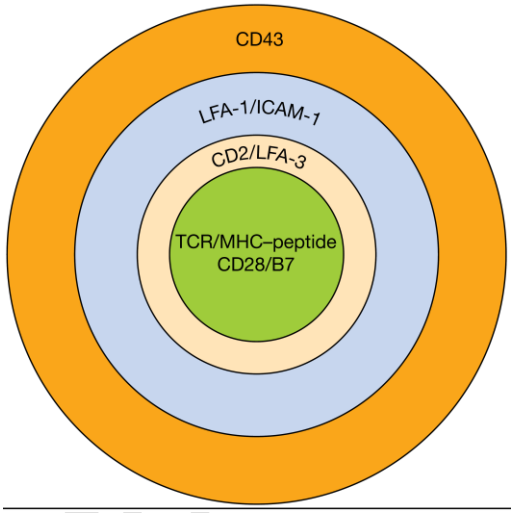


FIGURE 14-1 Lymphocyte recirculation routes. The percentage of the lymphocyte pool that circulates to various sites and the average transit times in the major sites are indicated. Lymphocytes migrate from the blood into lymph nodes through specialized areas in postcapillary venules called high-endothelial venules (HEVs).

1. CD4+ and CD8+T cells leave the thymus and enter the circulation as resting cells in the **G_0 stage** of the cell cycle. These **naïve** T cells are mature, but they have not yet encountered antigen. Their **chromatin is condensed**, they have very **little cytoplasm**, and they exhibit **little transcriptional activity**.
2. However, they are mobile cells and recirculate continually among the blood, lymph, and secondary lymphoid tissues, including lymph nodes, browsing for antigen.
3. It is estimated that each naïve T cell recirculates from blood through lymph nodes and back again every 12 to 24 hours. Because only about 1 in 10^5 naïve T cells is likely to be specific for any given antigen, this large-scale recirculation increases the chances that a T cell will encounter appropriate antigen.
4. If a naïve T cell does not bind any of the MHC-peptide complexes encountered as it browses the surfaces of stromal cells of a lymph node, it exits through the efferent lymphatics, ultimately draining into the thoracic duct and rejoining the blood.

5. However, if a naïve T cell does encounter an APC expressing an MHC-peptide to which it can bind, it will initiate an activation program that produces a diverse array of cells that orchestrate efforts to clear infection.
6. A successful ***T cell-APC interaction results*** in the stable organization of signaling molecules into an ***immune synapse***. The ***TCR/MHC-peptide complexes*** and ***coreceptors*** are aggregated in the central part of this synapse ***(central supra molecular activating complex, or cSMAC)***. The intrinsic affinity between the TCR and MHC-peptide surfaces is quite low (K_d ranges from 10^{-4} M to 10^{-7} M) and is stabilized by the activity of several molecules which together increase the **avidity** (the combined affinity of all cell-cell interactions) of the cellular interaction.



7. The coreceptors CD4 and CD8, which are found in the cSMAC, stabilize the interaction between TCR and MHC by binding MHC class II and MHC class I molecules, respectively. ***Interactions between adhesion molecules and their ligands (e.g., LFA-1/ICAM-1 and CD2/LFA-3)*** help to sustain the signals generated by allowing long-term cell interactions. These molecules are organized around the central aggregate, forming the ***peripheral or "p" SMAC***.
8. However, even the increased functional avidity offered by coreceptors and adhesion molecules is still not sufficient to fully activate a T cell. Interactions between costimulatory receptors on T cells (e.g., CD28) and costimulatory ligands on dendritic cells (e.g., CD80/86) provide a second, required signal. In addition, as you will see below, a third set of signals, provided by local cytokines (Signal 3), directs T-cell diff erentiation into distinct effector cell types.

COSTIMULATORY SIGNALS ARE REQUIRED FOR OPTIMAL T-CELL ACTIVATION AND PROLIFERATION

What evidence pointed to a requirement for a second, costimulatory signal?

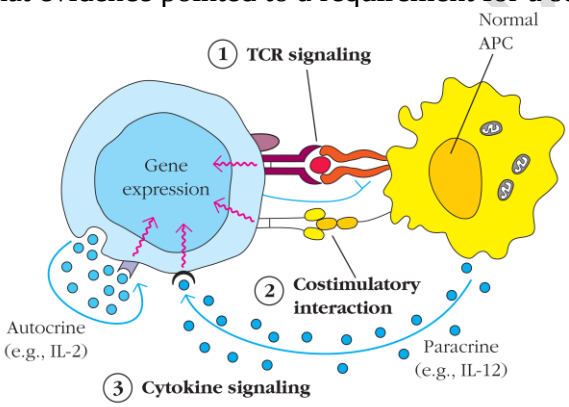


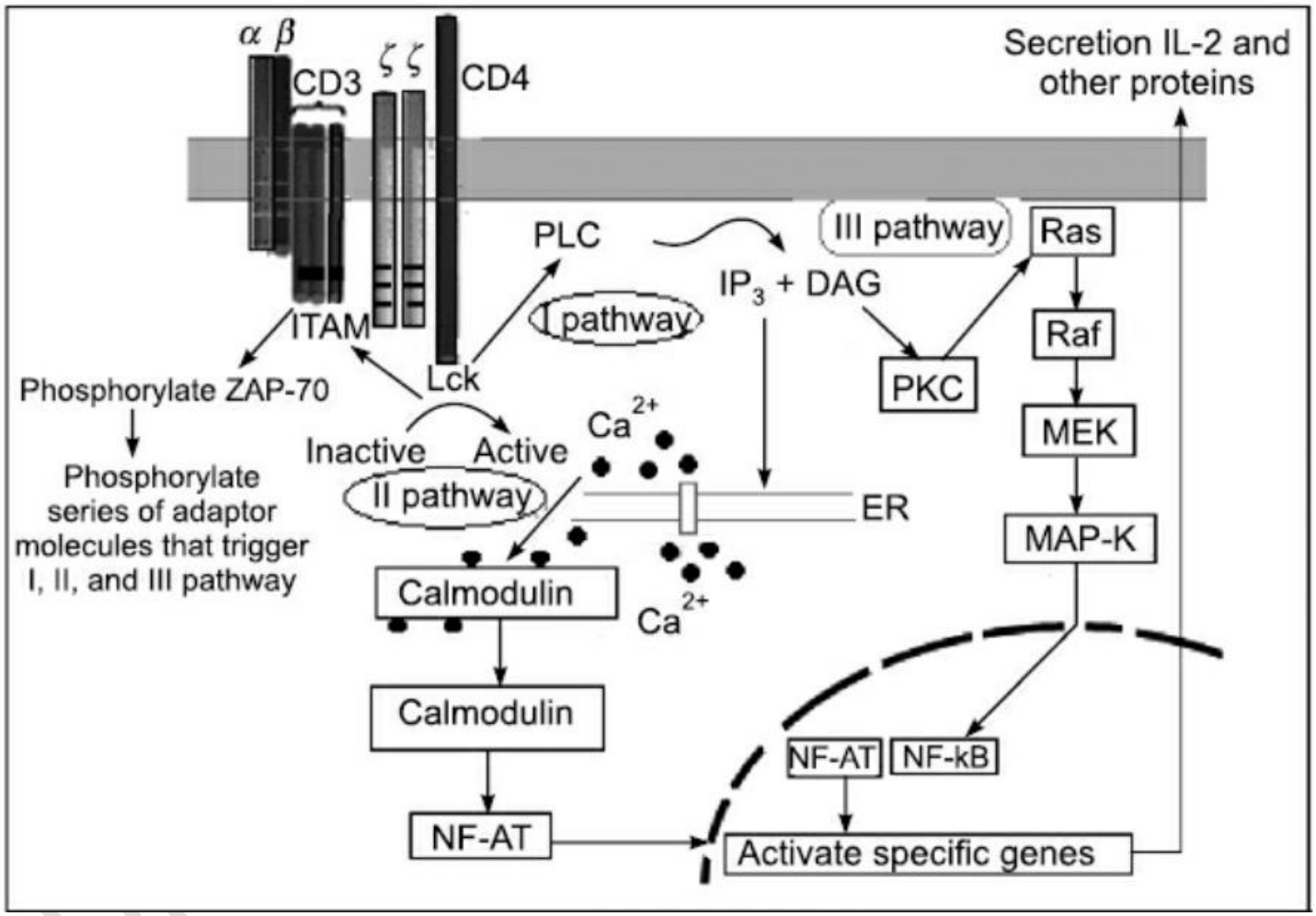
FIGURE 11-3 Three signals are required for activation of a naïve T cell. The TCR/MHC-peptide interaction, along with CD4 and CD8 coreceptors and adhesion molecules, provide Signal 1. Co-stimulation by a separate set of molecules, including CD28 (or ICOS, not shown) provide Signal 2. Together, Signal 1 and Signal 2 initiate a signal transduction cascade that results in activation of transcription factors and cytokines (Signal 3) that direct T-cell proliferation (IL-2) and differentiation (polarizing cytokines). Cytokines can act in an *autocrine* manner, by stimulating the same cells that produce them, or in a *paracrine* manner, by stimulating neighboring cells.

1. In **1987, Helen Quill** and **Ron Schwartz** recognized that, in the absence of functional APCs, isolated high affinity TCR-MHC interactions actually led to T-cell non responsiveness rather than activation—a phenomenon they
2. called T-cell **anergy**. Their studies led to the simple but powerful notion that not one but two signals were required for full T-cell activation:
3. ***Signal 1*** is provided by antigen-specific TCR engagement (which can be enhanced by coreceptors and adhesion molecules), and
4. ***Signal 2*** is provided by contact with functional APC. When a T cell receives both Signal 1 and Signal 2, it will be activated to produce cytokines that enhance entry into cell cycle and proliferation (Figure 11-3). It is now known that Signal 2 results from an interaction between specific **costimulatory receptors** on T cells and costimulatory ligands on dendritic cells

5. That dendritic cells and other APCs become activated by antigen binding to PRRs, to express costimulatory ligands (e.g., CD80 and CD86) and produce cytokines that enhance their ability to activate T cells. CD28 is the

6. most commonly cited example of a costimulatory receptor, but other related molecules that provide costimulatory signals during T-cell activation have since been identified and are also described below. Because these molecules enhance TCR signaling, They are collectively referred to as "positive" costimulatory receptors and ligands.
7. **Negative costimulatory receptors**, which inhibit TCR signaling, have also been identified. These play important roles in (1) maintaining peripheral T-cell tolerance and (2) reducing inflammation both after the natural course of an infection and during responses to chronic infection.
8. Naïve T cells, for example, do not express negative costimulatory receptors, allowing them to be activated in secondary lymphoid tissue during the initiation of an immune response. On the other hand, effector T cells up-regulate negative costimulatory receptors at the end of an immune response, when proliferation is no longer advantageous.

SIGNAL TRANSDUCTION PATHWAY OPERATIVE FOR THE 3 CONSECUTIVE SIGNALS OPERATIVE AFTER TCELL-PMHC INTERACTION



1. SIGNAL I TRANSDUCTION PATHWAYS

1. The signal I transduction is initiated by a **protein tyrosine kinase called Lck**. It is located in the cytoplasmic tail of co-receptor CD4. It is brought close to ITAMs of TCR complex. This creates a docking site for ZAP-70—another protein kinase—that becomes activated by phosphorylation. ZAP-70, in turn, phosphorylates adaptor molecules that trigger three pathways .
    a. **Pathway I:** The inactive **phospholipase C (PLC)** is phosphorylated to active PLC. Activation of PLC leads to hydrolysis of **phosphatidylinositol 4, 5-bisphosphate (PIP_2)**, generating **diacylglycerol (DAG)** and **inositol trisphosphate (IP_3)**.
    b. **Pathway II:** DAG activates protein kinase C (PKC) that, in turn, **phosphorylates Ras**, a GTPase that activates **Raf** leading to recruitment of the **MAP kinase cascade**. It also activate **Rac pathway**. They release the

inhibitory factor of a transcription factor **NF-kB** which enter into nucleus and promotes the expression of genes required for T cell activation.

c. **Pathway III:** The **Ca^{2+}** released from ER store by IP3 binds to **calmodulin** activates **calcineurin**, a Ca2+/calmodulin dependent protein phosphatase. Calcineurin dephosphorylates a transcriptional regulator **NF-AT** in the cytoplasm. On migration to the nucleus **induces the expression of the IL-2, IL-4 genes, and other growth promoting cytokines.**

2. SIGNAL II TRANSDUCTION:

1. The interaction of CD28 with B7 delivers a positive co-stimulatory signal. This is antigen non-specific.
2. This interaction determines whether T cell undergo clonal expansion or clonal anergy. Absence of this co-stimulatory signal results in the inability of cells to proliferate in response to peptide-MHC complex. There is a minimal production of cytokines specially IL-2. Another marker CTLA-4, whose competitive interaction with B7 is inhibitory and down regulates the activation of the T cell.
3. CTLA-4 is important in the regulation of T cell activation and proliferation. Both **signal I and II triggers** the entry of ***T cell into G1 phase*** of cell cycle. They ***divide two three times per day for 4 to 5 days*** generating large clones of T cells. A higher percentage of cells are converted to effector T cells and few are converted to memory cells.
4. The effector cells are short-lived and perform helper, cytotoxic or delayed type hypersensitivity functions. Memory cells are long-lived, quiescent in G_0 stage of cell cycle. With the subsequent challenge of same antigen, their reactivity is heightened, generating a secondary response.

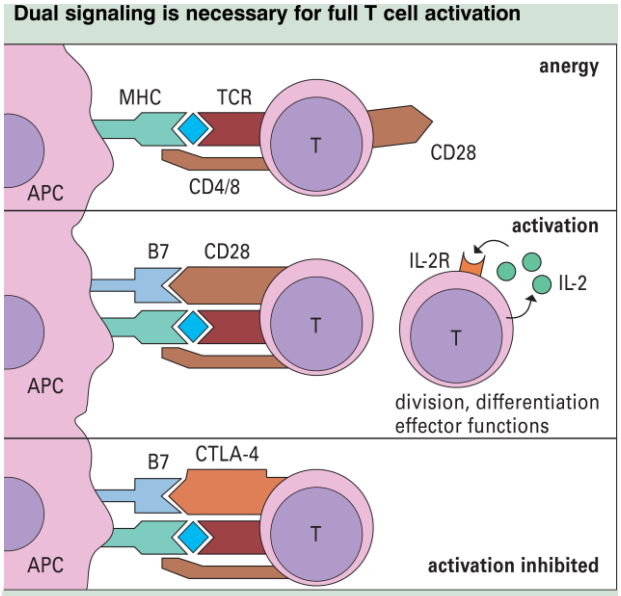
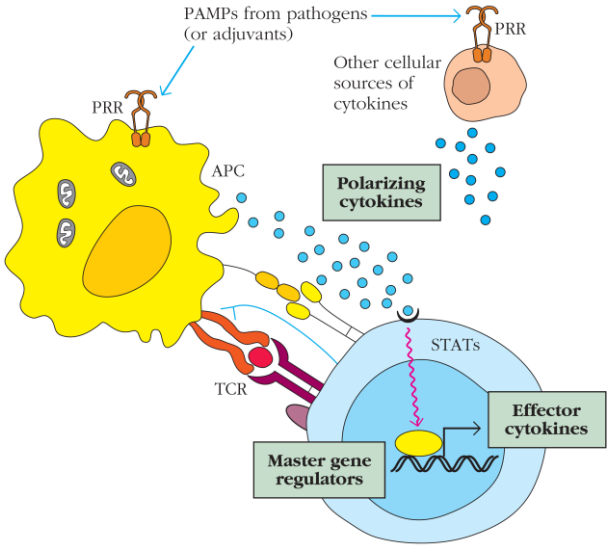


Fig. 8.16 A T cell requires signals from both the TCR and CD28 for activation. In the absence of costimulatory molecules inactivation or anergy results. If CD28 is bound by B7 on the surface of a professional APC, the T cell is activated and produces IL-2 and its receptor (IL-2R). The cell divides and differentiates into an effector T cell, which no longer requires signal 2 for its effector function. However, if CTLA-4 on the T cell binds to B7, activation is inhibited.

3. SIGNAL III TRANSDUCTION:

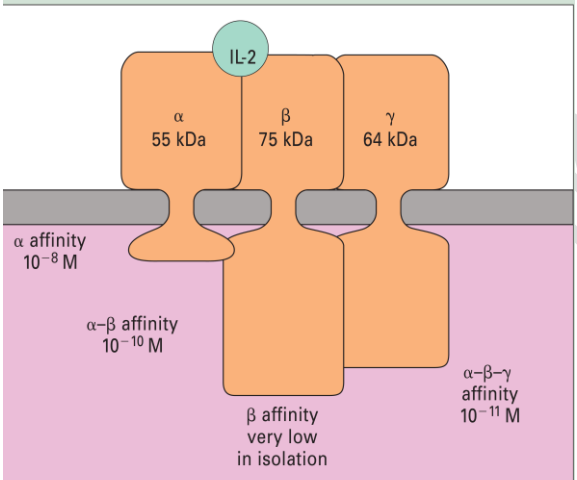
1. The nature of ***signal III depends on the activation of PRRs by PAMPs or tissue factors.***
2. Optimal expression of T-cell-polarizing factors often requires feedback stimulation by CD40 ligand (CD40L) expressed by T cells after activation by signals I and II. Various soluble and membrane-bound molecules act as T_H polarization factors which are not associated with APC. At an early phase of activation IL-12 produced by APC acts as T_H polarization factor. It up-regulates IFN-γ. T_H1 cell polarization occurs at high concentration of IL-12. IL-18 also helps in this by perpetuating IFN-g in these IL-12 activated cells.
3. IL-12 at lower concentrations polarizes T cells to T_H2 cells because of lack of IFN-γ. IL-4 autoactivate the transcription factor ***GATA-3*** that induces T_H2 development.
4. PGE2 and nitric oxide from APC or bystander cell induce directly or indirectly via inhibition of IL-12 production the T_H2 polarization. The cell surface expression of OX40 ligand and B71/2 also promote T_H2 signaling.



Costimulatory receptor on T cell	Costimulatory ligand	Activity
Positive costimulation		
CD28	CD80 (B7-1) or CD86 (B7-2) *Expressed by professional APCs, (and medullary thymic epithelium)*	Activation of naïve T cells
ICOS	ICOS-L *Expressed by B cells, some APCs, and T cells*	Maintenance of activity of differentiated T cells; a feature of T-/B-cell interactions
Negative costimulation		
CTLA-4	CD80 (B7-1) or CD86 (B7-2) *Expressed by professional APCs (and medullary thymic epithelium)*	Negative regulation of the immune response (e.g., maintaining peripheral T-cell tolerance; reducing inflammation; contracting T-cell pool after infection is cleared)
PD-1	PD-L1 or PD-L2 *Expressed by professional APCs, some T and B cells, and tumor cells*	Negative regulation of the immune response, regulation of T_{REG} differentiation
BTLA	HVEM *Expressed by some APCs, T and B cells*	Negative regulation of the immune response, regulation of T_{REG} differentiation (?)

6. INTERLEUKIN-2 DRIVES T CELL DIVISION

Expression of the high-affinity IL-2 receptor on T cells



approximate numbers per cell	α chain	β chain	γ chain
at rest	0	500	5000
maximum activation	50 000	5000	~5000

Fig. 8.18 The high-affinity IL-2R consists of three polypeptide chains, shown schematically. Resting T cells do not express the α chain, but after activation they may express up to 50 000 α chains per cell. Some of these associate with the β chain to form the high-affinity IL-2R. (The γc chain is a common signaling chain of several cytokine receptors.)

1. T cell activation leads to the production of IL-2 and IL-2 receptors, so a T cell can act on itself and surrounding cells. In most CD4+T cells and some CD8+T cells, there is a transient production of IL-2 for **1–2 days**. During this time the interaction of ***IL-2 with the high-affinity IL-2R results in T cell division.***
2. On resting T cells, the ***IL-2R is predominantly present as a low-affinity*** form consisting of two polypeptide chains, ***a β chain (p75) that binds IL-2*** and a common ***γ chain that signals to the cell.***
3. When the T cell is activated, it produces an ***α chain (CD25)***, which contributes to IL-2 binding and, together with the β and γ chains, forms the high affinity receptor.
4. IL-2 is ***internalized within 10–20 minutes*** and the ***β and γ chains are degraded in lysosomes*** while the ***α chain is recycled to the cell surface***. Sustained signaling by IL-2 over several hours is needed to drive T-cell division.
5. The transient expression of the ***high-affinity IL-2R for about 1 week after stimulation of the T cell***, together with the induction of CTLA-4, helps limit T cell division.
6. In the absence of positive signals, the T cells will start to die by apoptosis.
7. In view of the importance of IL-2 in T cell division, it was surprising that the rare patients who lack CD25 (and IL-2 receptor knockout mice) develop an immunoproliferative condition.
8. These observations lead to an awareness that ***IL-2 also has a regulatory function in T cell development*** –regulatory ***T***

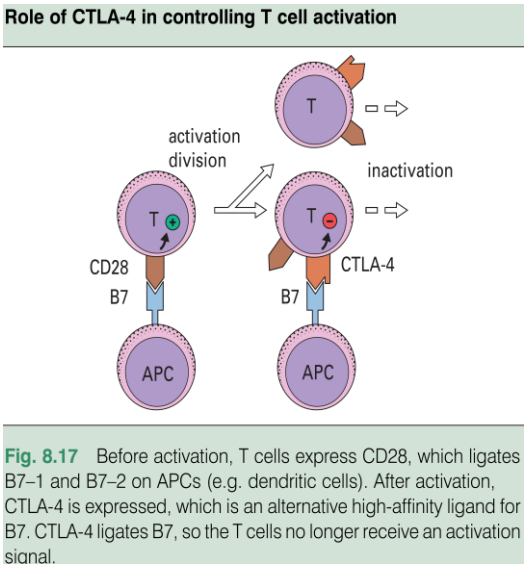
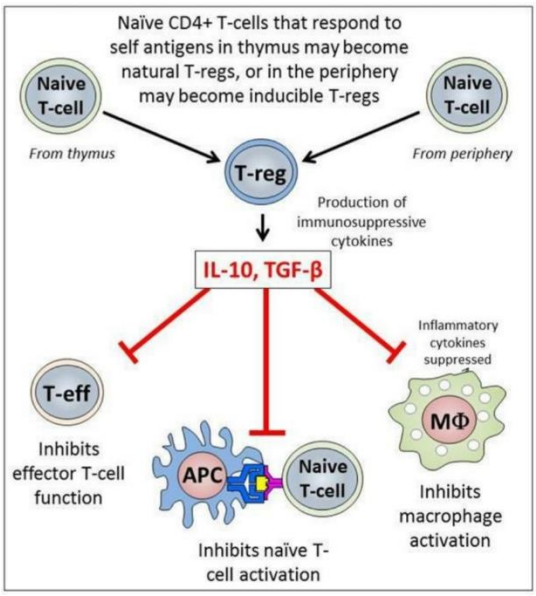


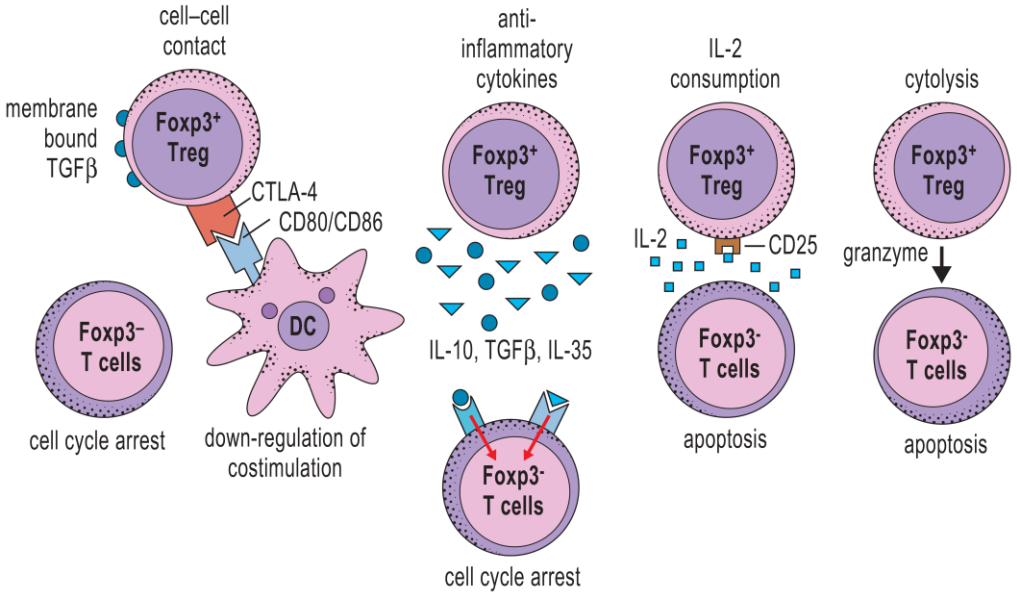
Fig. 8.17 Before activation, T cells express CD28, which ligates B7–1 and B7–2 on APCs (e.g. dendritic cells). After activation, CTLA-4 is expressed, which is an alternative high-affinity ligand for B7. CTLA-4 ligates B7, so the T cells no longer receive an activation signal.

cells (Tregs) are characterized by high CD25 expression, and IL-2 is required for their generation in the thymus and maintenance in the periphery

7. LYMPHOCYTES; POLARIZING SIGNALS.

1. **There are three types of lymphoid cells: B cells, T cells, and natural killer (NK) cells.** B and T cells are members ofclonal populations distinguished by antigen receptors of unique specificity.
    a. **B cells synthesize and display membrane antibody, and**
    b. **T cells synthesize and display T-cell receptors (TCRs).**
    c. **NK cells do not synthesize antigen specific receptors;**
    d. **a small population of TCR expressing T cells have features of NK cells and are called NKT cells.**
2. T cells can be further subdivided into
    a. **helper T cells, which typically express CD4 and recognize pMHC class II, and**
    b. **cytotoxic T cells, which typically express CD8 and recognize pMHC class I.**
3. In broad terms, **T helper type 1 (T_H1) cells** and **T helper type 17 (T_H17) cells** (the latter so named because they secrete IL-17) regulate our response to intracellular pathogens, and
4. **T helper type 2 (T_H2) cells** and **T follicular helper (T_{FH}) cells** regulate our response to extracellular pathogens, such as bacteria and parasitic worms.
5. Each $CD4^+$ **T_H**-cell subtype produces a different set of cytokines that enable or "help" the activation of B cells, T cells, macrophages, and various other. **Which helper subtype dominates a response depends largely on what type of pathogen (intracellular versus extracellular, viral, bacterial, fungal, helminth) has infected an animal.**
6. Another type of $CD4^+$ T cell, the **regulatory T cell (T_{REG})**, has the unique capacity to inhibit immune responses.
7. These cells, called natural T cells, arise during maturation in the thymus from cells that bind self proteins with high affinity (autoreactive cells).
8. They can also can be induced at the site of an immune response in an antigen-dependent manner **(iT_{REG} cells).**
9. Regulatory T cells are identified by the presence of CD4 and CD25 on their surfaces, as well as by the expression of the internal transcription factor FoxP3.
10. **T_{REG}** cells quell autoreactive responses and play a role in limiting our normal T-cell responses to pathogens.





TREGS MAY SUPPRESS BY A VARIETY OF MECHANISMS.

1. Via cell-to-cell contact
2. (secreted or cell surface molecules such as CTLA-4 expression, or membrane bound TGFβ).
3. Release of suppressor cytokines such as IL-10, TGFb and IL-35.
4. IL-2 consumption (Tregs can express high levels of CD25, the IL-2 receptor).
5. Cytolysis, akin to CD8+ T cell killing.

THE DIFFERENTIATION OF T HELPER CELL SUBSETS IS REGULATED BY POLARIZING CYTOKINES

a. As you know, T-cell activation requires TCR and costimulatory receptor engagement, both of which are supplied by an activated APC. It is now clear that the functional fate of activated T cells is determined by signals they receive from additional cytokines generated during the response. These cytokines **(Signal 3)** are referred to as ***polarizing* cytokines** because they are responsible for guiding a helper T cell toward one of several different effector fates.

b. Polarizing cytokines can be generated by the stimulating APC itself, or by neighboring immune cells that have also been activated by antigen. Which cytokines are produced during an immune response depends on
   (1) the cell of origin (DC, macrophage, B cell, NK cell, etc.),
   (2) its maturation and activation status,
   (3) which pathogens and other inflammatory mediators it encounters, and
   (4) in what tissue environment it encounters that pathogen.

c. Innate interactions therefore have a critical role in shaping adaptive responses. Specifically, by influencing APC secretions and the surface and the microenvironmental landscape that a T cell encounters, innate immune responses directly influence the functional fate of helper T cells.

d. For example, double-stranded RNA, a product of many viruses, binds TLR3 receptors on dendritic cells, initiating a signaling cascade that results in production of IL-12, which directly promotes T_H1 diff erentiation. On the other hand, worms stimulate PRRs on innate immune cells, including mast cells, which generate IL-4. IL-4 directly promotes polarization of activated T cells to the T_H2 subset, which coordinates the IgE response to helminths. In this case, the main polarizing cytokine is not made by the activating dendritic cell, but is generated by a neighboring immune cell.

	Polarizing cytokines	Master gene regulators	Effector cytokines	Functions
T_H1	IL-12 IFN-γ IL-18	T-Bet	IFN-γ TNF	Enhances APC activity Enhances T_C activation Protects against intracellular pathogens Involved in delayed type hypersensitivity, autoimmunity
T_H2	IL-4	GATA-3	IL-4 IL-5 IL-13	Protects against extracellular pathogens (particularly IgE responses) Involved in allergy
T_H17	TGF-β IL-6 (IL-23)	RORγ	IL-17A IL-17F IL-22	Protects against some fungal and bacterial infections Contributes to inflammation, autoimmunity
T_{REG}	TGF-β IL-2	FoxP3	IL-10 TGF-β	Inhibits inflammation
T_{FH}	IL-6 IL-21	Bcl-6	IL-4 IL-21	B cell help in follicles and germinal centers

EFFECTOR T HELPER CELL SUBSETS ARE DISTINGUISHED BY THREE PROPERTIES

Each helper T-cell subset is defined by an array of features

1. Each of the major T helper cell subsets is characterized by
   (1) a distinct set of *polarizing cytokines* that induce the expression of
   (2) a *master gene regulator* that regulates expression of
   (3) a signature set of *effector cytokines* the T-cell population produces once it is fully differentiated
2. Which effector subset an activated helper cell becomes depends on the quality and quantity of signals its naïve
3. cell precursor receives from APCs in a secondary lymphoid organ; that activity, in turn, depends on the nature of the pathogen the APC encountered at the site of infection.
4. Broadly speaking, T_H1 and T_H17 cells regulate cell mediated immunity ($CD8^+$ T cells and macrophages) and T_H2 and T_{FH} cells regulate humoral immunity (B cells). However, it is important to recognize that all $CD4^+$ effector T-cell subsets may have the potential to provide help to B cells.
5. T_H1 and T_H17 subsets generally encourage B cells to produce antibodies that contribute to cell-mediated immunity. T_H2 cells encourage B cells to produce antibodies that mediate the clearance of extracellular pathogens (e.g.isotypes like IgE that induce the release of molecules that harm extracellular parasites).

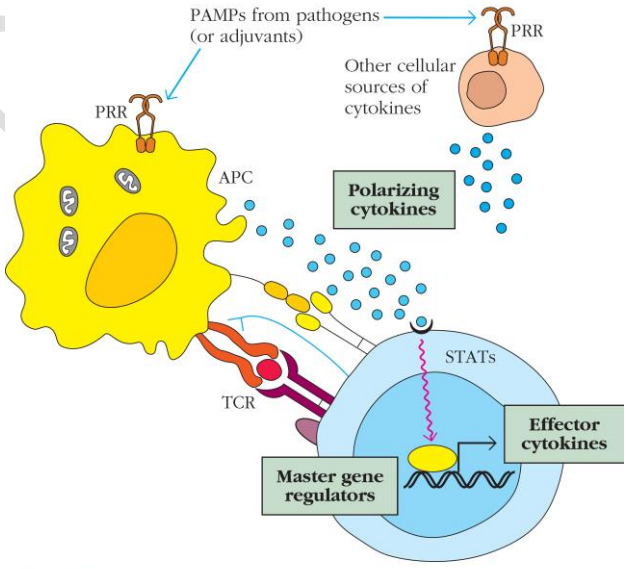


FIGURE 11-8 General events and factors that drive T_H subset polarization. Interaction of pathogen with pattern recognition receptors (PRRs) on dendritic cells and other neighboring immune cells determines which polarizing cytokines are produced and, hence, into which T helper subset a naïve cell will differentiate. In general, polarizing cytokines that arise from dendritic cells or other neighboring cells interact with cytokine receptors and generate signals that induce transcription of unique master gene regulators. These master regulators, in turn, regulate expression of various genes, including effector cytokines, which define the function of each subset.

6. Helper T-cell subsets often "cross-regulate" each other. The cytokines they secrete typically enhance their own differentiation and expansion and inhibit commitment to other helper T-cell lineages. This is particularly true of the T_H1 and T_H2 pair, as well as the T_H17 and T_{REG} pair.

7. Helper cell lineages may not be fixed; some subsets can assume the cytokine secretion profile of other subsets if
8. exposed to a different set of cytokines, particularly early in the differentiation process.
9. The precise biological function and sites of differentiation and activity of each subset continue to be actively investigated. Much remains unknown.

THE DIFFERENTIATION AND FUNCTION OF T_H1 AND T_H2 CELLS

A. The key polarizing cytokines that induce differentiation of naïve T cells into **T_H1 CELLS** are **IL-12**, **IL-18**, and **IFN-γ**.

1. **IL-12** is produced by dendritic cells after an encounter with pathogens via PRRs (e.g., TLR4, TLR3). It is also up-regulated in response to IFN-γ, which is generated by activated T cells and activated NK cells.
2. **IL-18**, which is also produced by dendritic cells, promotes proliferation of developing T_H1 cells and enhances their own production of IFN-γ. These polarizing cytokines trigger signaling pathways that up-regulate the expression of the ***master gene regulator T-Bet***. This master transcription factor induces commitment to the ***T_H1 lineage, inducing expression of the signature T_H1 effector cytokines, including IFN-γ and TNF***.
3. **IFN-γ** is a particularly potent effector cytokine. It activates ***macrophages***, stimulating these cells to increase microbicidal activity, ***up-regulate the level of class II MHC***, and, as mentioned above, secrete cytokines such as IL-12, which further enhance T_H1 differentiation.
    a. IFN-γ secretion also induces antibody class switching in B cells to IgG classes (such as IgG2a in the mouse) that support phagocytosis and fixation of complement.
    b. Finally, IFN-γ secretion promotes the differentiation of fully cytotoxic TC cells from $CD8^+$ precursors by activating the dendritic cells that engage naïve TC cells.
4. These combined effects make the T_H1 subset particularly suited to respond to viral infections and other intracellular pathogens. They also contribute to the pathological effects of T_H1 cells, which are also involved in the delayed type hypersensitivity response to poison ivy.

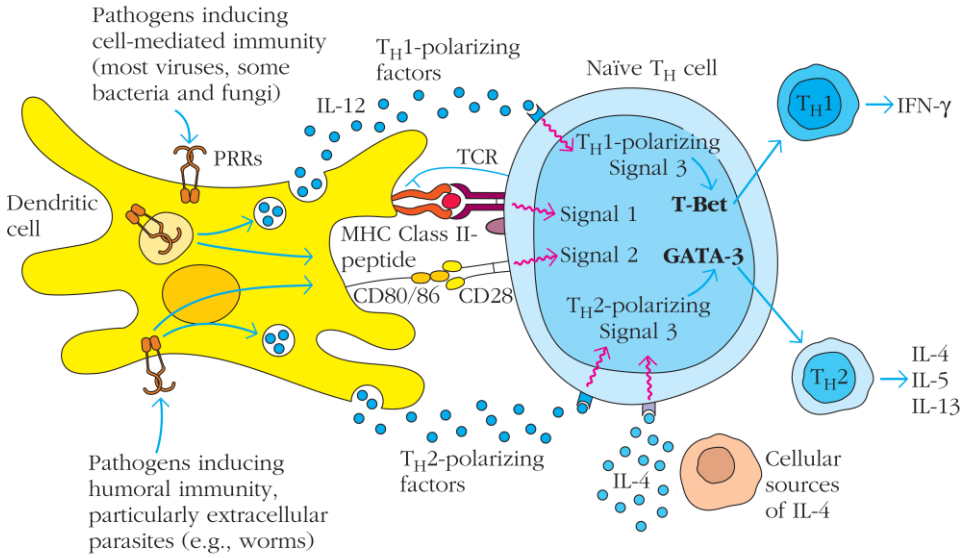


FIGURE 11-9 Regulation of T_H1 and T_H2 subset differentiation. This figure depicts some of the cellular events that drive T_H1 and T_H2 lineage commitment in more detail. Intracellular pathogens activate a cascade of signals that polarize cells to the T_H1 lineage. For example, viruses interact with PRRs (e.g., TLR-3) that induce dendritic cells to generate IL-12. This binds to receptors on naïve T cells, activating a signal transduction pathway mediated by STAT4 that induces expression of the transcription factor T-Bet. T-Bet, in turn, activates expression of effector cytokines, including IFN-γ, which define the T_H1 subset's functional capacities (and can also enhance T_H1 polarization). On the other hand, extracellular pathogens activate signal cascades that can polarize naïve T cells to the T_H2 lineage. Parasitic worms interact with PRRs on neighboring immune cells (such as mast cells, basophils, or germinal center B cells), triggering the release of the signature T_H2 polarizing cytokine IL-4. This interacts with receptors on T cells that activate STAT6, up-regulating expression of the transcriptional regulator GATA-3. GATA-3, in turn, induces expression of the T_H2 effector cytokines, including IL-4, IL-5, and IL-13. *[Adapted from M. L. Kapsenberg, 2003, Dendritic-cell control of pathogen-driven T-cell polarization,* Nature Reviews Immunology *3:984.]*

B. Differentiation to the T_H2 subset is promoted by a defining polarizing cytokine, **IL-4.**

1. Exposing naïve helper T cells to IL-4 at the beginning of an immune response causes them to differentiate into T_H2 cells. Interestingly, T_H2 development is greatly favored over T_H1 development. Even in the presence of IFN-γ and IL-12, naïve T cells will differentiate into T_H2 effectors if IL-4 is present.
2. IL-4 triggers a signaling pathway within the T cell that up-regulates the ***master gene regulator GATA3***, which, in turn, regulates expression of T_H2-specifi c cytokines, including **IL-4, IL-5,** and **IL-13**.
3. **Mast cells**, **basophils**, and **NKT** cells can be induced to make IL-4 after exposure to pathogens and could influence helper T cell fate in the periphery.
4. **Germinal-center B cells** and **T_{FH}** cells can also produce IL-4, which could influence helper T-cell polarization in the lymph nodes and spleen. And **T_H2 cells** themselves are an excellent source of additional IL-4 that can enhance polarization events.

8. T_H1 and T_H2 Cross-regulation

1. The major effector cytokines produced by T_H1 and T_H2 subsets (IFN-γ and IL-4, respectively) not only influence the overall immune response, but also influence the helper T cell subsets.
2. First, they promote the growth (and in some cases even the polarization) of the subset that produces them; second, they inhibit the development and activity of the opposite subset, an eff ect known as *cross-regulation*. ***For instance, IFN-γ (secreted by the T_H1 subset) inhibits proliferation of the T_H2 subset, and IL-4 (secreted by the T_H2 subset) downregulates the secretion of IL-12 by APCs, thereby inhibiting T_H1 differentiation.*** IL-4 enhances T_H2 cell development by making T_H cells less susceptible to the T_H1 promoting cytokine signals (and vice versa).
3. **IL-10** secreted by T_H2 cells also inhibits (cross-regulates) T_H1 cell development, but not directly. Instead, IL-10 acts on monocytes and macrophages, interfering with their ability to activate the T_H1 subset by abrogating their activation, specifi cally by
   a. inhibiting expression of class II MHC molecules,
   b. suppressing production of bactericidal metabolites (e.g., nitric oxide) and various inflammatory mediators (e.g., IL-1**, IL-6, IL-8, GM-CSF, G-CSF, and TNF-α),** and even by
   c. inducing apoptosis.
4. The master regulators ***T-Bet*** and ***GATA-3*** also play an important role in cross-regulation. Specifically, the expression of **T-Bet** drives cells to differentiate into T_H1 cells and suppresses their differentiation along the T_H2

pathway. Expression of **GATA-3** does the opposite, promoting the development of naïve T cells into T_H2 cells while suppressing their differentiation into T_H1 cells. Consequently, cytokine signals that induce one of these transcription factors sets in motion a chain of events that represses the other.

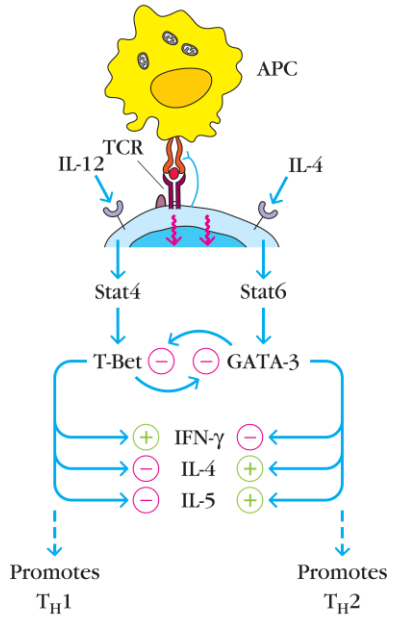


FIGURE 11-10 Cross-regulation of T helper cell subsets by transcriptional regulators. GATA-3 and T-Bet reciprocally regulate differentiation of T_H1 and T_H2 lineages. IL-12 promotes the expression of the T_H1-defining transcription factor, T-Bet, which induces expression of T_H1 effector cytokines, including IFN-γ. At the same time, T-Bet represses the expression of the T_H2 defining master transcriptional regulator, GATA-3, as well as expression of the effector cytokines IL-4 and IL-5. Reciprocally, IL-4 promotes expression of GATA-3, which up-regulates the synthesis of IL-4 and IL-5, and at the same time represses the expression of T-Bet and the T_H1 effector cytokine IFN-γ. *[Adapted from J. Rengarajan, S. Szabo, and L. Glimcher, 2000, Transcriptional regulation of Th1/Th2 polarization*, Immunology Today 21:479.*]*

9. T_H17 Cells

1. T_H17 cells are generated when naïve ***T cells are activated in the presence of IL-6 and TGF-β,*** the key polarizing cytokine for iT_{REG} differentiation.
2. IL-23 also plays a role in finalizing the commitment to the T_H17 fate and is induced in APCs by interactions with PAMPs including fungal wall components, with TLR2 and the CLR Dectin-1.
3. Like T_H1 and T_H2 differentiation, T_H17 cell differentiation is also controlled by a ***master transcriptional regulator*** whose expression is induced by polarizing cytokines. In this case the master regulator is the ***orphan steroid receptor RORγt***, which also plays a role in T-cell development.
4. T_H17 cells are so named because they produce ***IL-17A, a cytokine associated with chronic inflammatory and autoimmune responses,*** including those that result in inflammatory bowel disease, arthritis, and multiple sclerosis. T_H17 cells are the dominant inflammatory cell type associated with chronic autoimmune disorders. They also produce **IL-17F** and **IL-22**, cytokines associated with tissue inflammation.

1O. (Induced)T_{REG} Cells

1. Another major $CD4^+$ T-cell subset negatively regulates T-cell responses and plays a critically important role in peripheral tolerance by limiting autoimmune T-cell activity.This subset of T cells, designated ***induced T_{REG}* (iT$_{REG}$) cells,** is similar in function to the *n*atural T_{REG} cells (nT_{REGs}) that originate from the thymus.
2. Induced T_{REG} cells, however, do not arise in the thymus, but from naïve T cells that are activated in secondary lymphoid tissue in the presence of TGF-β.
3. TGF β induces expression of ***FoxP3***, the ***master transcriptional regulator responsible*** for iT_{REG} commitment.
4. The iT_{REG} cells secrete the effector cytokines **IL-10** and **TGF-β**, which down-regulate inflammation via their inhibitory effects on APCs, and can also exert their suppressive function by interacting directly with T cells.
5. The depletion of iTREG cells in otherwise healthy animals leads to **multiple autoimmune** outbreaks, revealing that even healthy organisms are continually warding off autoimmune responses.
6. Recent data also indicate that ***iTREG cells are critically important for maintaining a mother's tolerance to her fetus.***

11. T_H17 and T_{REG} Cross-Regulation

1. **T_{REG}** and **T_H17** cells also cross-regulate each other. TGF-β induces T_{REG} differentiation; however, when accompanied by **IL-6, TGF-β** induces T_H17 differentiation. Specifically TGF-β appears to up-regulate both **FoxP3** and **RORγ** (which control T_{REG} and T_H17 differentiation, respectively).
2. In combination with signals generated by IL-6,signals generated by ***TGF-β inhibit FoxP3 expression***, letting RORγ dominate and induce T_H17 development.
3. The T_H17 versus iT_{REG} relationship may be very adaptive. At rest, a healthy organism may favor the development of an anti-inflammatory iT_{REG} population, which would be reinforced by the iT_{REG} cell's own production of TGF-β.
4. Inflammation, however, would induce the generation of acute response proteins, including IL-6. In the presence of **IL-6, TGF-β activity** would shift development of T cells away **from iTREGs toward the pro-inflammatory T_H17**, so a proper defense could be mounted.

12. T_{FH} Cells

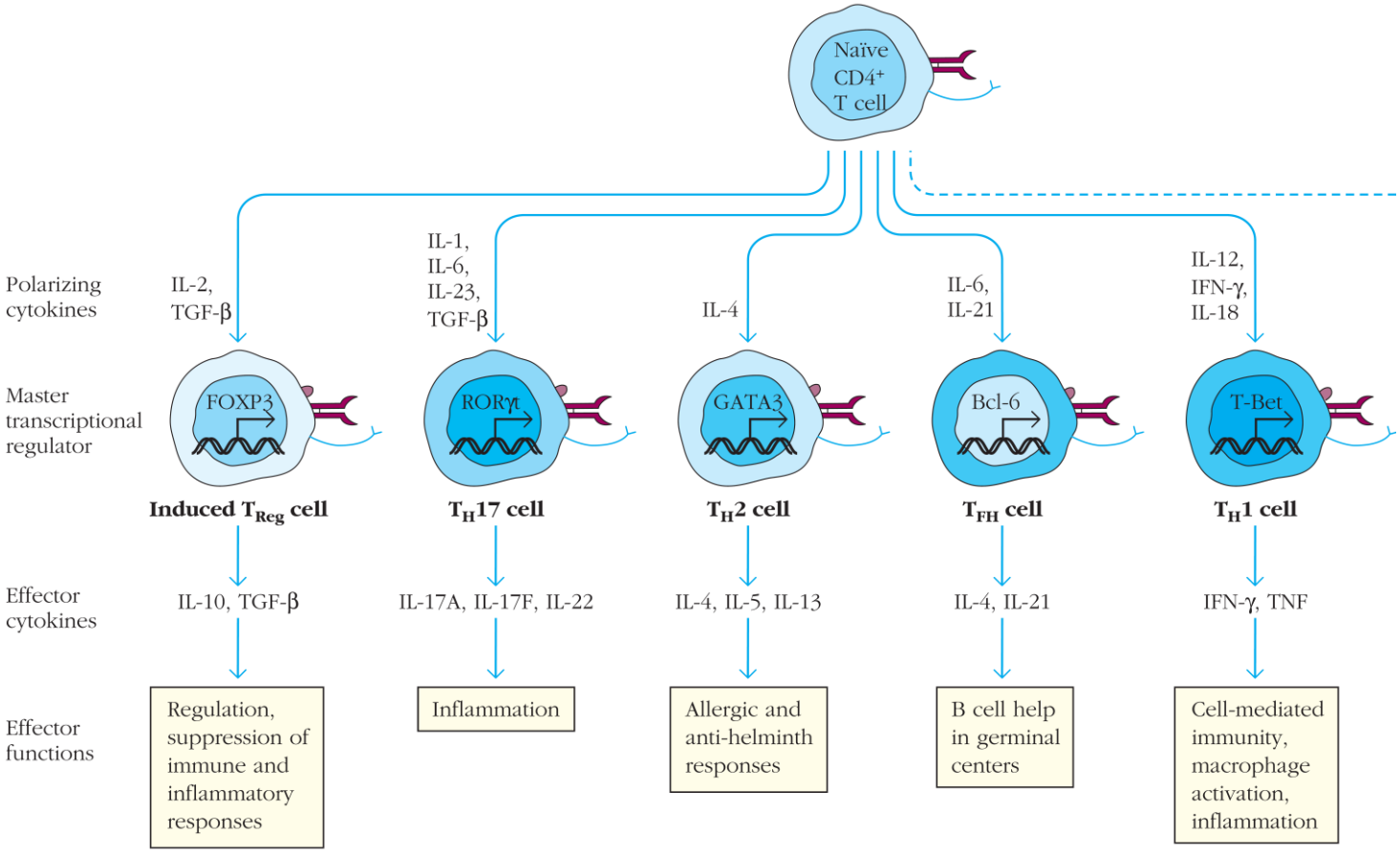
1. **Follicular helper T (T_{FH}) cells** are a very recent addition to the helper T-cell subset family. T_{FH} cells play a central role in mediating B-cell help and are found in B-cell follicles and germinal centers.
2. Cytokines that polarize activated T cells toward the T_{FH} lineage include **IL-6** and **IL-21**. These polarizing cytokines induce the expression of **Bcl-6**, a transcriptional repressor that is thought to be T_{FH}'s master transcriptional regulator.
3. Cross-regulation is also a feature of TFH function: Bcl-6 expression inhibits **T-bet**,
4. **GATA3**, and **RORγt** expression, thus inhibiting T_H1, T_H2, and T_H17 differentiation, respectively, while inducing T_{FH} polarization. Although both T_{FH} and T_H2 cells secrete IL-4, T_{FH} cells are best characterized by their secretion of IL-21, which induces B-cell differentiation.

5. Interestingly, they can also produce IFN-γ (the defining T_H1 cytokine). How **T_{FH}** and **T_H2** cells divide responsibilities for inducing B-cell antibody production is still an open question.

13. Other Potential Helper T-Cell Subsets

1. Other T-cell subsets with distinct polarizing requirements and unique cytokine secretion profiles have been identified (e.g.,**T_H9** cells, which secrete IL-9 and IL-10).
2. However, because these subpopulations secrete cytokines that are also produced by T_H1, T_H2, T_H17, or iT_{REG} cells, some speculate that these cell types do not represent distinct subclasses but rather are developmental or functional variants of one of the major subpopulations.
3. This perspective has, indeed, been applied to the follicular helper T-cell (T_{FH}) subset, which also expresses cytokines shared by several other subtypes. However, this subset has a distinct gene signature and a distinct ***master regulator (bcl-6)***, so most now consider it a bona fi de independent lineage.

T Helper Subset Differentiation



This figure synthesizes current information about the distinguishing features of T helper subset differentiation and activity. Polarizing cytokines, master transcriptional regulators, effector cytokines, and broad functions in health and disease are depicted for each of the major helper subsets. Neither cross-regulation nor the potential plasticity in differentiation among subsets is depicted, but both are described in the text. *[Adapted from S. L. Swain, K. K. McKinstry, and T. M. Strutt, Expanding roles for CD41 T cells in immunity to viruses,* Nature Reviews. Immunology 12:*136–148.]*

13. T CELL MEMORY

1. A small proportion (<10%) of the progeny of a naïve cell that has proliferated robustly in response to antigen differentiates into T_{CM} and T_{EM} cells.
2. In general, **T_{CM} cells** reside in and travel between secondary lymphoid tissues. They live longer and have the capacity to undergo more divisions than their T_{EM} counterparts.When they reencounter their cognate

pathogen in secondary lymphoid tissue, they are rapidly activated and have the capacity to differentiate into a variety of effector T-cell subtypes, depending on the cytokine environment.

3. On the other hand, **T_{EM}** cells travel to and between tertiary tissues (including skin, lung, liver, and intestine). They are arguably better situated to contribute to the first line of defense against reinfection because they have already committed to an effector lineage during the primary response and exhibit their effector function course of an immune response (e.g., within 3 days), but their cell of origin remains controversial. Some investigations suggest that memory cells arise as soon as naïve T cells are activated.

TABLE 11-4 Surface proteins that are used to distinguish naïve, effector, and memory T cells

Cell type	CD44	CD62L	CCR7
Naïve T cell	low	+	+
Effector T cell	+	low	–
Effector memory T cell	+	variable	–
Central memory T cell	+	+	+

4. Others suggest that memory cells arise from more fully differentiated naïve T cells. Still others raise the intriguing possibility that naïve T-cell activation generates a "memory stem cell" that is self-renewing and gives rise to memory effector cell populations. These models are not mutually exclusive, and it is possible that memory cells can arise at several different stages of T-cell activation throughout a primary response.
5. The relationship between **T_{CM}** and **T_{EM}** cells is also debated. They may originate independently from naïve and effector cells, respectively, or may give rise to each other.
6. Studies suggest, in fact, that **T_{CM} cells** arise from **T_{EM} cells**, and one possible model of relationships is shown in Figure 11-13.

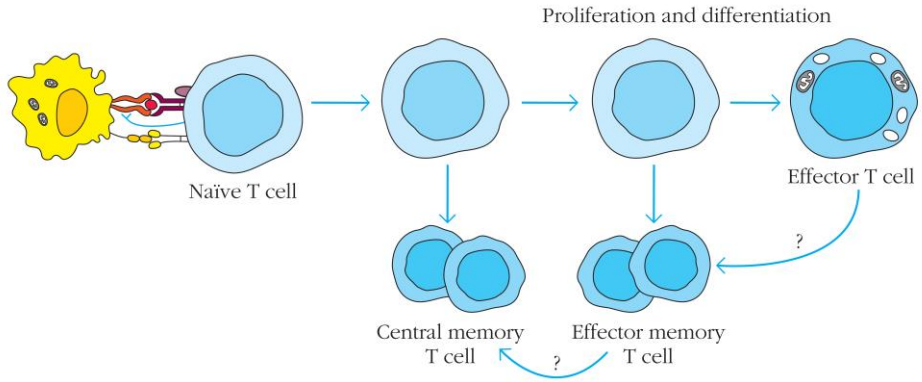


FIGURE 11-13 One possible model for the development of central and effector memory T cells. This model, only one of several that have been advanced, suggests that central memory cells arise early after naïve T-cell activation, perhaps from the first divisions. Effector memory T cells may arise later, after the progeny have divided more and have assumed at least some effector cell features. The model also includes the possibilities that (1) some effector memory T cells arise from fully differentiated effector T cells and (2) effector memory T cells can develop into central memory T cells. [*Adapted from D. Gray, 2002, A role for antigen in the maintenance of immunological memory,* Nature Reviews Immunology 2:60.]

7. Here, investigators speculate that T_{CM} cells arise prior to T_{EM} cells, from cells at an earlier stage of differentiation into effector (helper or cytotoxic) T cells. T_{EM} cells arise late, and also may develop from fully differentiated effector cells.
8. The model also suggests that effector cells can replenish central memory cells. Memory $CD8^+$T cells are clearly more prevalent than memory $CD4^+$T cells. This is partly because $CD8^+$T cells proliferate more robustly and therefore generate proportionately more memory T cells.
9. It may also be due to differences in the life span of memory T cells: $CD4^+$ memory T cells may not be as long-lived as $CD8^+$ memory T cells. Both **IL-7** and **IL-15** appear important in enhancing homeostatic proliferation, but $CD4^+$ and $CD8^+$ memory T-cell requirements may differ.

14. TCR GENES

Functional TCR α and β chain genes as well as γ- and δ- chain genes are expressed as polypeptides only in cells of T-lymphocyte lineage. The functional TCR genes of α, β or γ and δ are formed by somatic rearrangement of germ line DNA sequences, a process that is similar to immunoglobulin gene rearrangement.

1. The **α chain** can be compared to a **light chain of immunoglobulin** as it is coded by **V, J and C segments**.
2. The **β chain** can be compared to a **heavy chain** as it is coded, by **V, D, J and C gene segments**.
3. Similarly the **γ chain** is encoded by the **V and J and C** segments while
4. the **δ chain** is encoded by **V, D, J and C** segments.

HUMAN TCR α -GENE LOCUS

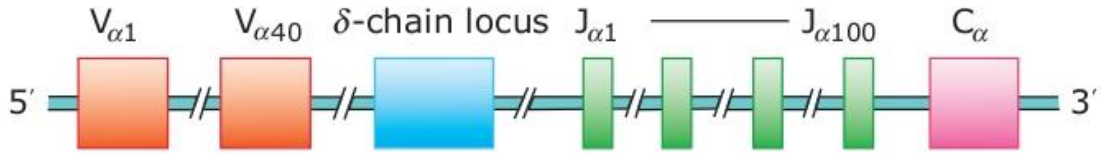
The chief features of the human TCR α-gene locus are as follows.

1. It is located on chromosome 14.
2. There are about 40 Vα gene segments located 5′ of about 75–100 Jα gene segment.
3. There is only one Cα gene segment that has four exons of which the last one is entirely non-coding.

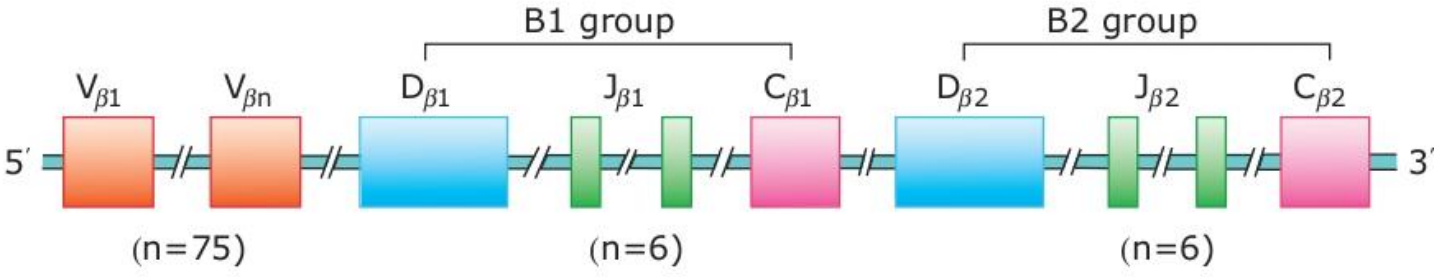
HUMAN TCR β -GENE LOCUS

The chief features of the human TCR β-gene locus are as follows. •

1. It is located on chromosome 7. •
2. There are about 57 Vβ gene segments. D, J and C segments are present in two sets **B1** and **B2** as in mouse TCR β-gene locus.
   a. The **B1** segment comprises a single Dβ1 segment. Downstream of this region is located the 6 Jβ1 segment followed by a single Cβ1 segment. The **B2** segment has a single Dβ2 segment, 6 Jβ2 segments and a single Cβ2 segment. The whole B2 unit is located downstream of B1 unit.
3. The Vβ segments are shared between B1 and B2 gene segments.



Human TCR α-gene locus



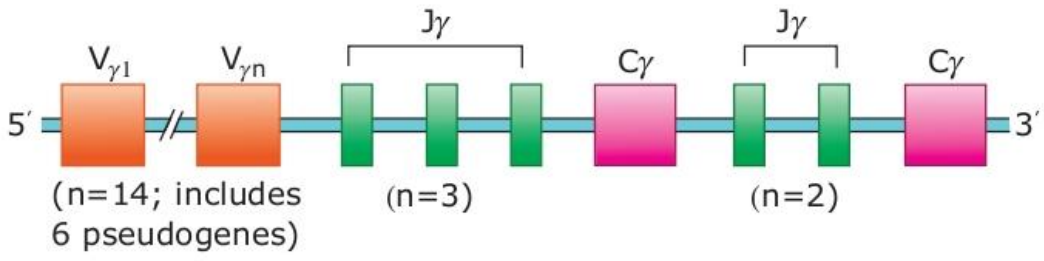
Human TCR β-gene locus

HUMAN TCR γ -GENE LOCUS

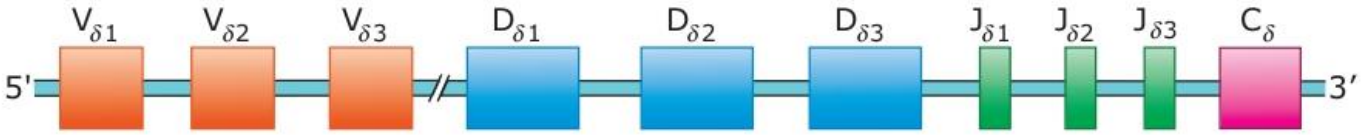
1. It is located on chromosome 7 in humans.
2. There are 14 Vγ gene segments (including six non-functional pseudogenes).
3. There are five Jγ gene segments and two Cγ gene segments. They are arranged as Vγ –3Jγ –Cγ– 2Jγ – Cγ – 2JC clusters.

HUMAN TCR δ -GENE LOCUS

1. The δ-chain gene locus is located on chromosome 14 between Vα and Jα segments.
2. There are about three Vδ gene segments and three, Dδ and three Jδ segments.
3. It contains only one Cδ gene segment.



Human γ-chain locus



Human δ-chain locus

Gene	Chromosome	Gene Segment			
		V	D	J	C
α	14	40		75–100	1
β	7	57	2	13	2
γ*	7	14•		5	2
δ	1 4	3	3	3	1

*D, J, C segments of β chain genes are present in two sets. There are 6 non-functional pseudogenes.

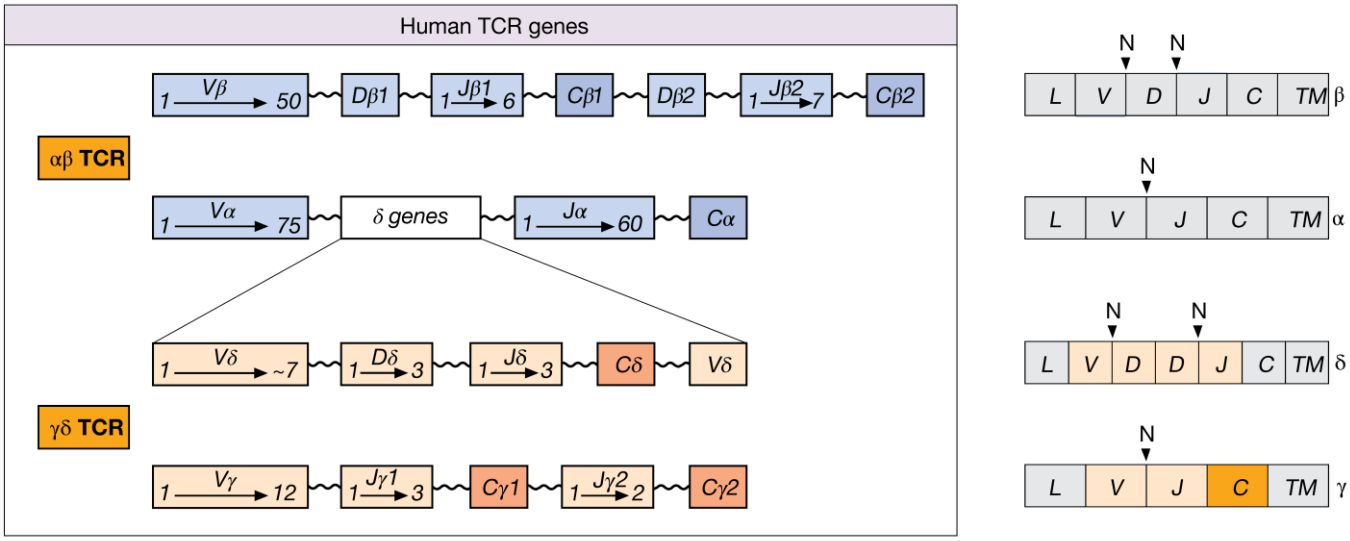
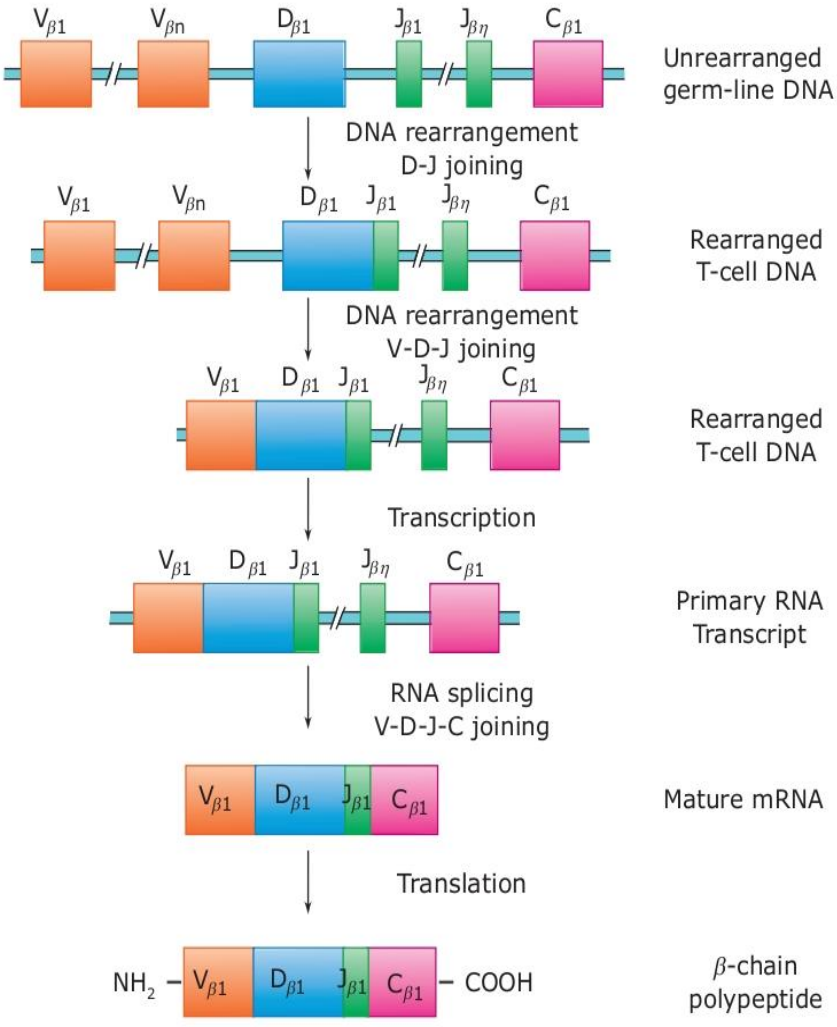


Figure 4.9 Genes encoding αβ and γδ T-cell receptors (TCRs). Genes encoding the δ chains lie between the *Vα* and *Jα* clusters and some *V* segments in this region can be used in either δ or α chains (i.e., as either *Vα* or *Vδ*). TCR genes rearrange in a manner analogous to that seen with immunoglobulin genes, including N-region diversity at the *V(D)J* junctions. One of the *Vδ* genes is found downstream (3′) of the *Cδ* gene and rearranges by an inversional mechanism.

15. GENE REARRANGEMENT TO FORM MATURE TCR β GENE

1. The β-chain genes are rearranged prior to α-chain genes.
2. The functional, rearranged β chain gene is needed for signalling the subsequent maturation of T cells.
3. First, one **Dβ segment joins** one **Jβ segment** then **Vβ adds** to the **DβJβ complex** forming the **VβDβJβ** rearranged gene. The Vβ and Dβ segments located between the rearranged Vβ and Dβ gene segments are deleted and so are extra the Jβ located 5′ to the rearranged Jβ gene when VβDβ joins the Jβ gene.
4. The rearranged gene with the VβDβJβ complex, along with Jβ and Cβ segments present on the 3′ side, is transcribed to give a primary RNA transcript for the TCR β chain. The β chain promoters have been identified in the 5′ flanking regions the of V genes.

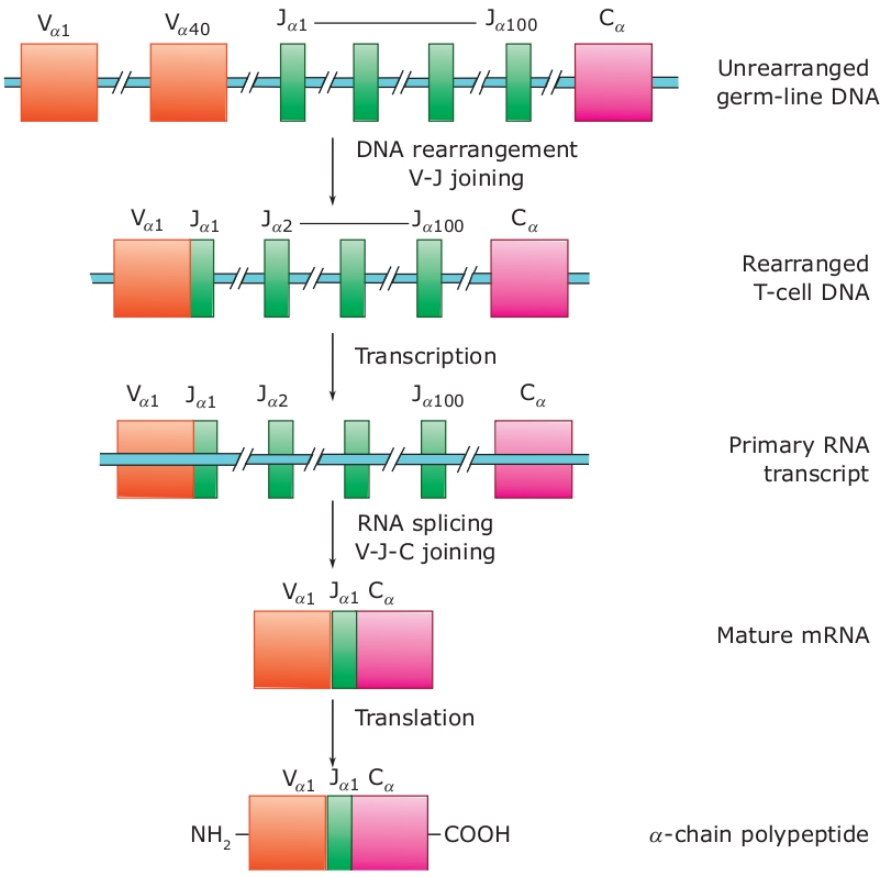
SPLICING AND MATURATION OF PRIMARY TRANSCRIPT



1. The primary nuclear transcript of the β chain of the TCR contains the rearranged VβDβJβ gene complex followed by the Jβ segments (if any) on the 3′ side, intron and Cβ genes.
2. The genomic sequences between the VDJ complex and the Cβ gene are then spliced out to form mature mRNA having VDJCβ segments.
3. The rearrangement uses the Cβ1 gene segment; if a non-productive gene rearrangement occurs, a subsequent rearrangement involving Cβ2 can occur.
4. The use of Cβ1 or Cβ2 is completely random and there are no reports that a T cell can ever switch from one C gene to other.
5. A functional β chain is formed prior to the formation of an α chain.
6. The newly formed β chain pairs with a molecule called pre-T α chain (pTα); pTαβ heterodimers are expressed on the surface of the thymocytes in association with CD3 proteins.
7. The pTαβ receptor (pre-TCR) is unable to recognize and bind any antigen (antigen–MHC) but is supposedly involved in initiating intracellular events which lead to β chain allelic exclusion (discussed later).
8. The gene arrangements and RNA processing that occur during the synthesis of a human β chain is shown in Figure.

16. GENE REARRANGEMENT TO FORM MATURE TCR αGENE

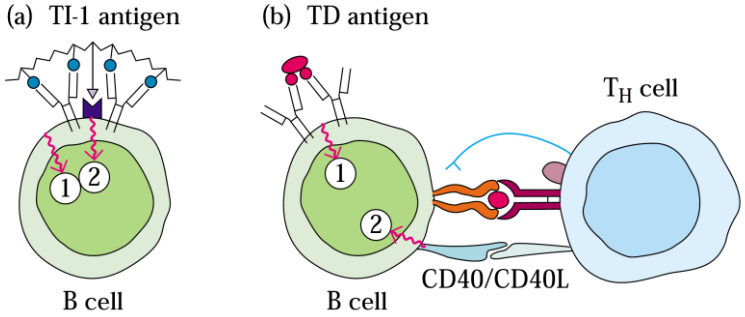
1. The rearrangement of α-chain genes occurs as follows.
2. One of the consequences of the expression of pre-TCR is that it signals the cells to start dividing or proliferating. Once this proliferative phase of pTαβ cells is over, α-chain gene rearrangement starts.
3. Once α-chain gene rearrangement starts, it proceeds for four days. It is not clear whether pre-TCR signal contributes to gene rearrangements at the α-chain locus.
4. The α-chain gene rearrangements consists of the joining of **one Vα gene** segment and **one Jα gene** segment. All the Vα and Jα segments between the rearranged Vα and Jα segments are deleted. Th e presence of a large number of Jα segments allows several attempts to produce a productive VαJα segment, thereby increasing the chances that a functional α chain is formed.



5. Once the VJ joining has occurred, α chain genes are transcribed.
6. The primary RNA transcript contains VJ segment + unrearranged Jα segments + intron + one Cα segment.
7. Since there is only one Cα gene, the RNA processing of a primary transcript gives rise to only one possible complete α chain mRNA, which is translated to give a mature α chain.
8. These α and β chains of the TCR which are translated and transferred to the endoplasmic reticulum are packaged and sent to the cell surface plasma membrane where the α-β heterodimer is expressed in membrane-bound form. Only membranebound TCRs are produced.

17. THYMUS INDEPRMDENT ANTIGENS AND THYMUS DEPENDENT

Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon TH cells, the other not. The B-cell response to **thymus-dependent (TD) antigens** requires direct contact with TH cells, not simply exposure to TH-derived cytokines.



Thymus independent antigens

1. Antigens that can activate B cells in the absence of this kind of direct participation by TH cells are known as **thymus-independent (TI) antigens.**
2. **TI antigens are divided into types 1 and** 2, and they activate B cells by different mechanisms.
   a. Some bacterial cell-wall components, including lipopolysaccharide (LPS), function as *type 1 thymus-independent (TI-1) antigens.*
   b. *Type 2 thymus-independent (TI-2) antigens* are highly repetitious molecules such as polymeric proteins (e.g.,bacterial flagellin) or bacterial cell-wall polysaccharides with repeating polysaccharide units.TI-2 antigens activate B cells by extensively crosslinking the mIg receptor.

Difference between *type 1 thymus-independent (TI-1) antigens* and *Type 2 thymus-independent (TI-2) antigens*

However, TI-2 antigens differ from TI-1 antigens in three important respects.

1. First, they are not B-cell mitogens and so do not act as polyclonal activators.
2. Second,TI-1 antigens will activate both mature and immature B cells,but TI-2 antigens activate mature B cells and inactivate immature B cells.
3. Third, although the B-cell response to TI-2 antigens does not require direct involvement of TH cells, cytokines derived from TH cells are required for efficient B-cell proliferation and for class switching to isotypes other than IgM.

Thymus-dependent (TD) antigens

(a) Antigen crosslinks mIg, generating signal ①, which leads to increased expression of class II MHC and co-stimulatory B7. Antigen–antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide–MHC complexes.

(b) T_H cell recognizes antigen–class II MHC on B-cell membrane. This plus co-stimulatory signal activates T_H cell.

(c) 1. T_H cell begins to express CD40L.
2. Interaction of CD40 and CD40L provides signal ②.
3. B7-CD28 interactions provide co-stimulation to the T_H cell.

(d) 1. B cell begins to express receptors for various cytokines.
2. Binding of cytokines released from T_H cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.