

Lecture 11

T-cell Polarization & Cytokine Signaling

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What controls T-cell maturation and activity?

- Antigen Presenting Cells (APCs), which present peptide fragments in MHC I or MHC II.
- Co-receptors (e.g., CD28, CD84 & CD86)
- Cytokines (and chemokines).

What are cytokines & chemokines?

- Small (10-30 kDa), usually secreted and usually glycosylated peptides.
- They bind specific, high affinity (e.g., K_d of 10^{-10} - 10^{-12} M) receptors found on target cells.
- Expression of cytokines and their receptors is usually tightly regulated (i.e., temporally/ transiently and geographically).
- Cytokine receptors define the specific type of biological response cytokines stimulate.
- Other more anachronistic terms include monokines and lymphokines. The term interleukin (IL) is now commonly used (e.g., IL-1, IL-2, ...).

What do cytokines, chemokines and growth factors do?

- They direct the development, maturation, localization, interactions, activation and life span of immune cells.
- Thus they play an essential role in regulating both immunity adaptive and innate.

Cytokines & Chemokines can be grouped into families (providing insight into what they do)

- Cytokines can be divided into six functionally distinct groups.
- There are significant functional similarities within each receptor family. The same is true for corresponding ligands.
- There are important functional differences between receptor families.

Six Functional Cytokine Groups*

- Growth Factors (e.g., CSF-1, SCF, RANKL, Flt3L)
- IL-1 Family (e.g., IL-1, IL-18 & "Toll-like")
- TNF Family (e.g., TNF- α , CD40L, FasL, LT- α , LT- β , BAFF)
- TGF- β Family (e.g., TGF- β)
- Chemokines (e.g., CC and CXC families -->more later)
- Type I & II Cytokines (4 Helix Bundle Cytokines; e.g., IL-2, IL-4, IL-6, IL-10, IL-12, IL-23, GM-CSF, IFN- γ , IFN- α/β)
- Also steroid hormones, prostaglandins and IL-17

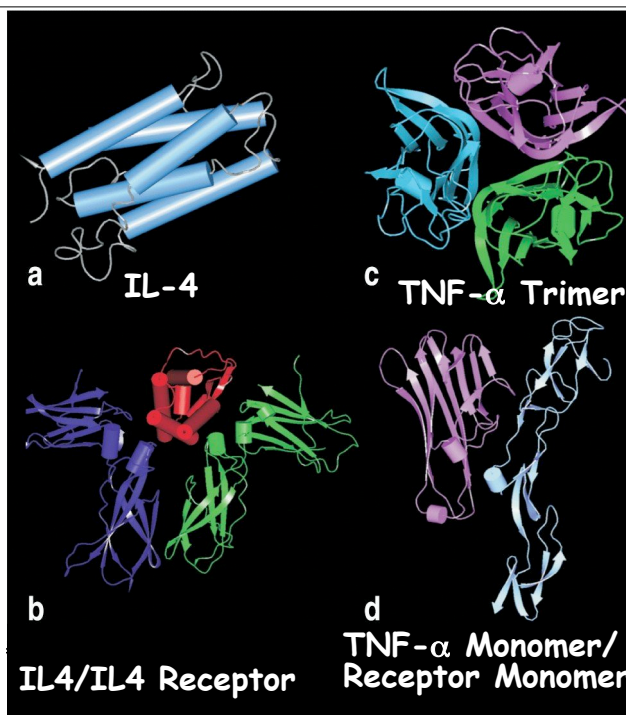
*Underlined cytokines are of particular importance

Cytokine Receptor Classes (by the book)

Table 11-2. Signal Transduction Mechanisms of Cytokine Receptors

Signal transduction pathway	Cytokine receptors using this pathway	Signaling mechanism
JAK/STAT pathway	Type I and type II cytokine receptors	JAK-mediated phosphorylation and activation of STAT transcription factors (see Box 11-2)
TNF receptor signaling by TRAFs	TNF receptor family: TNF-RII, CD40	Binding of adapter proteins, activation of transcription factors (see Box 11-1)
TNF receptor signaling by death domains	TNF receptor family: TNF-RI, Fas	Binding of adapter proteins, caspase activation (see Box 11-1)
Receptor-associated tyrosine kinases	M-CSF receptor, stem cell factor receptor	Intrinsic tyrosine kinase activity in receptor
G protein signaling	Chemokine receptors	GTP exchange and dissociation of $G\alpha \cdot GTP$ from $G\beta\gamma$, $G\alpha \cdot GTP$ activates various cellular enzymes

Consistent with their significant functional differences both IL-4 & TNF- α , and their corresponding receptors, are structurally very distinct.



Each class stimulates a biological response in target cells through a distinct pathway

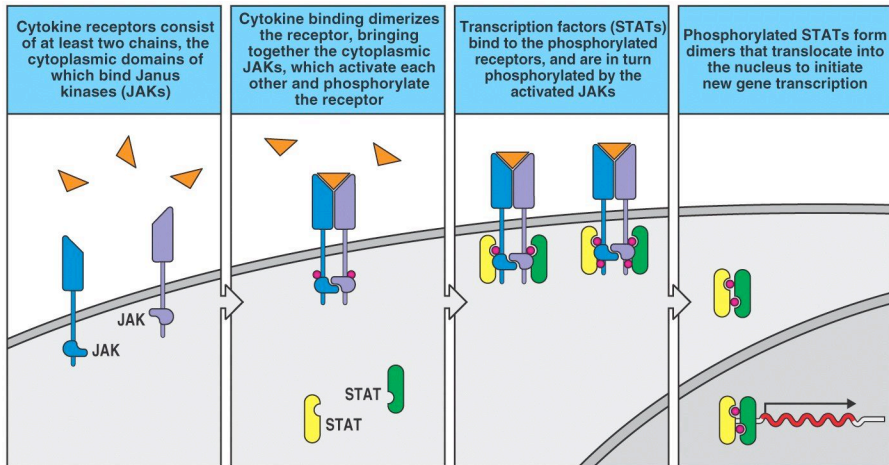


Figure 6-23 Immunobiology, 6/e. (© Garland Science 2005)

This diagram shows how Type I and II cytokines signaling the JAK-STAT pathway. Most of this signal culminate in the expression of new genes.

Localized release of IL-4 in the cleft between T cell and APC ("The immunological synapse")

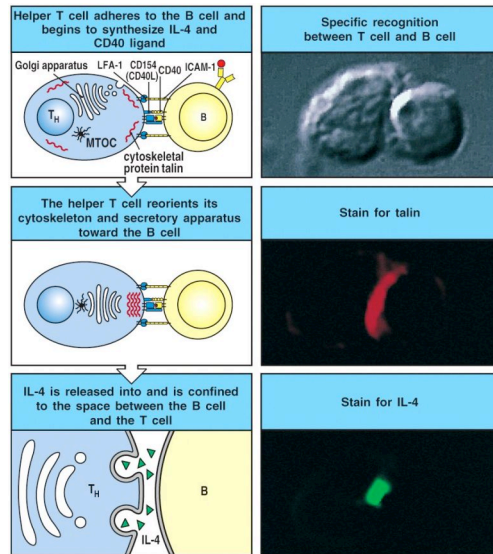
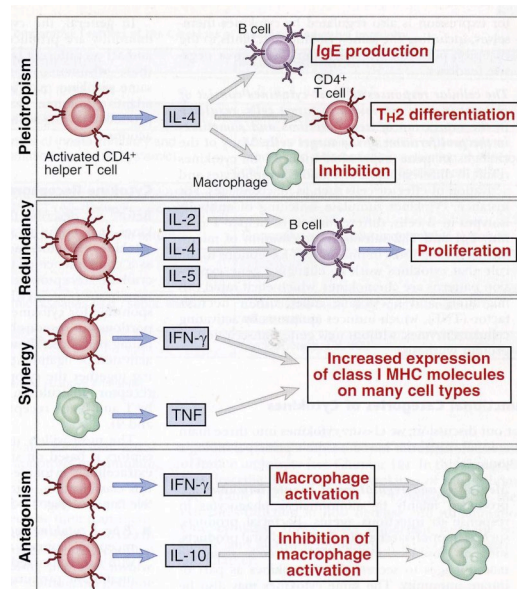


Figure 9-6 Immunobiology, 6/e. (© Garland Science 2005)

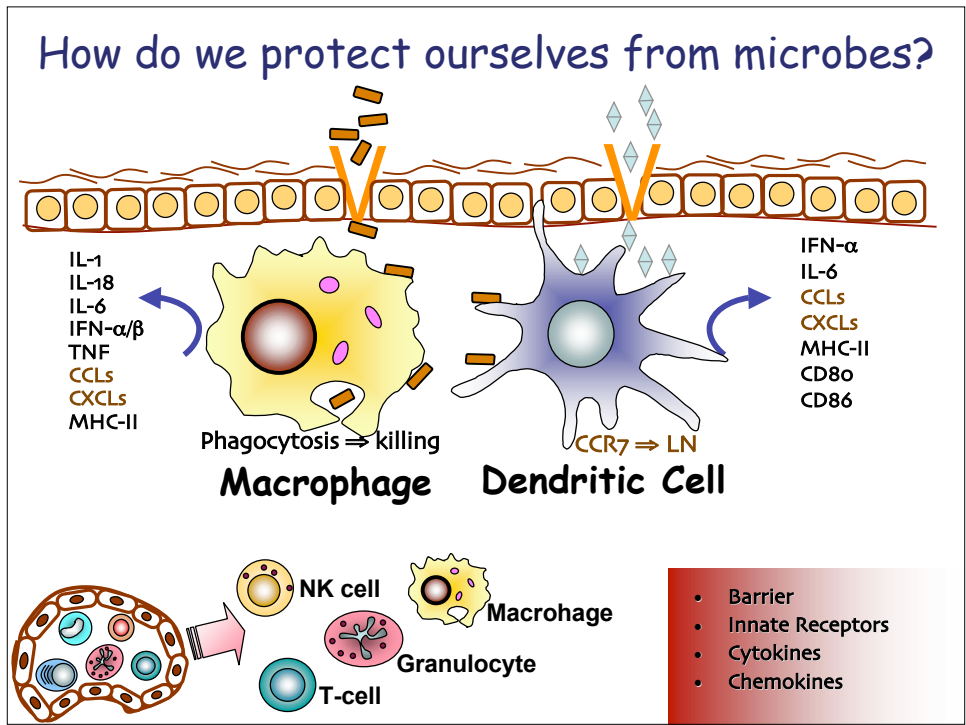
Important general properties of Cytokines and Chemokines

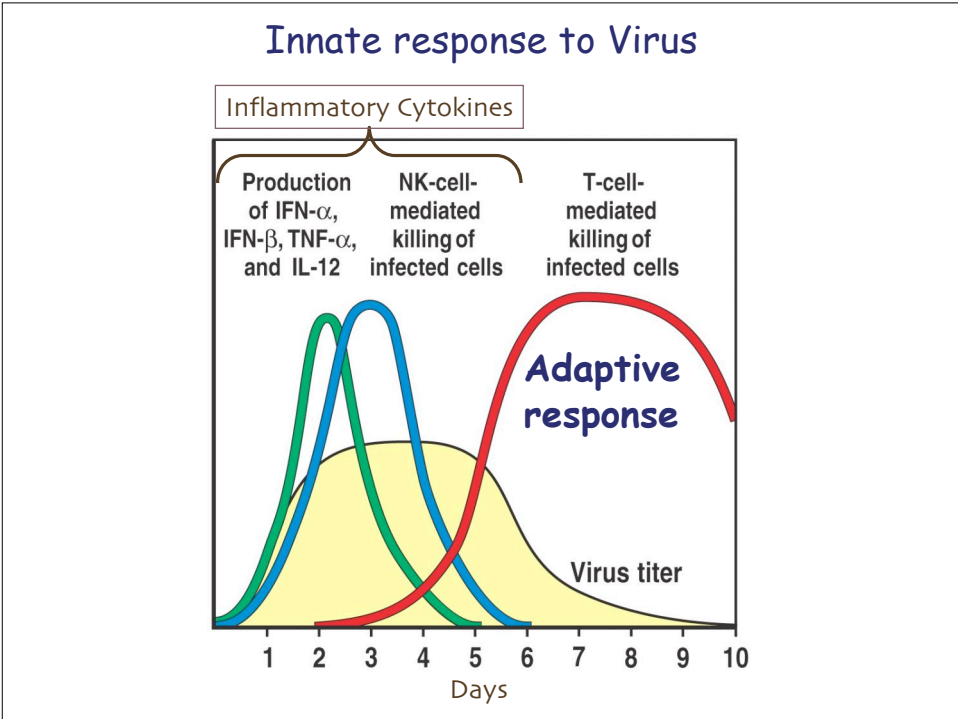
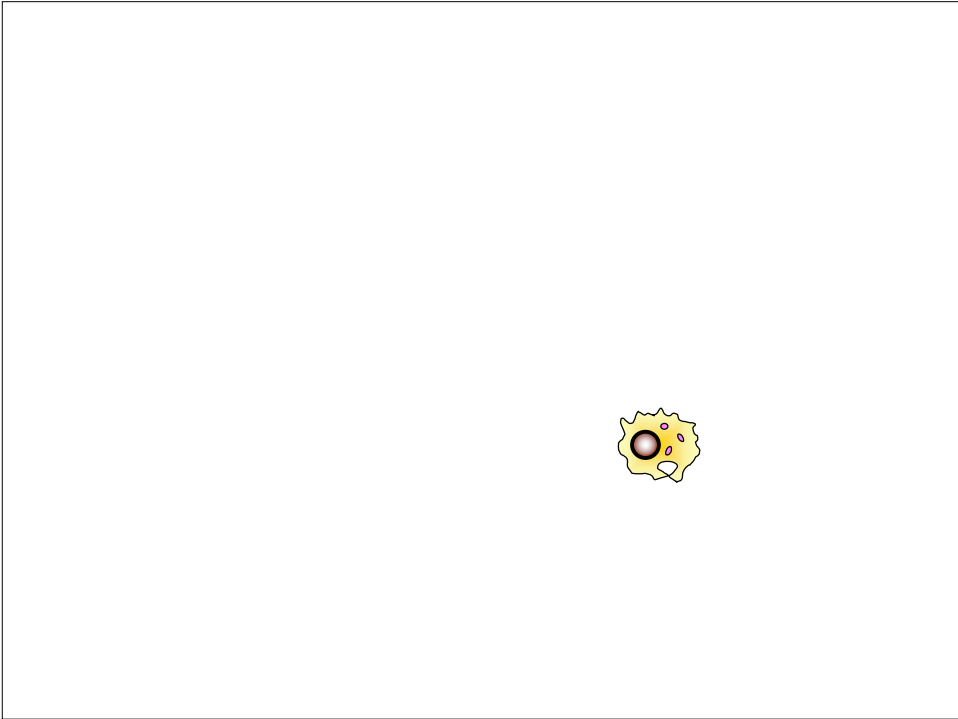
- Usually stimulate transient responses.
- Function at three ranges:
 - Autocrine - "self"
 - Paracrine - adjacent cells
 - Endocrine - through circulatory system (e.g., septic shock: IL-1 and TNF)
- **Pleitropism** - one ligand activate numerous types of responses (e.g., differentiation, growth & activation).
- **Redundancy** - two or more ligands exhibit functional overlap.
- **Synergy** - two or more ligands synergize to mount a single response.
- **Antagonism** - two or more cytokines mediating opposite responses to either limit a response or achieve balance (e.g. Feedback loops).

Properties of Cytokines



Some Biology





Cytokines and the evolving Th1-Th2 paradigm

Dendritic Cells pick up antigen, get activated and migrate to lymph nodes

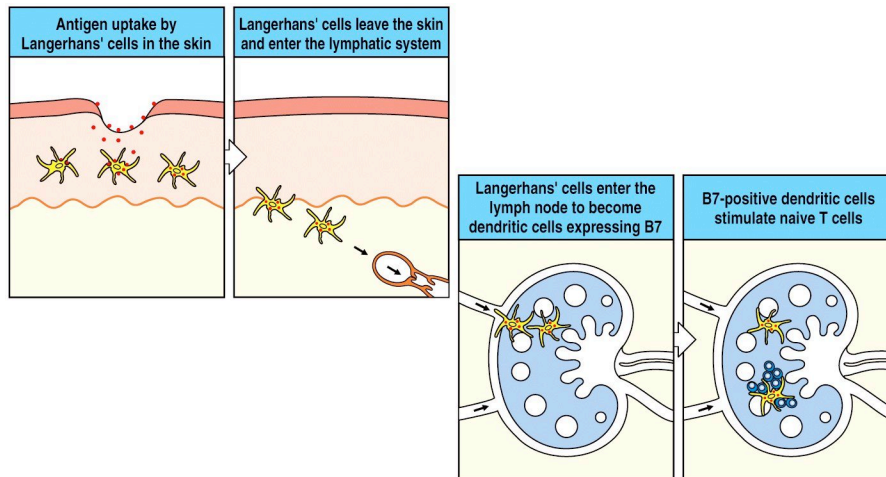


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Naïve CD4 cells kiss APCs to sample them for right antigen ($1 \times 10^4 - 10^6$)

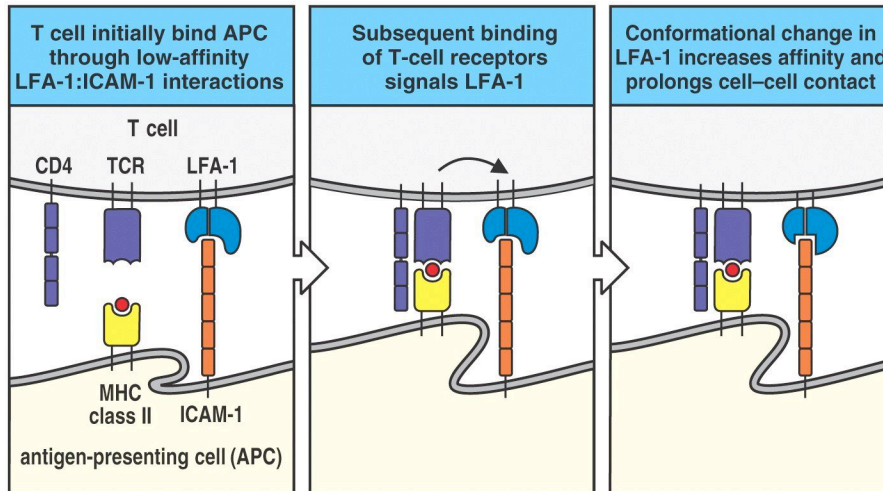


Figure 8-9 Immunobiology, 6/e. (© Garland Science 2005)

Signal #1 (TCR) and Signal #2 (co-receptors) direct activation of naïve T-cells.

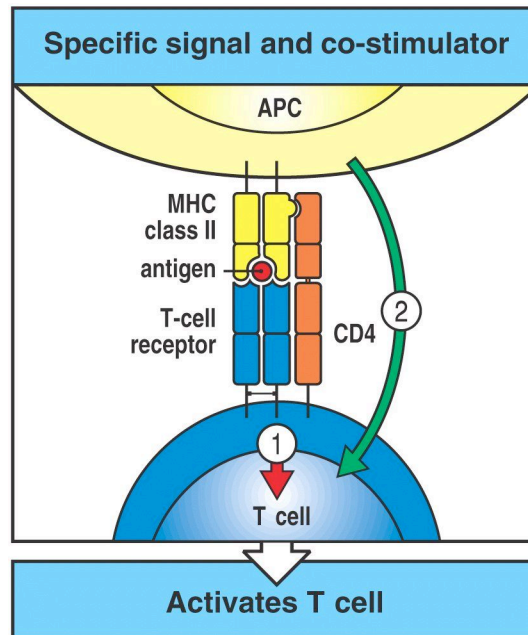
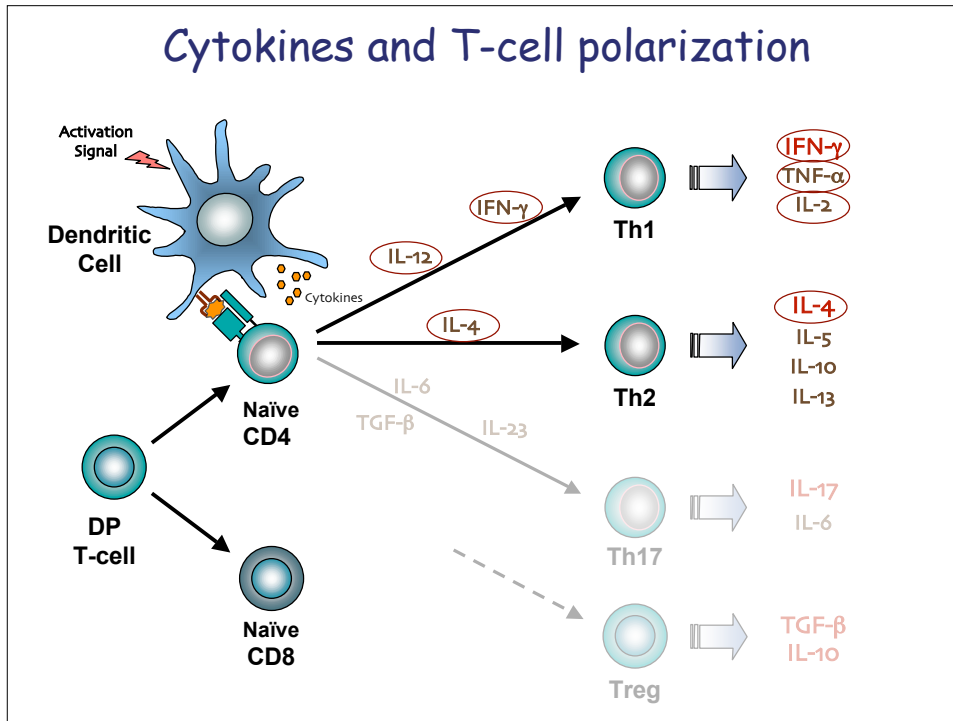
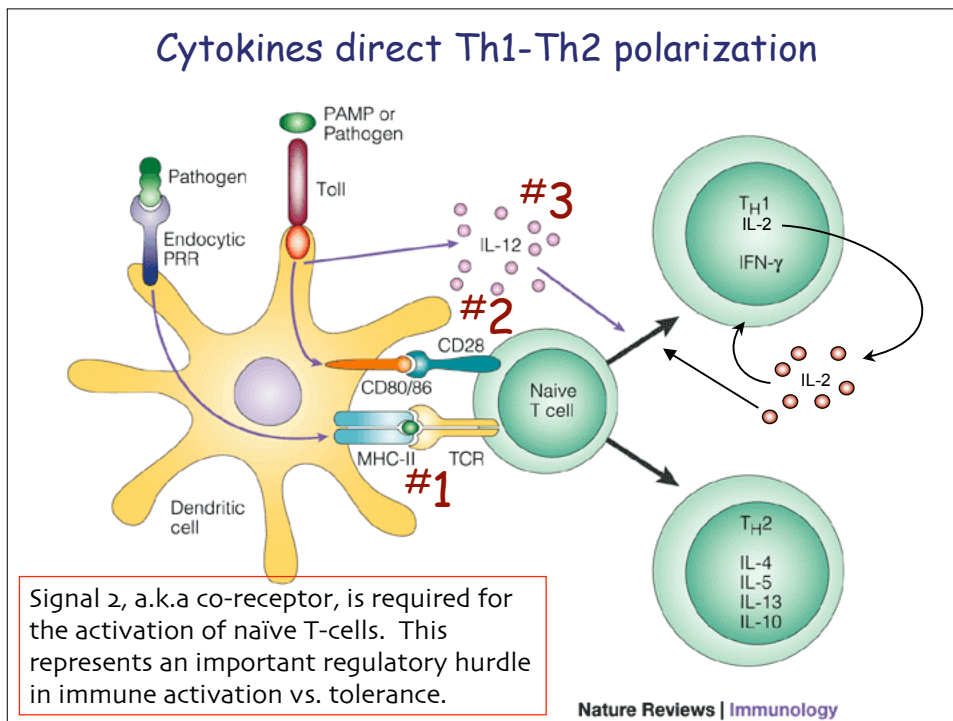


Figure 8-10 Immunobiology, 6/e. (© Garland Science 2005)

Cytokines and T-cell polarization



Cytokines direct Th1-Th2 polarization



Signal 2, a.k.a co-receptor, is required for the activation of naïve T-cells. This represents an important regulatory hurdle in immune activation vs. tolerance.

Nature Reviews | Immunology

The important IL-2 "autocrine loop"

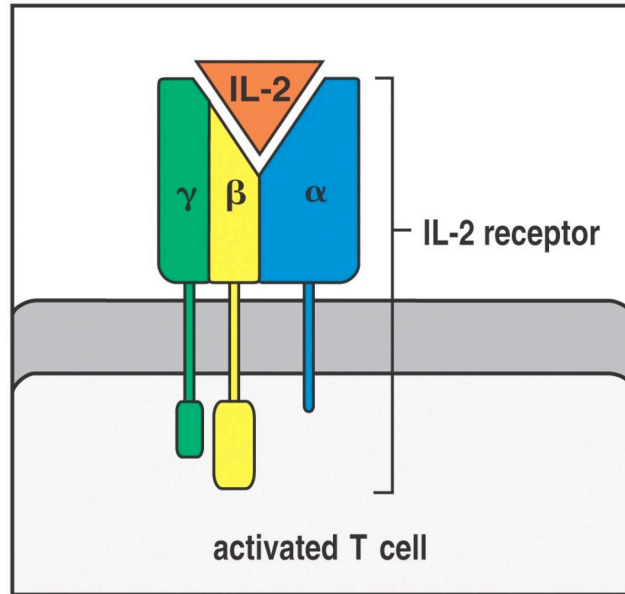
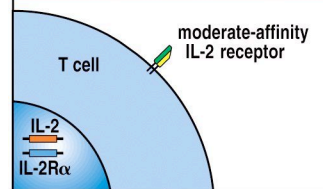


Figure 8-19 Immunobiology, 6/e. (© Garland Science 2005)

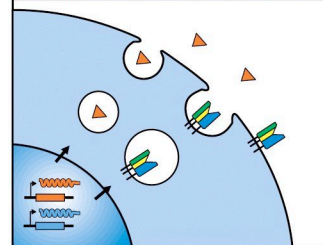
The IL-2 autocrine loop

TCR stimulation leads to induction of IL-2 & IL-2 receptor α -chain --> generating a high affinity receptor & culminating in potent T-cell proliferation.

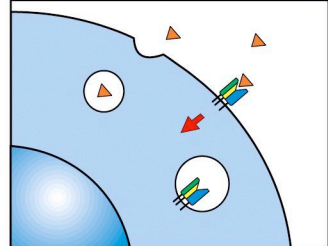
Resting T cells express only a moderate-affinity IL-2 receptor (IL-2R β and γ chains only)



Activated T cells express a high-affinity IL-2 receptor (IL-2R α , β and γ chains) and secrete IL-2



Binding of IL-2 to its receptor signals the T cell to enter the cell cycle



IL-2 induces T-cell proliferation

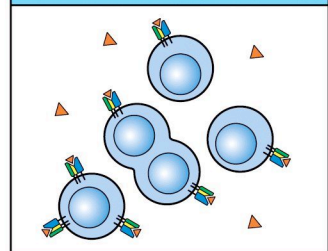


Figure 8-20 Immunobiology, 6/e. (© Garland Science 2005)

Now let's digress to review how TCR signaling directs cytokine production . . .
 it's an important drug target!

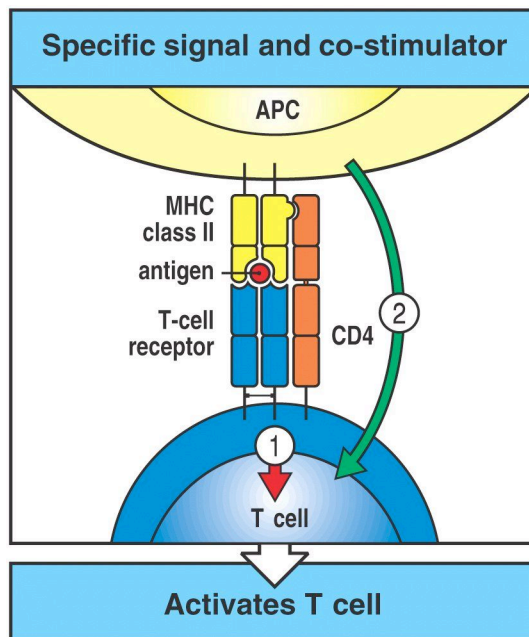
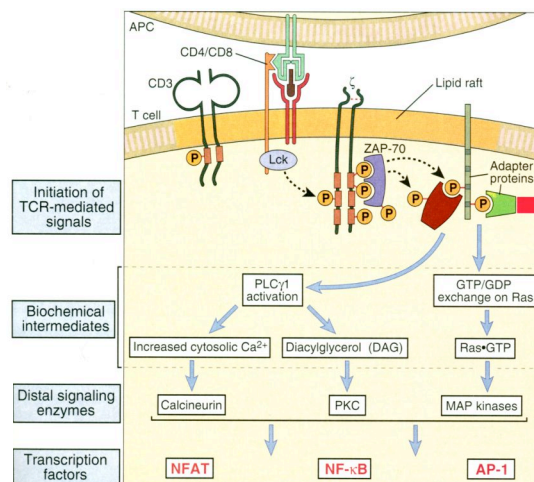
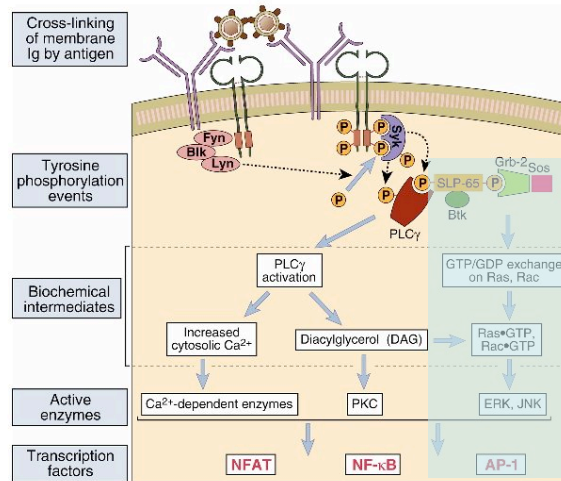


Figure 8-10 Immunobiology, 6/e. (© Garland Science 2005)

TCR-mediated Signal Transduction: A Tyrosine Kinase Cascade



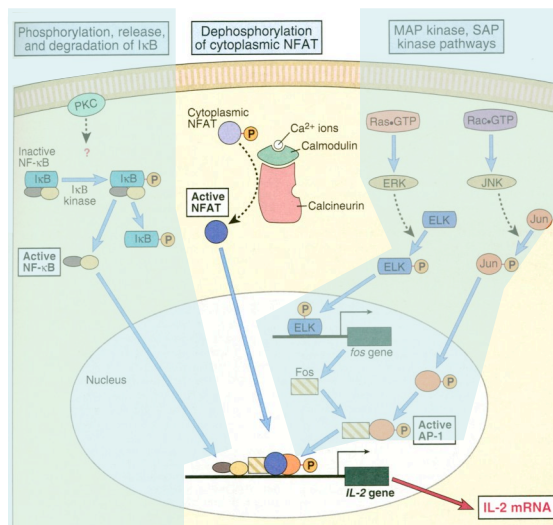
BCR-mediated Signal Transduction: A Tyrosine Kinase Cascade



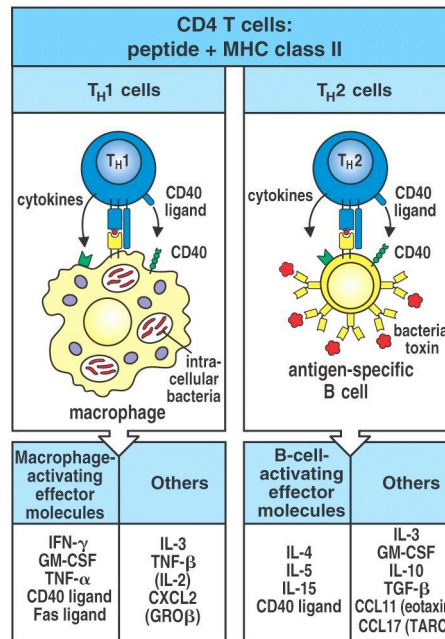
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NFAT & TCR-mediated signal transduction culminate in cytokine production

Cyclosporin A (CyA) & Tacrolimus (FK506) are two important drugs that block calcineurin and therefore the activation NFAT and the subsequent expression of **IL-2** production! Thus, they are potent immunosuppressive drugs.



Th1 and Th2 cells each secrete signature cytokines & chemokines that define their effector functions.



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Important Th1 effector cytokines

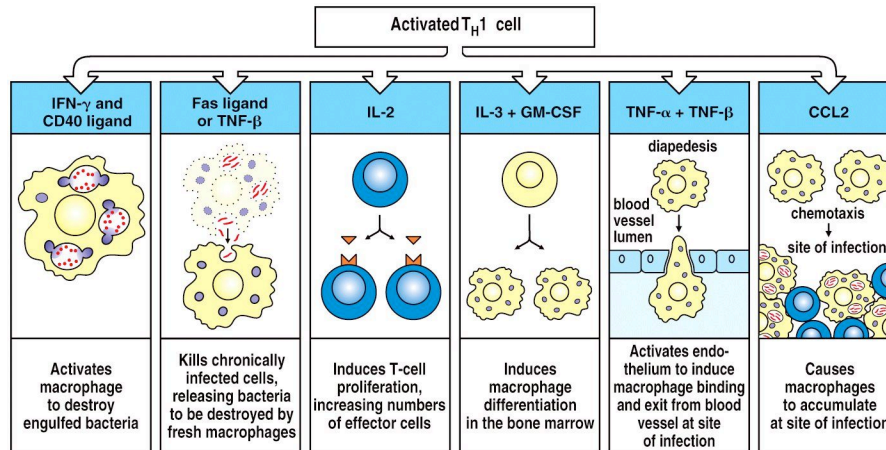


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IFN- γ is the Th1 signature cytokine

Important Th2 effector cytokines IL-4, IL-5 & IL-6 promote humoral immunity

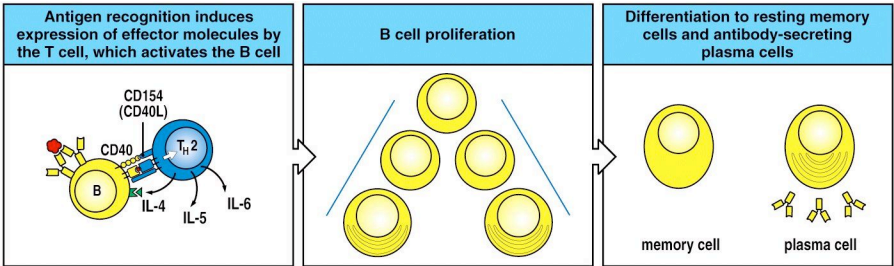
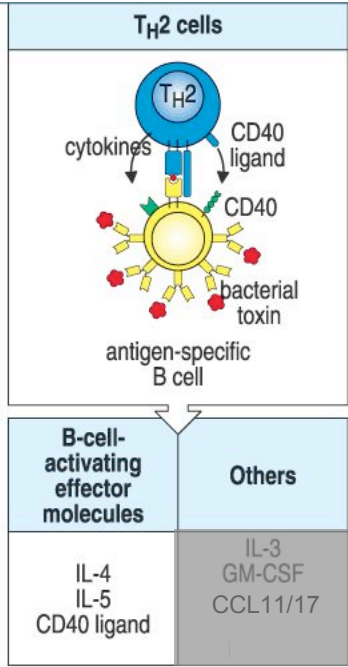
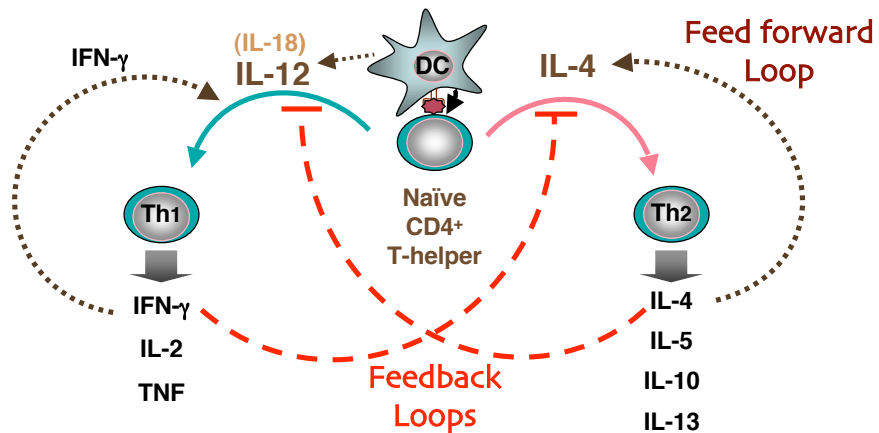


Figure 9-5 Immunobiology, 6/e. (© Garland Science 2005)

IL-4, which regulates B-cells, is the signature Th2 effector cytokine



Polarization of CD4⁺ T-cells into Th1 & Th2 subsets, but more later . . .



Other factors that influence the decision to become Th1 vs. Th2 include co-stimulators and nature of peptide:MHC interaction

The emerging story of a new T-cell effector pathway. Th17 cells.

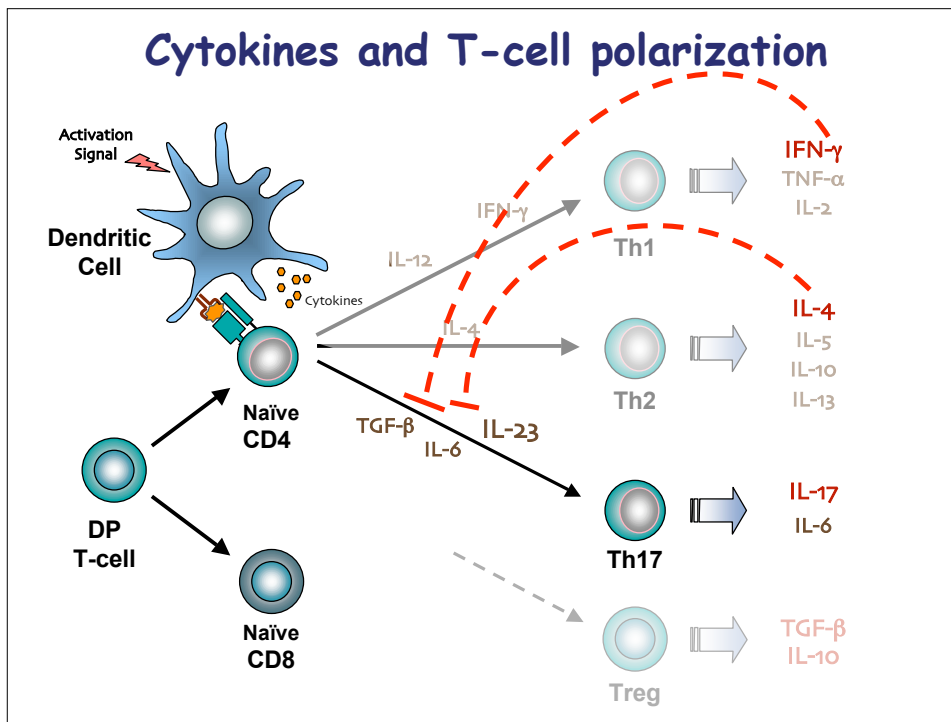
The Th17 Cell

- A CD4+ T-cell that arises from naïve CD4 cell.
- Secretes IL-6 and prodigious quantities of IL-17.
- Th17 cells probably evolved to combat pathogens not covered by Th1 (intracellular) or Th2 (helminths) cells.
- IL-17 deficient mice are highly susceptible to extra-cellular pathogens including *Klebsiella*, *Borrelia* and *Citrobacter*).
- IL-17 binds to a unique receptor expressed on many cell types
 - IL-17 stimulates fibroblasts, endothelial cells, macrophages, and epithelial cells to produce multiple pro-inflammatory mediators, e.g., IL-1, IL-6, TNF- α , NOS-2, metalloproteases, and chemokines.
 - IL-17 activates enhance granulocytes (innate immunity)
 - IL-17 promotes cellular immunity by activating CD8 T-cells, NK cells and macrophages.
- Implicated in autoimmune diseases (e.g., MS and RA).

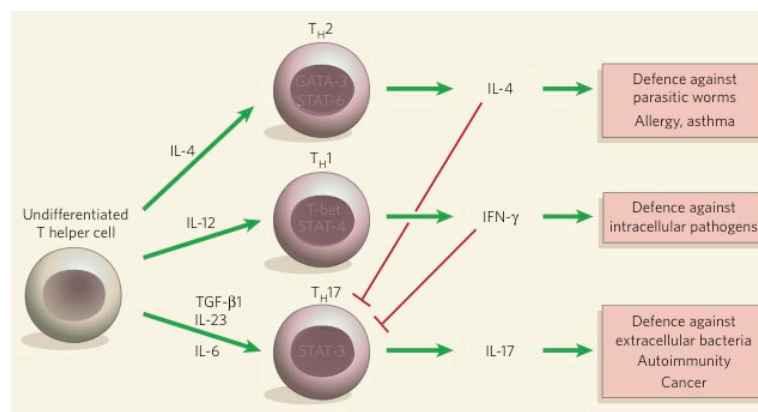
Th17 Cell Maturation

- Antigen plus TGF- β and IL-6 direct CD4 T-cells into the Th17 lineage.
- IL-23 is also essential for Th17 maturation /activity.
- IL-23 shares a 40 kDa subunit with IL-12 and binds to a related but distinct receptor.
 - IL-12 = p40 + p35
 - IL-23 = p40 + p17
- Thus Th17 cells “appear” to be more closely related to Th1 cells
- IFN- γ and IL-4 inhibit Th17 maturation.

Cytokines and T-cell polarization

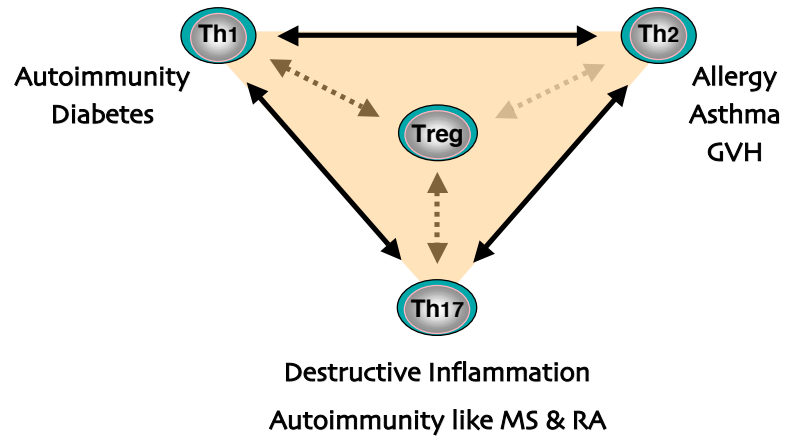


IL-17 Plays Important Roles in Autoimmunity and Cancer



From: Tato and O'Shea *Nature* 441:166 (2006)

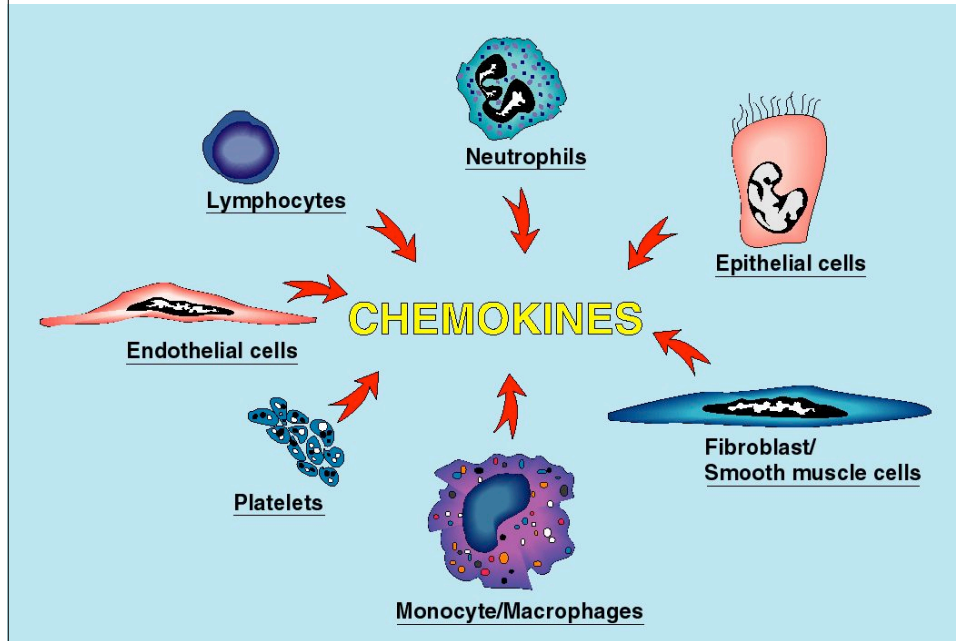
Failure to balance Th1, Th2 and Th17 cells



RA: Rheumatoid arthritis MS: Multiple sclerosis DM: Diabetes mellitus GVHD: Graft-vs-Host disease

Chemokines

Chemokines are secreted by many cell types



Chemokines signal through G-protein coupled receptors making them desirable drug targets

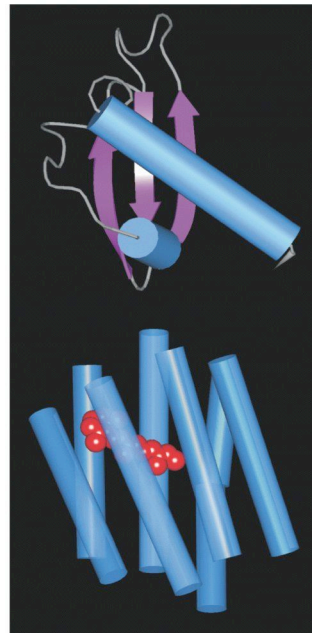
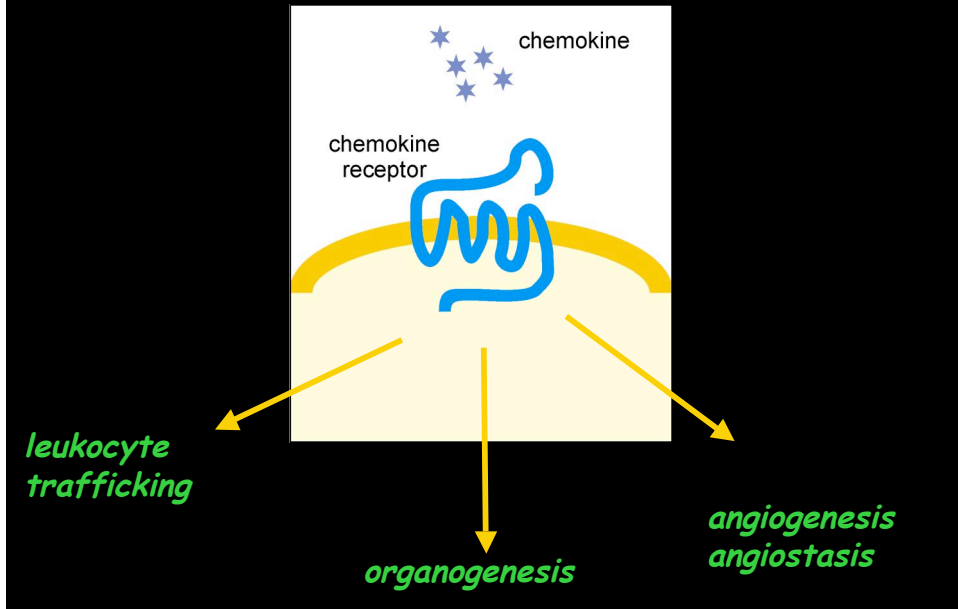
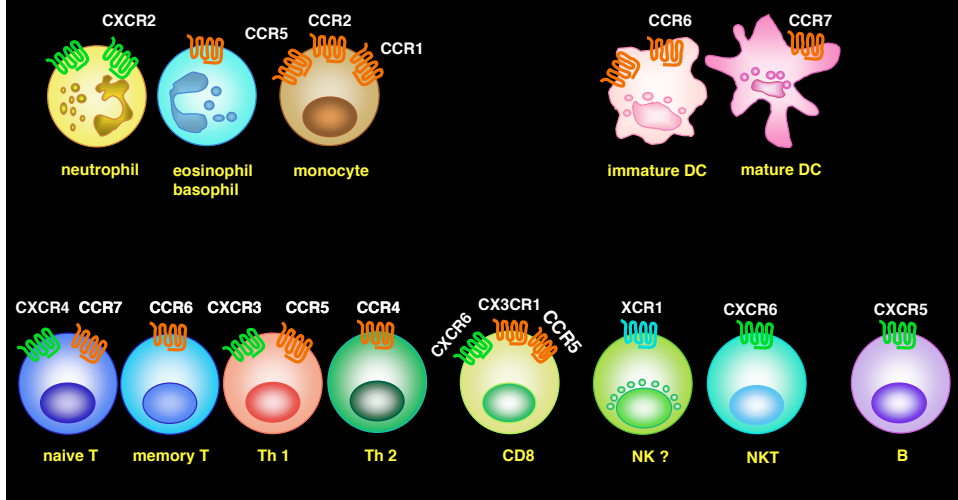


Figure 2-40 Immunobiology, 6/e. (© Garland Science 2005)

Chemokines are more than chemoattractants



Leukocytes express unique sets of chemokine receptors that direct them to the appropriate target tissue



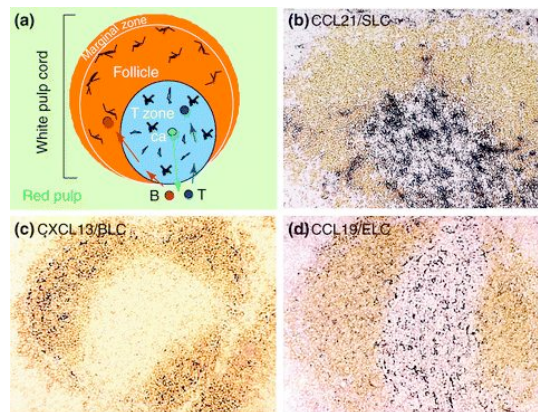
Functional Classification of Chemokines

- **Homeostatic Chemokines** - Development of immune tissues
 - These chemokines direct the basal or homeostatic distribution of leukocytes to immune tissues.
 - Homeostatic chemokines include: S1P,* CCL19, CCL21, CXCL12, CXCL13
- **Inflammatory Chemokines** - Acute and chronic inflammation
 - e.g., Danger signals, many chemokines are involved in directing leukocyte traffic during infection & inflammation (chronic & acute).
 - Inflammatory chemokines include: CCL2, CCL5, CCL11, CXCL8, CXCL9, CXCL10
- There are some “double dippers”

*S1P, sphingosine-1-phosphate, is a lipid mediator, not a true chemokine.

Homeostatic chemokines target cells to appropriate compartment in the absence of inflammation

e.g. lymphocytes and APCs to spleen or LN

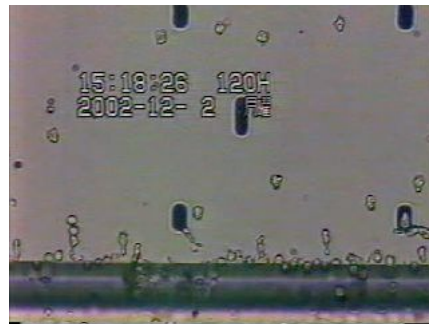


TRENDS in Cell Biology

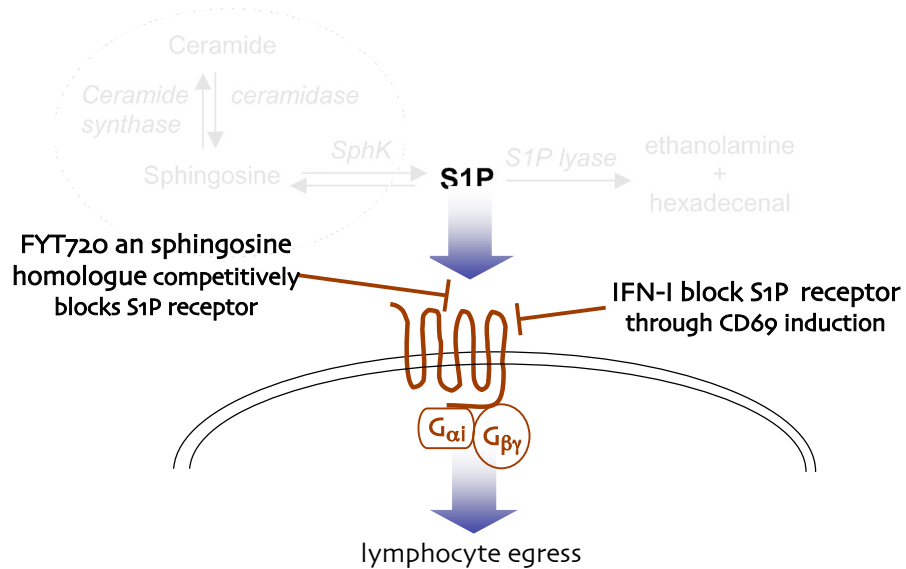
Cell	Chemokine receptor	Chemokine sensed
DC	CCR7	CCL19, CCL21
naïve T	CCR7	CCL19, CCL21
naïve B	CXCR5	CXCL13

IL-8 / CXCL8 (human) is a potent inflammatory chemoattractant for PMNs

CXCL8



Sphingosine-1P promotes lymphocyte egress from secondary lymphoid tissues



Of Note

- Two chemokine receptors serve as co-receptors for HIV infection (CXCR4 and CCR5)

Chemokine Summary*

- 8-12 kDa proteins secreted by WBCs, platelets, epithelial, endothelial, smooth muscle and fibroblast cells.
- Form **gradients** that act as chemoattractants for WBCs expressing the corresponding receptors
 - Inflammatory Chemokines - CCL2-5, CCL11, CCL17, CXCL8 (IL-8), CXCL9, CXCL10
 - Homeostatic Chemokines - S1P, CCL19, CXCL12, CCL21, CXCL13
- Bind GPCRs (G-protein coupled receptors).
- Chemokines also regulate the growth and development of some immune and non-immune tissues.
- There are several families of non-classical chemokines:
 - Lipid-based, e.g., sphingosine-1-phosphate (S1P; blocked by FTY720), LTB₄, PGD₂
 - Peptide-based, e.g., fMLP (bacterial-derived), C3a, C5a

***Do not memorize the list of individual chemokines, only the functional classes!**

Summary

1. Naive CD4+ T-cells differentiate into several distinct T cell subsets, a process that is driven by antigen and cytokines. These subsets include Th1, Th2, Th17 and Treg cells. Critical feed forward and feedback regulators assure an important balanced expansion of these subsets.
2. Th1 cells secrete IFN- γ and IL-2. IFN- γ potently activates macrophages to secrete pro-inflammatory cytokines and kill microbes. Th1 cells are also important for "Delayed Type Hypersensitivity," or DTH. IL-2 directs the proliferation of T-cells. Pathways leading to IL-2 production, especially those that activate NFAT, are important targets of immunosuppressive drugs (e.g., cyclosporin and FK506).
3. Th2 cells, which secrete IL-4, potently activate B-cells, via CD40 and MHC-peptide and cytokines (e.g., IL-4). IL-4 also stimulates immunoglobulin class-switch IgG and IgE. Finally, Th2 cells, IL-4 and IL-5 afford important immunity against parasites (e.g., helminths).
4. Th17 cells stimulate neutrophils during acute bacterial infections and many other cells during chronic inflammation (e.g., in autoimmunity).
5. Chemokines are small proteins that activate G protein-coupled receptors and are essential for homeostatic and inflammatory leukocyte trafficking. They also regulate additional activities in target cells.