Lecture 10

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.
- Bind specific, high affinity (e.g., K_d of 10^{-10}-10^{-12} M) receptors found on target cells.
- Expression of cytokines and their cognate receptors is usually tightly regulated (i.e., temporally & spatially).
- **Cytokine receptors define the specific type of biological response a cytokine stimulates.**
- Four helix bundle cytokines are usually referred to interleukins (ILs; e.g., IL-2, IL-3 …). Anachronistic terms include monokines & lymphokines.

What do cytokines do?

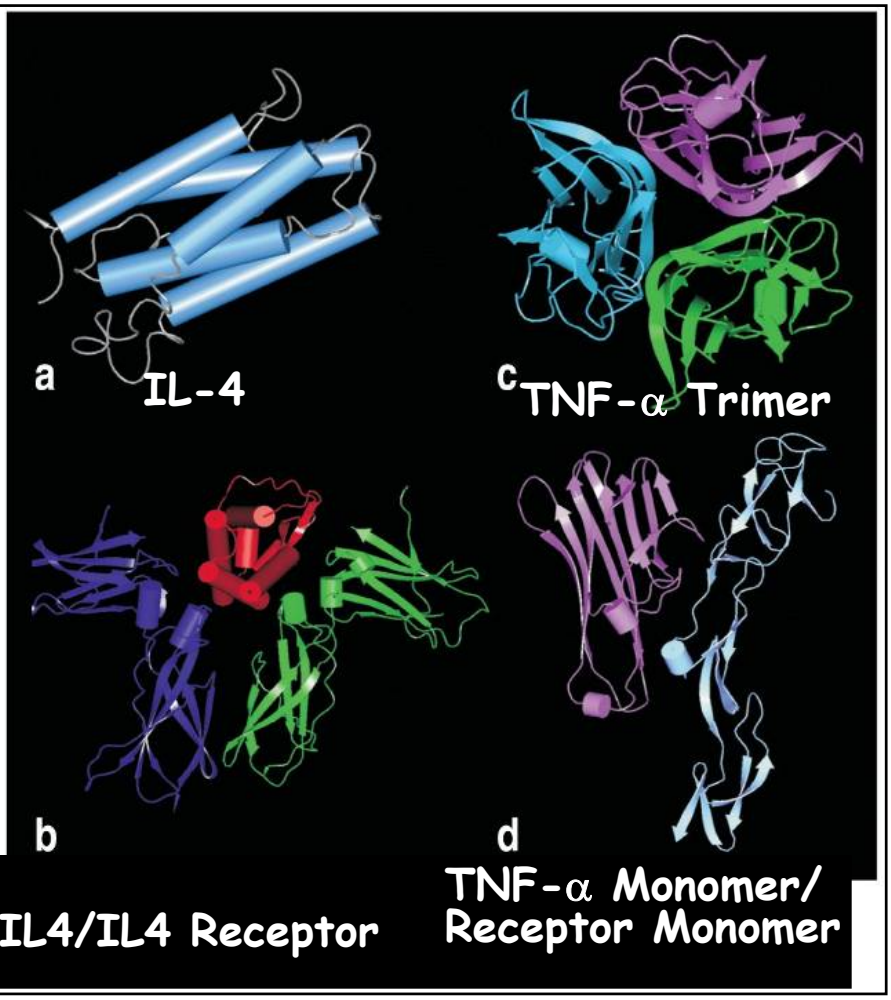
- They direct the development, maturation, localization, interactions, activation and life span of immune cells.
- Thus they play an essential role in regulating both immunity adaptive and innate.
- (Chemokines and Growth Factors also participate).

Cytokines subfamilies are functionally distinct

- Cytokines can be divided into functionally distinct groups based on the receptors they bind.
  - Growth Factors (e.g., CSF-1, SCF, RANKL, Flt3L)
  - IL-1 Family (e.g., IL-1, IL-18 & natural products/PAMPs)
  - TNF Family (e.g., TNF-α, CD40L, FasL, LT, TRAIL, BAFF)
  - TGF-β Family (e.g., TGF-β)
  - **Type I & II Cytokines (4 Helix Bundle Cytokines; e.g., IL-2, IL-4, IL-6, IL-7 IL-10, IL-12, IL-21, IL-22 IL-23, IL-27, G-CSF, GM-CSF, IFN-γ, IFN-α**
  - Chemokines (e.g., CC and CXC families)
  - Other (e.g., steroid hormones, prostaglandins and IL-17)
- There are significant functional similarities within each receptor family. The same is true for corresponding ligands.
- There are important functional differences between between receptor families.

***Underlined cytokines are of particular importance**

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



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

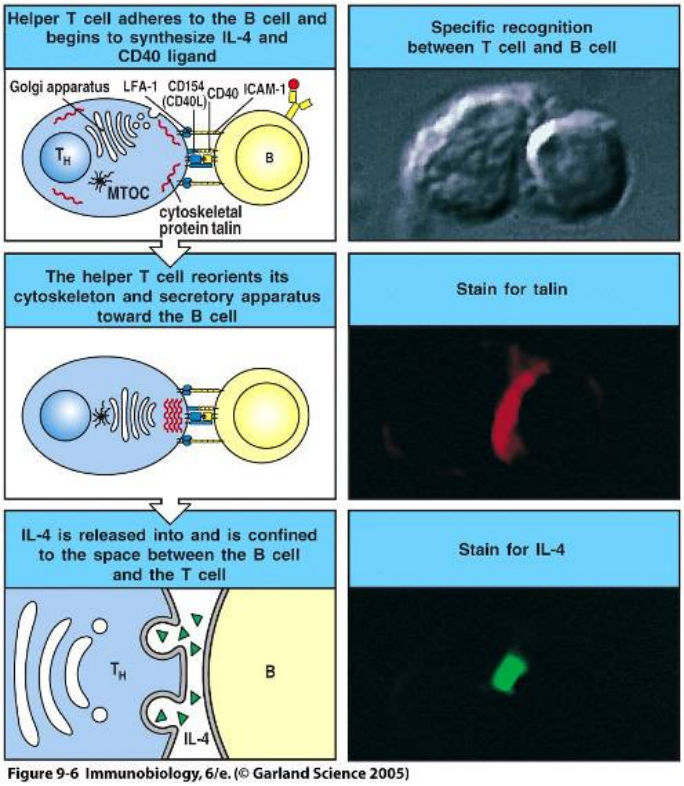
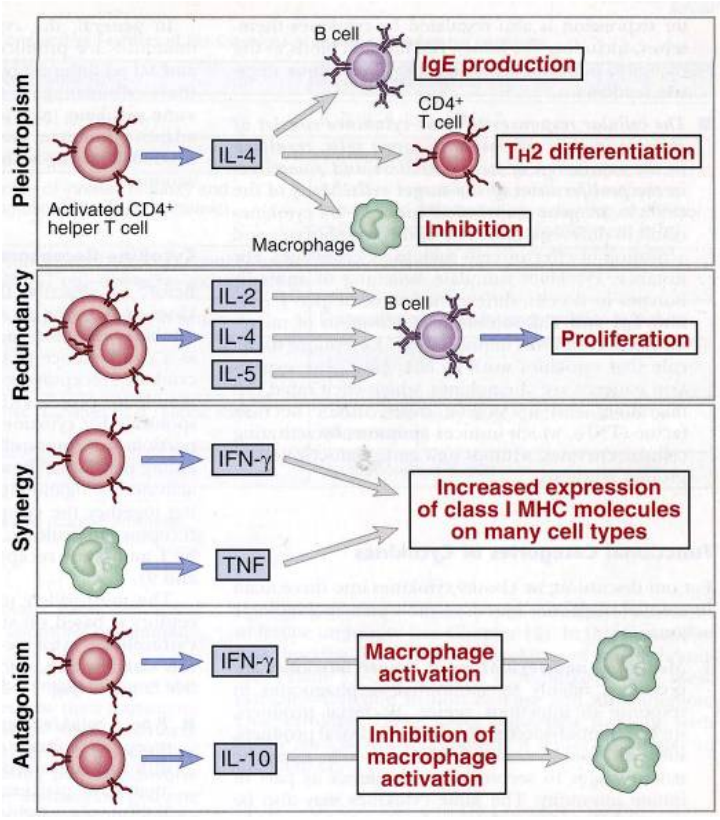


Figure 9-6 Immunobiology, 6/e. (© Garland Science 2005)

Important general properties of Cytokines and Chemokines

- Stimulate transient response in target cells.
- 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).

General Properties of Cytokines



Some Biology . . . . . .

How do we protect ourselves from microbes?

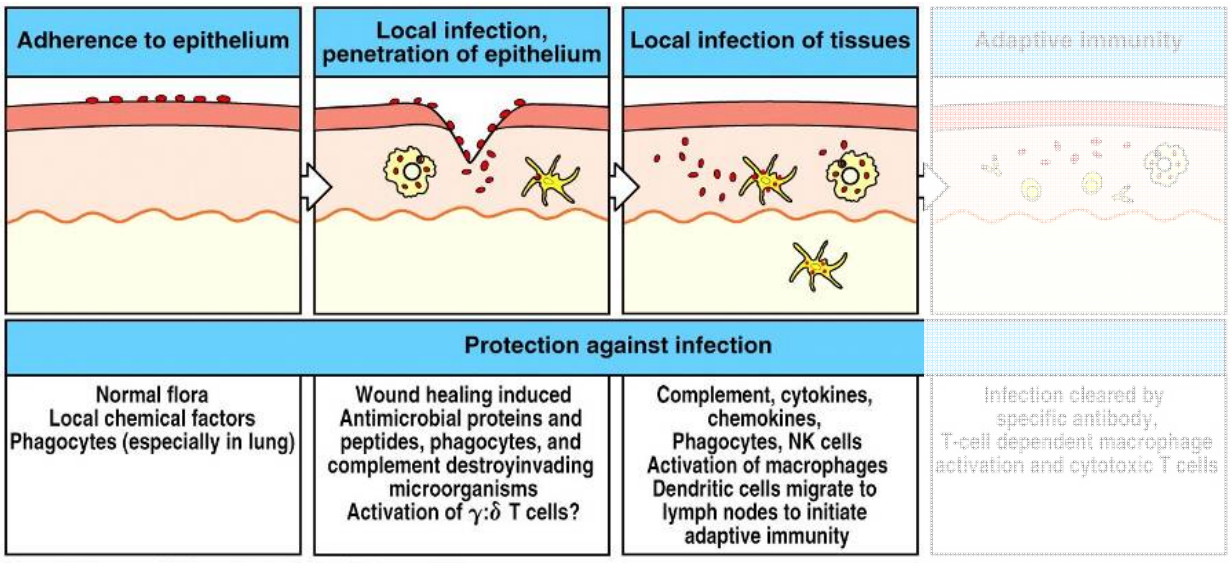
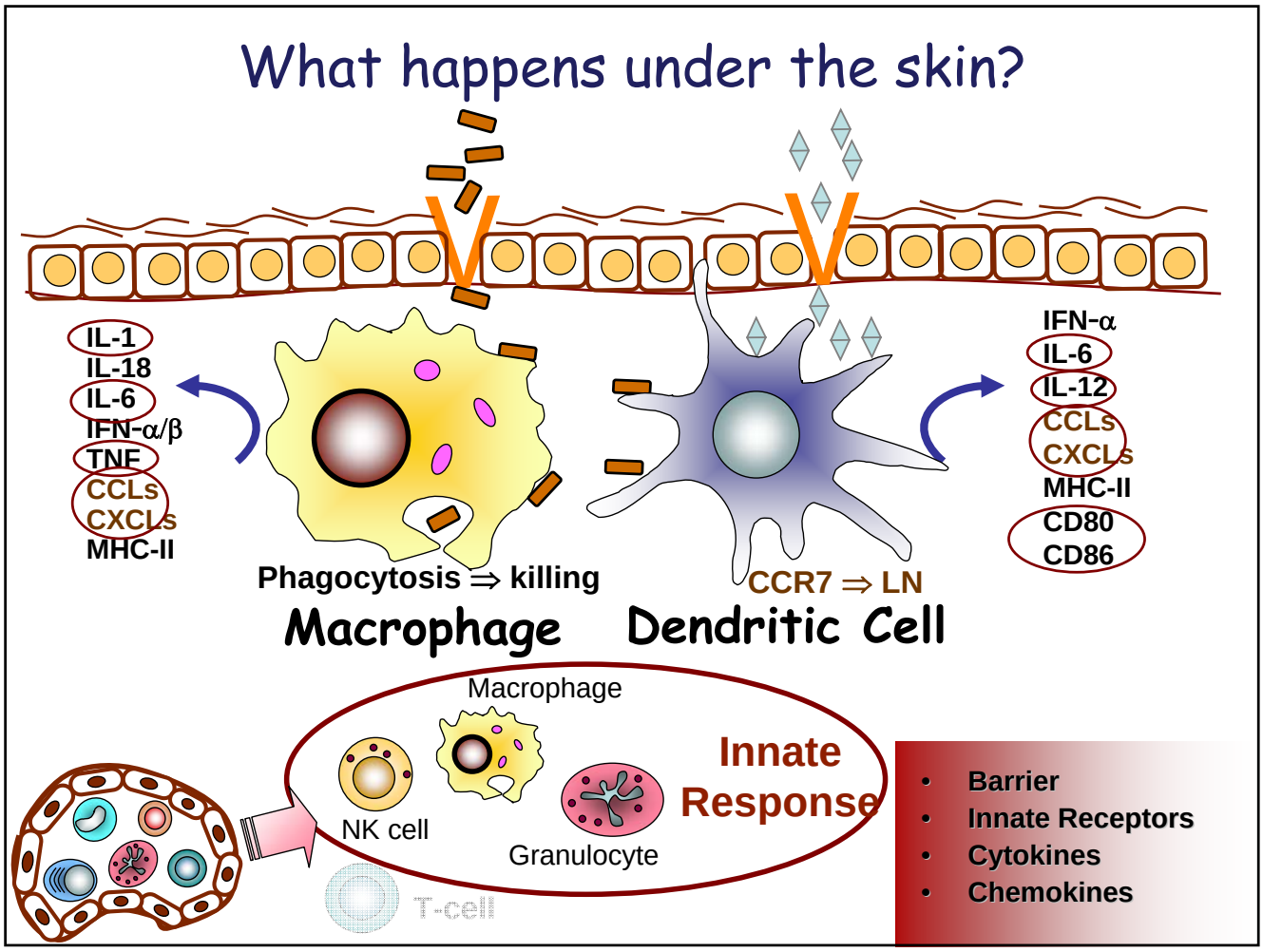


Figure 2-3 Immunobiology, 6/e. (© Garland Science 2005)

Wound Infection: Innate ➲ Adaptive


What happens under the skin?
IL-1
IL-18
IL-6
IFN-α/β
TNF
CCLs
CXCLs
MHC-II
Phagocytosis ⇒ killing
Macrophage
CCR7 ⇒ LN
Dendritic Cell
IFN-α
IL-6
IL-12
CCLs
CXCLs
MHC-II
CD80
CD86
Macrophage
NK cell
Granulocyte
T-cell
Innate Response
Barrier
Innate Receptors
Cytokines
Chemokines

PRRs detect the infection

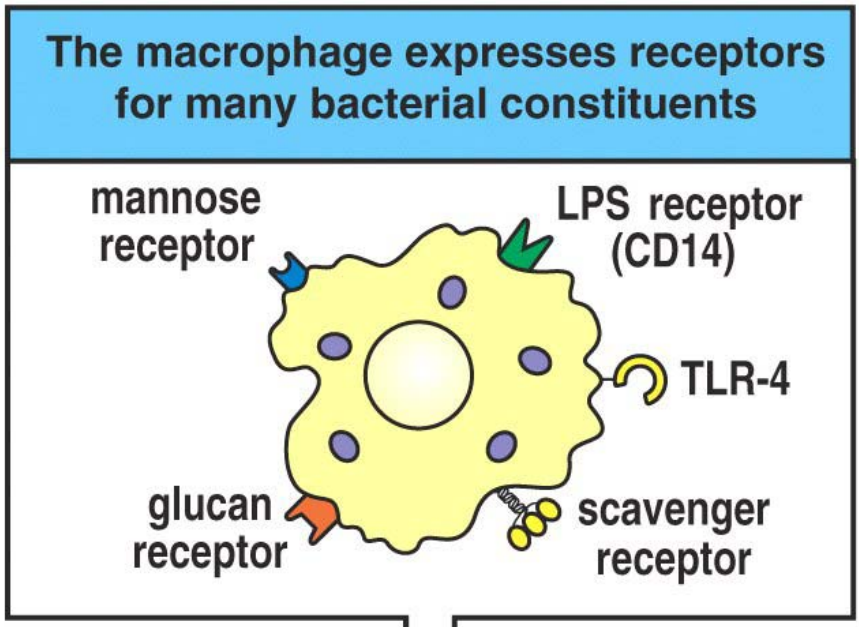


Figure 2-5 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Activated macrophages secrete cytokines & chemokines, Directing the ensuing response

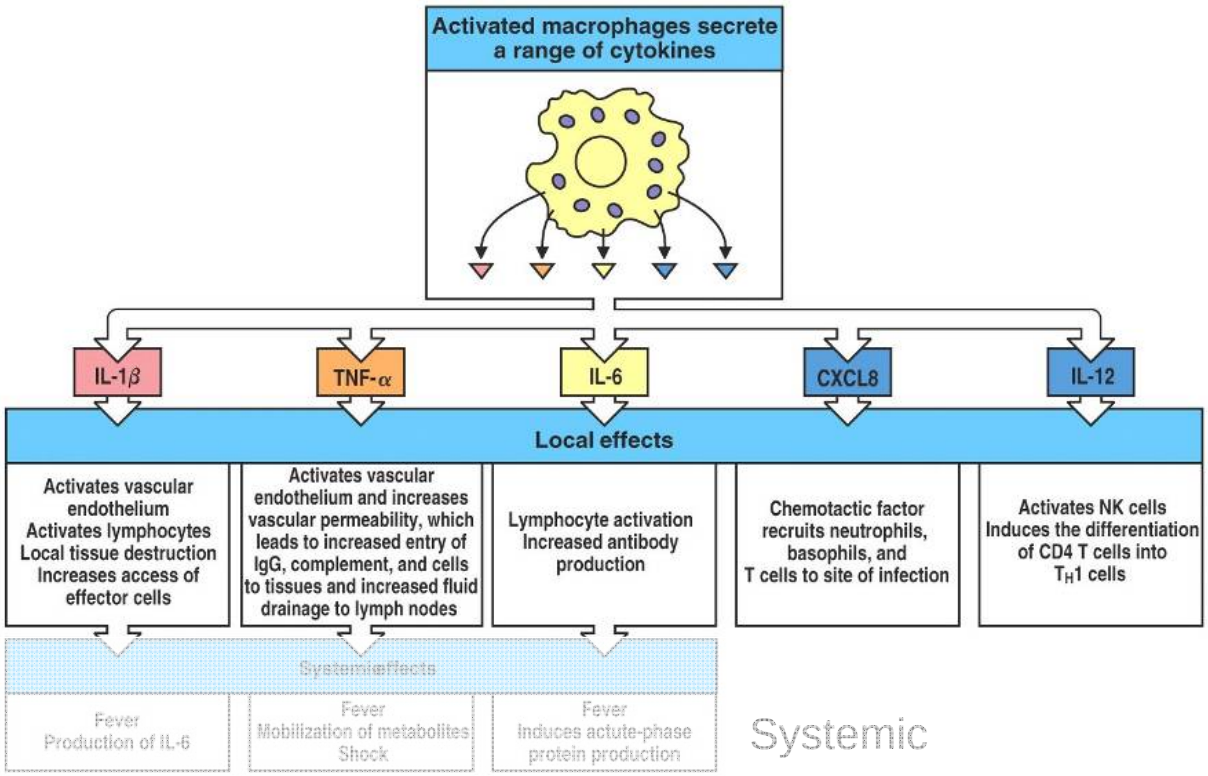


Figure 2-39 Immunobiology, 6/e. (© Garland Science 2005)

Local vs. systemic infection

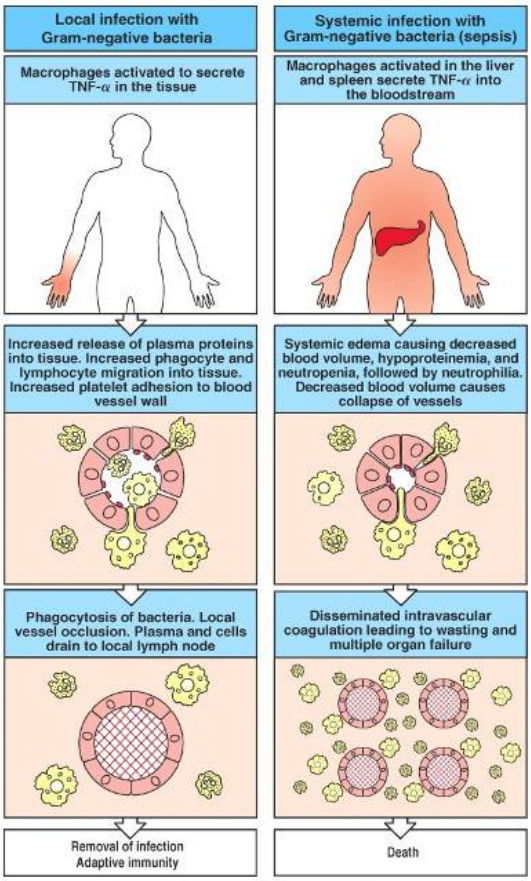
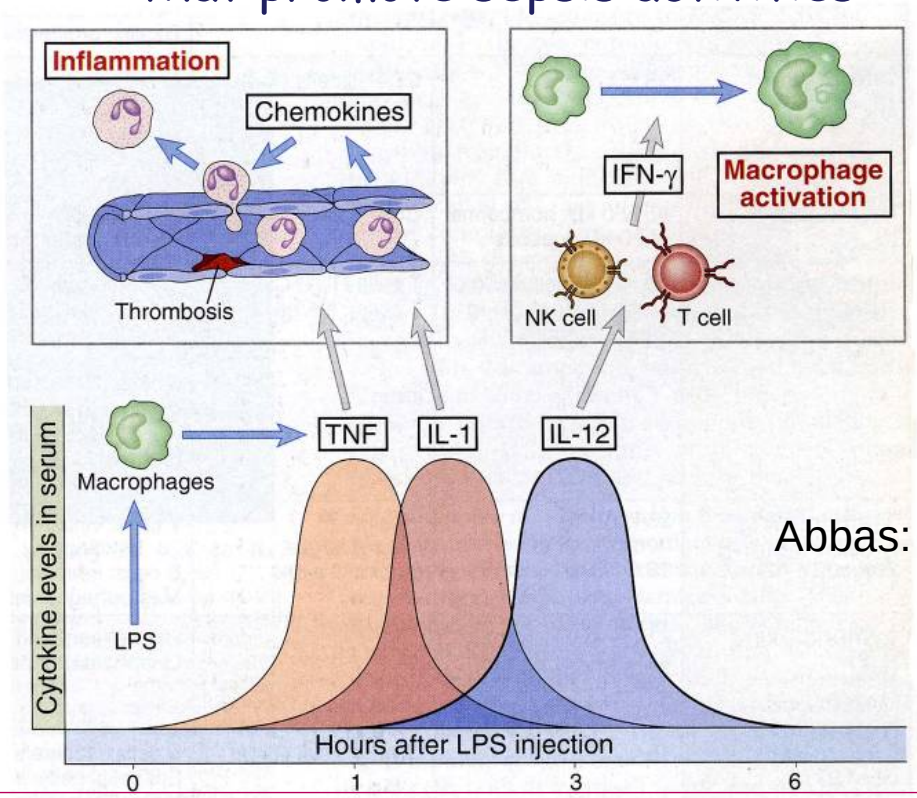


Figure 2-45 Immunobiology, 6/e. (© Garland Science 2005)

LPS stimulates macrophages to secrete cytokines that promote sepsis activities



Abbas: Chpt. 11

Note, one of the few times you can meaningfully measure serum cytokine levels!!

Cytokines and the septic constellation

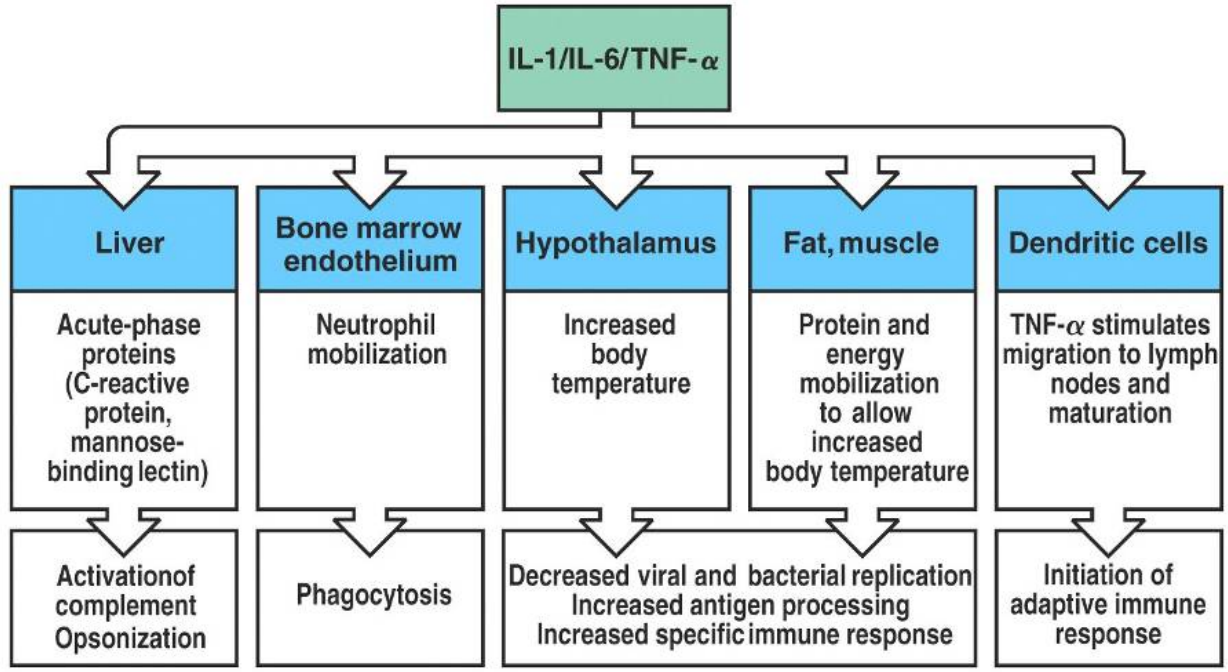
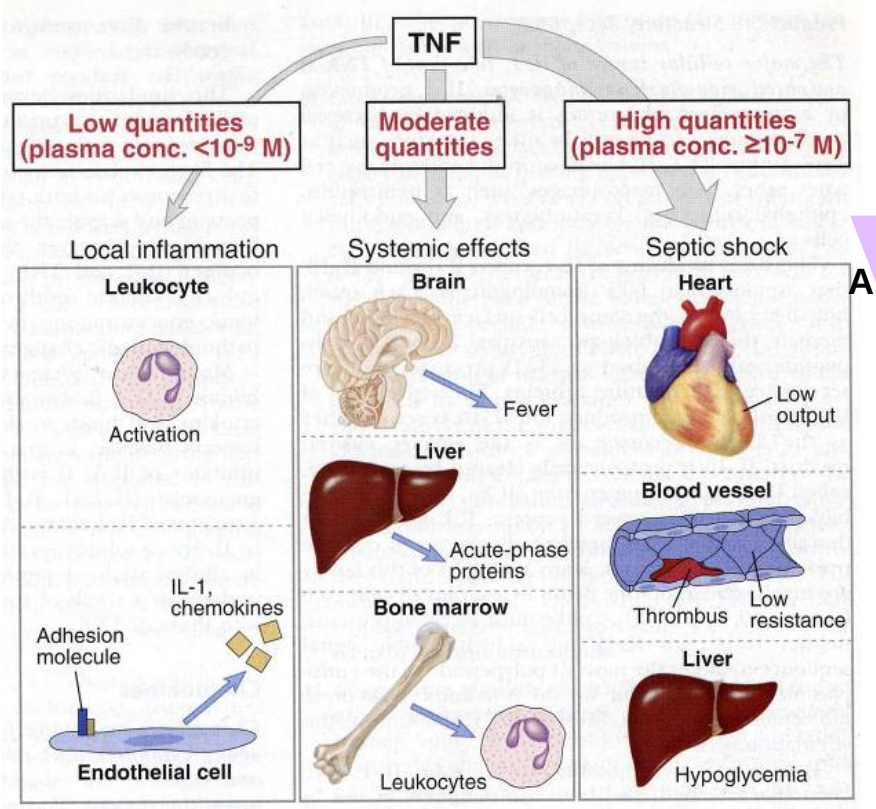


Figure 2-46 Immunobiology, 6/e. (© Garland Science 2005)

Biological actions of TNF



New Anti-inflammatory Agents

Remicade (infliximab)
Anti-TNF

Enbrel (etanercept)
TNF-Trap

Kineret (anakinra)
rIL1-RA

Abbas: Chpt. 11

Cytokines and T-cell subsets

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

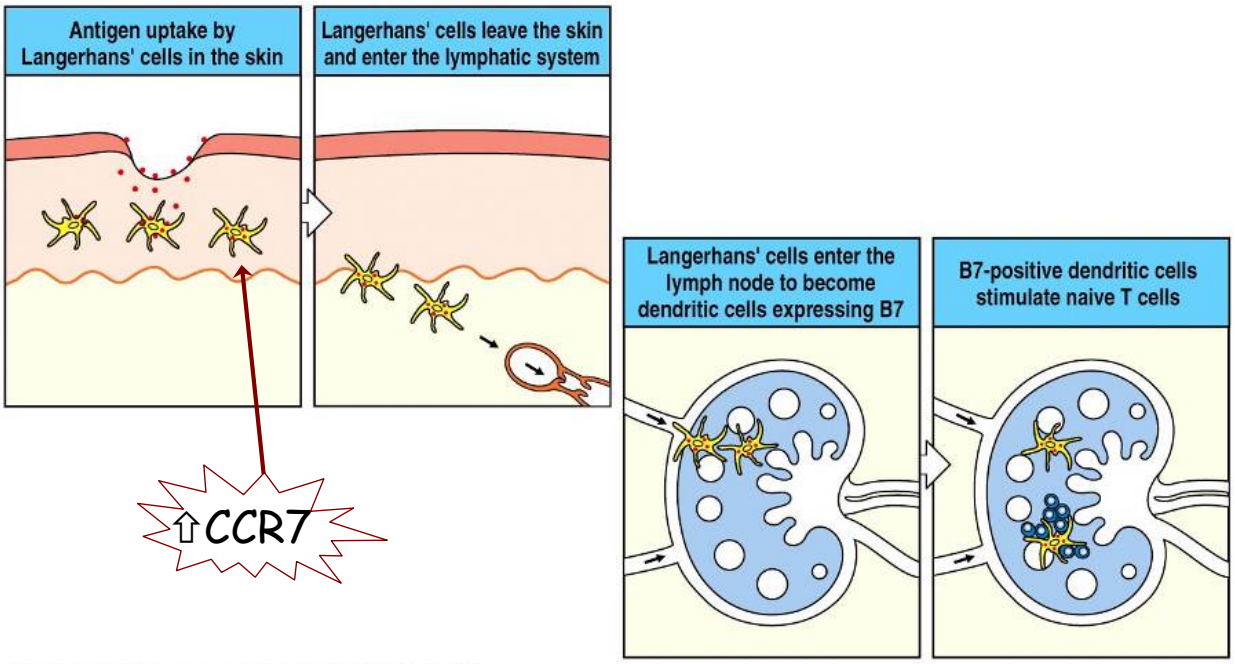


Figure 8-15 Immunobiology, 6/e. (© Garland Science 2005)

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

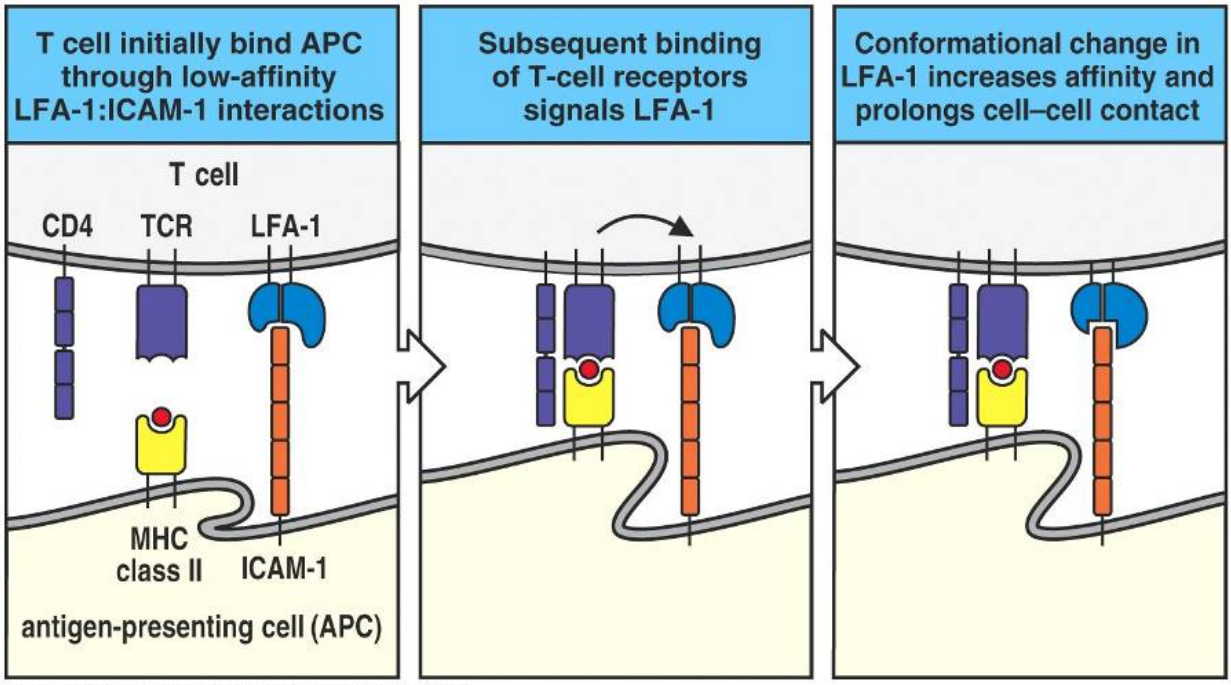


Figure 8-9 Immunobiology, 6/e. (© Garland Science 2005)

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

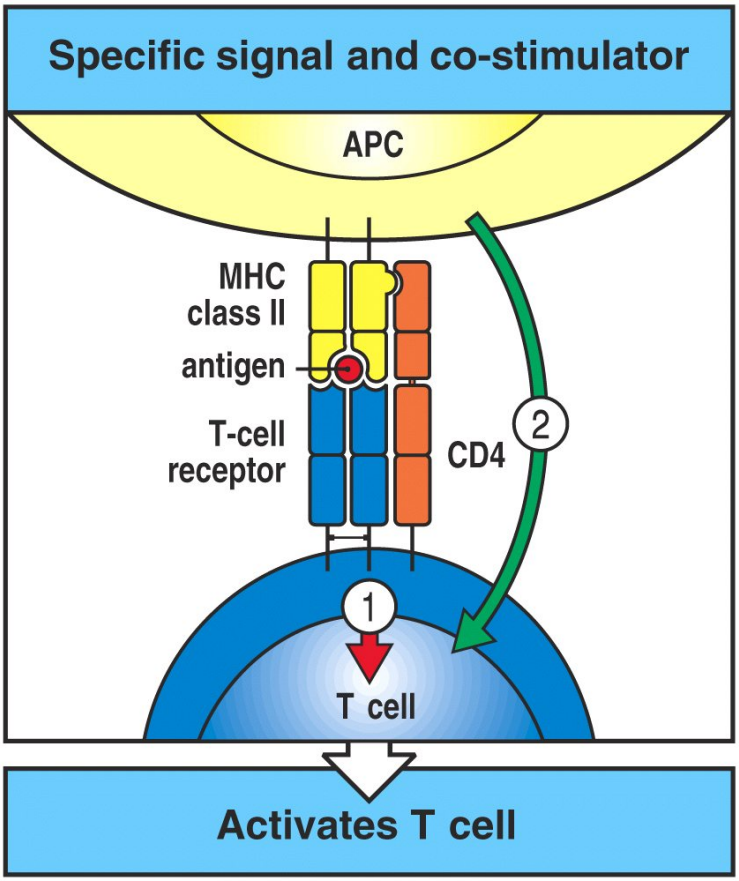
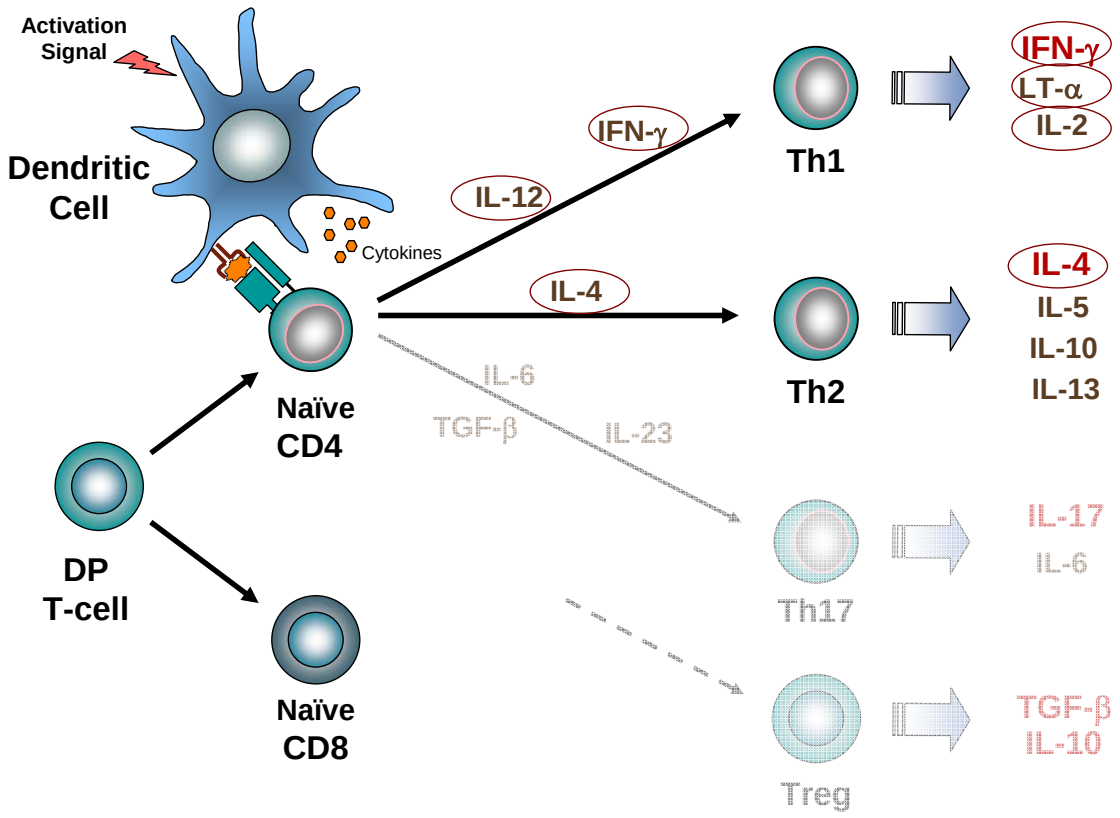
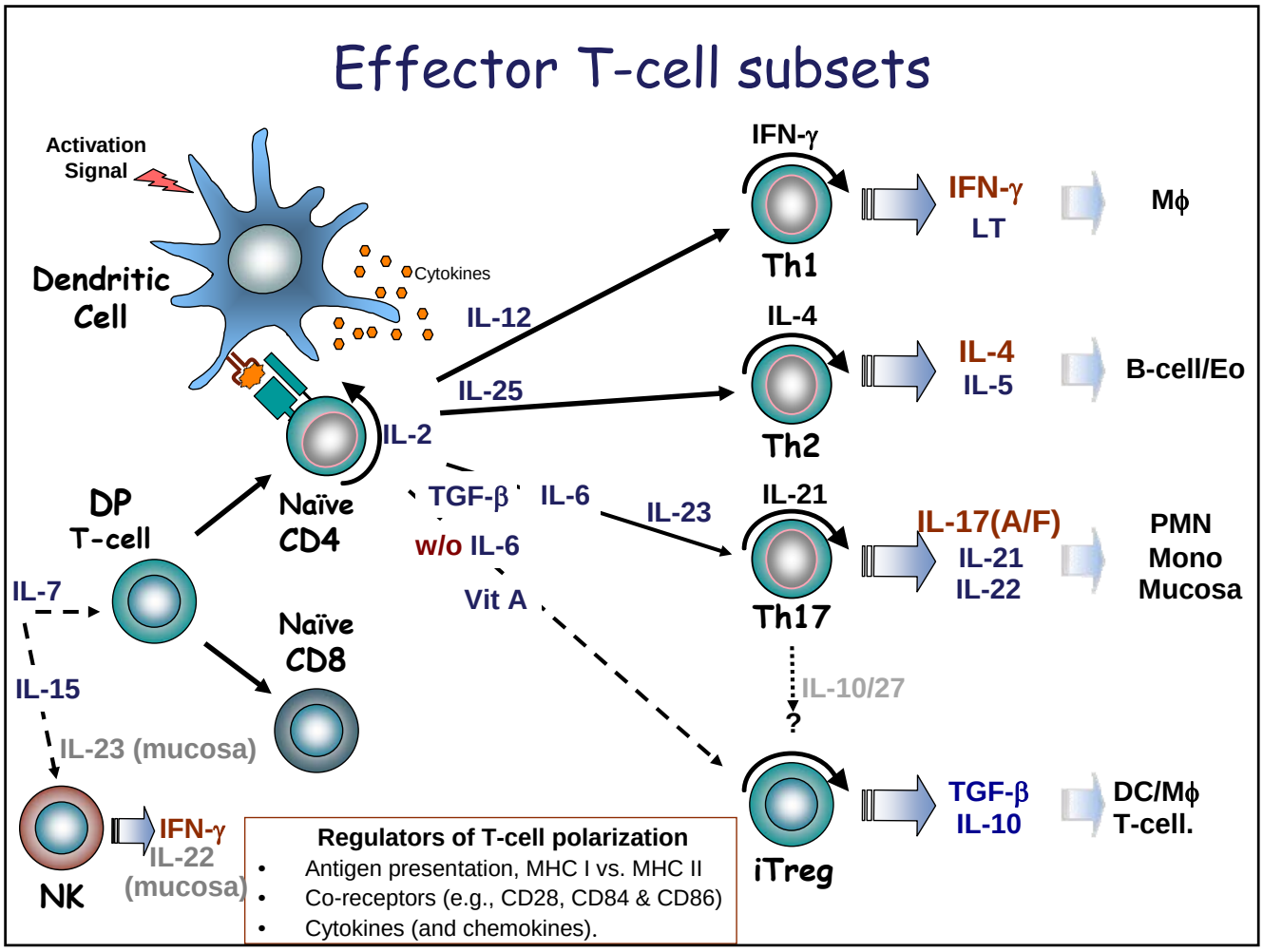


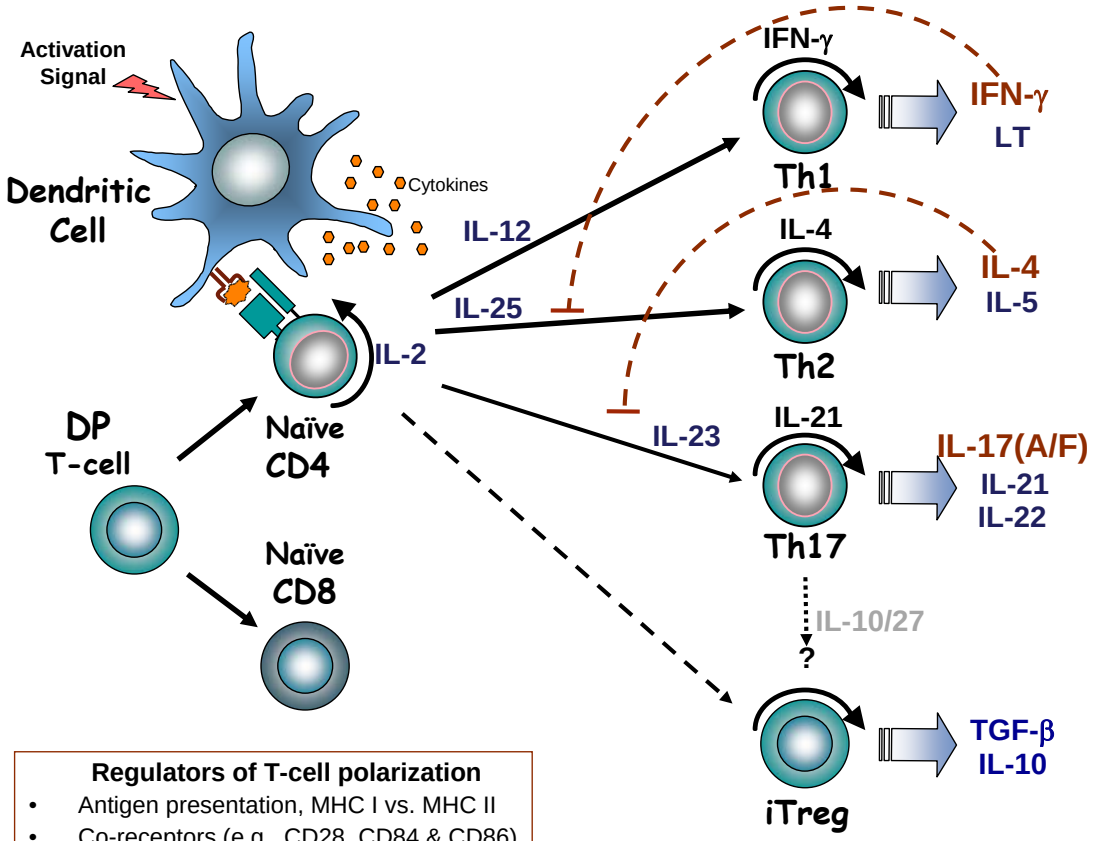
Figure 8-10 Immunobiology, 6/e. (© Garland Science 2005)

Cytokines and T-cell polarization




Effector T-cell subsets
Activation Signal
Dendritic Cell
Cytokines
IL-12
IL-25
IL-2
IFN-γ
Th1
IFN-γ
LT
Mϕ
IL-4
Th2
IL-4
IL-5
B-cell/Eo
DP T-cell
Naïve CD4
TGF-β
IL-6
w/o IL-6
IL-23
IL-21
Th17
IL-17(A/F)
IL-21
IL-22
PMN
Mono
Mucosa
IL-7
Naïve CD8
IL-15
Vit A
IL-10/27
?
IL-23 (mucosa)
NK
IFN-γ
IL-22
(mucosa)
iTreg
TGF-β
IL-10
DC/Mϕ
T-cell.
Regulators of T-cell polarization
• Antigen presentation, MHC I vs. MHC II
• Co-receptors (e.g., CD28, CD84 & CD86)
• Cytokines (and chemokines).

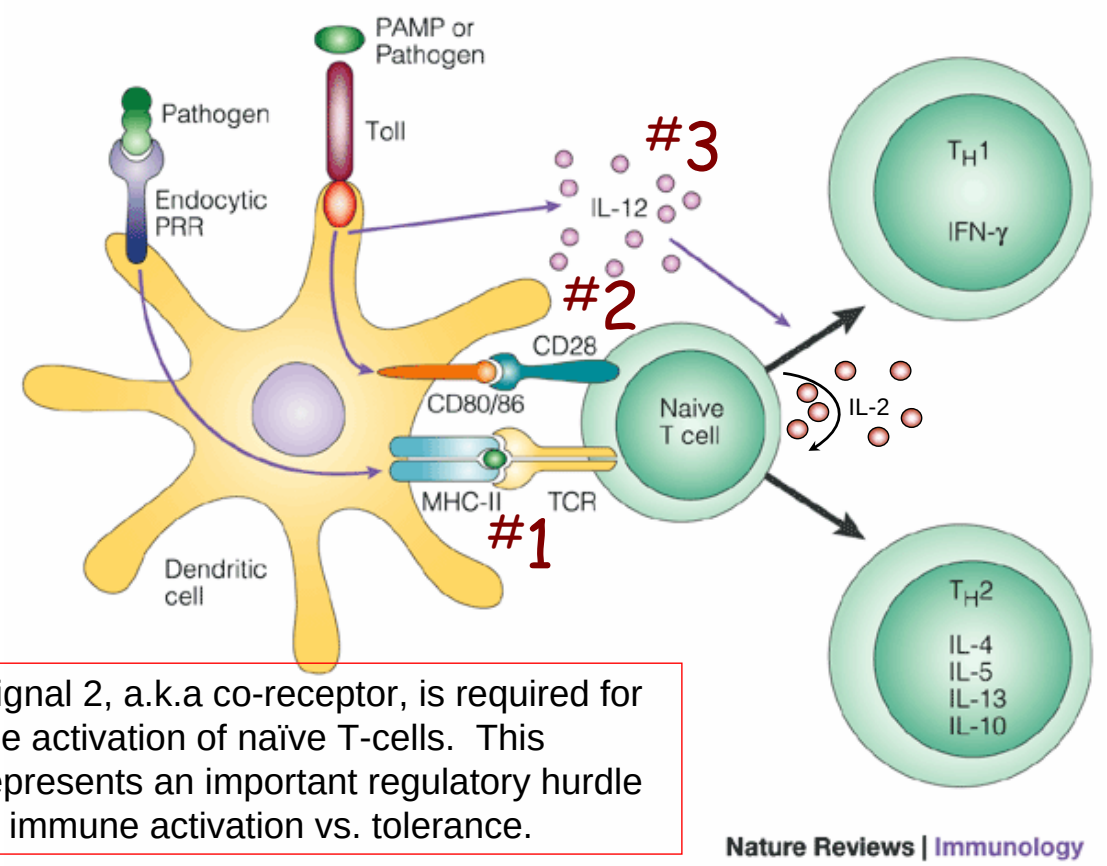
Negative Feedback Loops



Regulators of T-cell polarization

- Antigen presentation, MHC I vs. MHC II
- Co-receptors (e.g., CD28, CD84 & CD86)
- Cytokines (and chemokines).

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.

IL-2 activity is important in the activation
and medicinal suppression of immune response

The important IL-2 "autocrine loop"

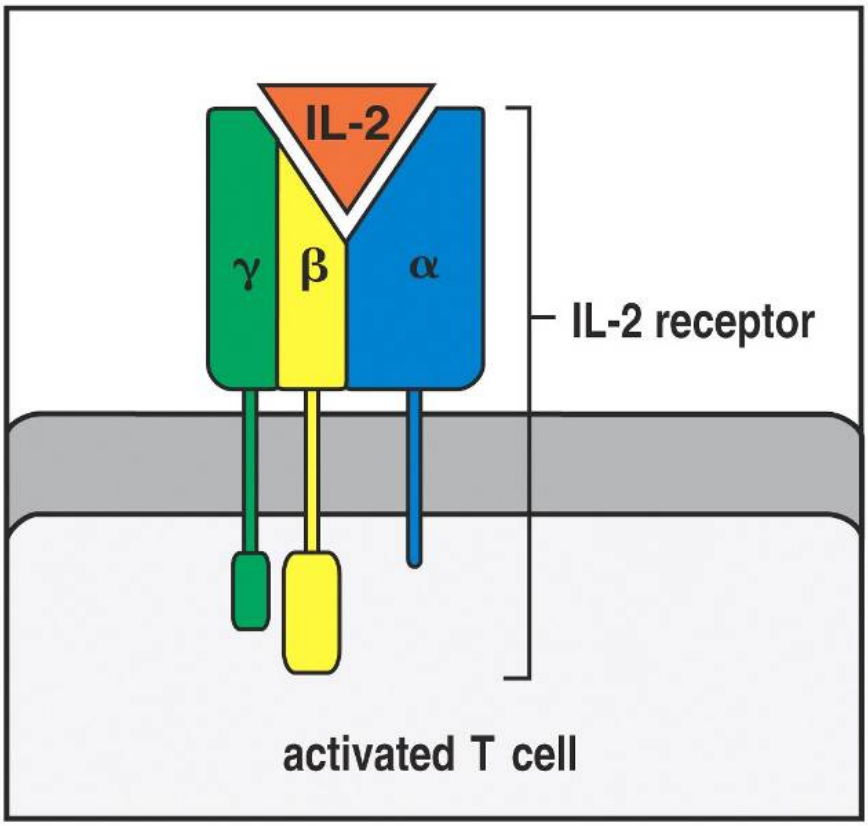


Figure 8-19 Immunobiology, 6/e. (© Garland Science 2005)

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.

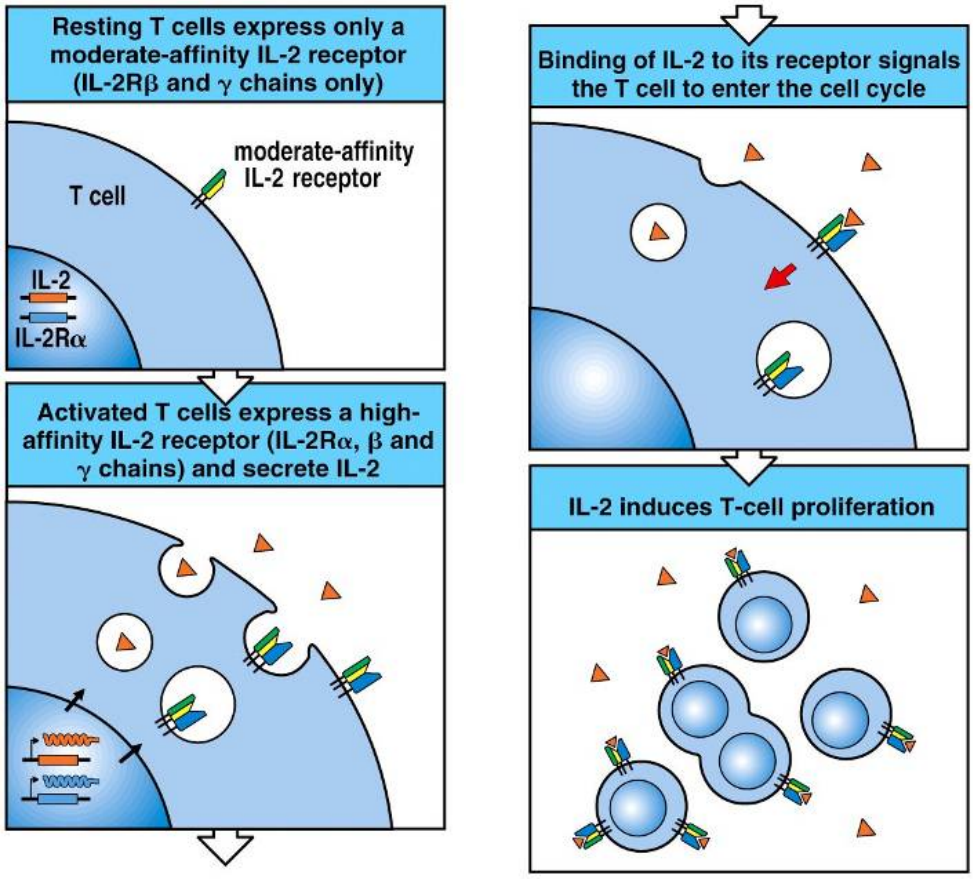


Figure 8-20 Immunobiology, 6/e. (© Garland Science 2005)

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

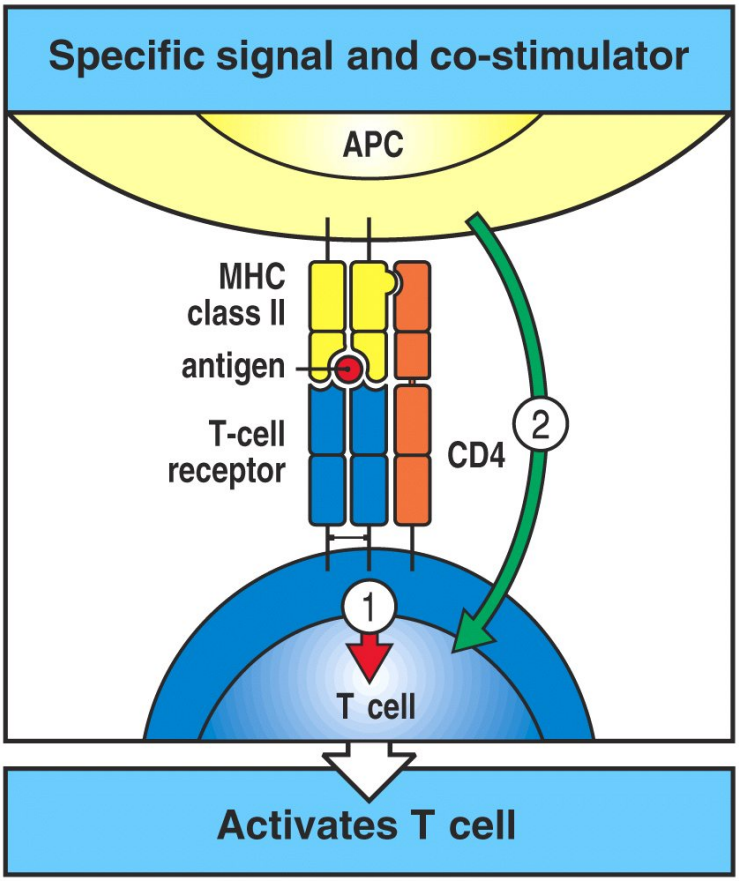
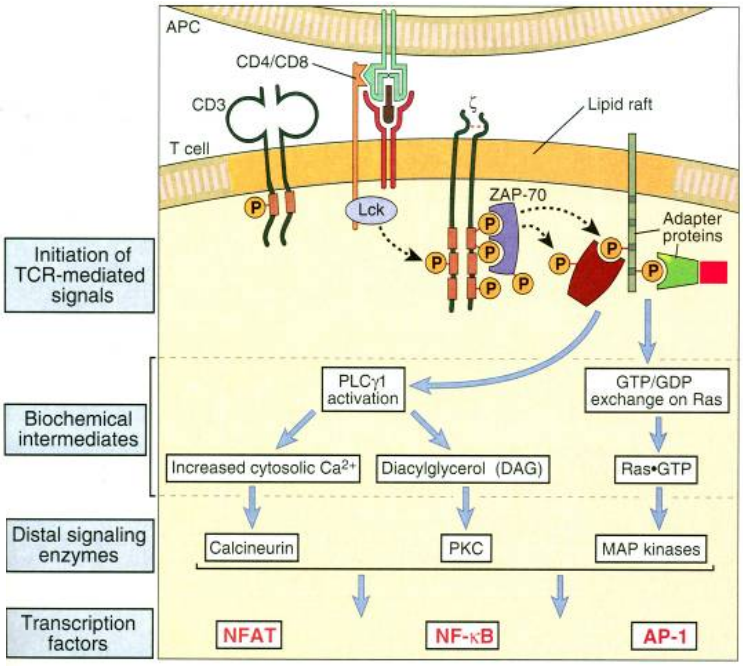
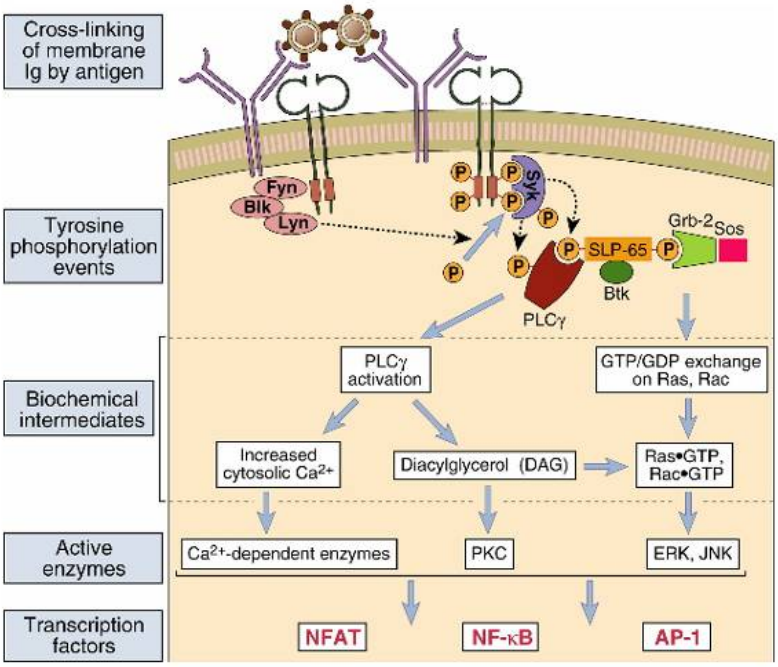


Figure 8-10 Immunobiology, 6/e. (© Garland Science 2005)

TCR-mediated Signal Transduction: A Tyrosine Kinase Cascade

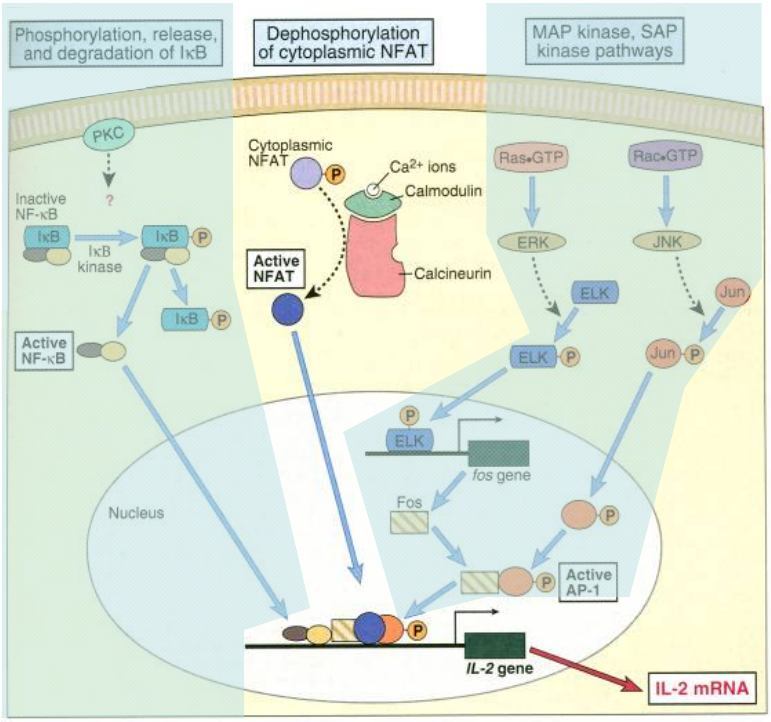


BCR-mediated Signal Transduction: A Tyrosine Kinase Cascade

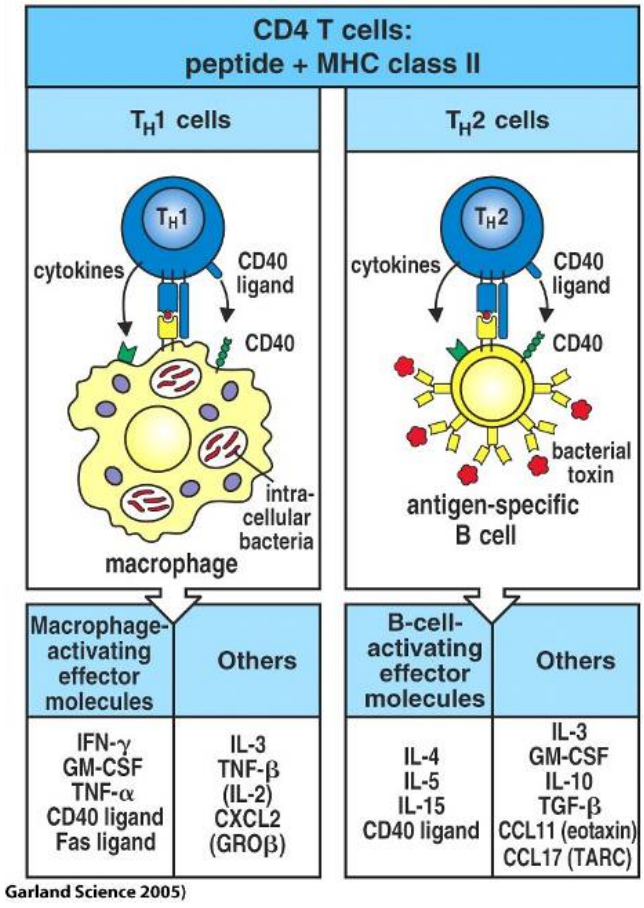


NFAT & TCR-mediated signal transduction culminate in cytokine production

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 immuno-suppressive drugs.



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



Garland Science 2005)

Other (T-cell) effector cytokines . . .

Important Th1 effector cytokines

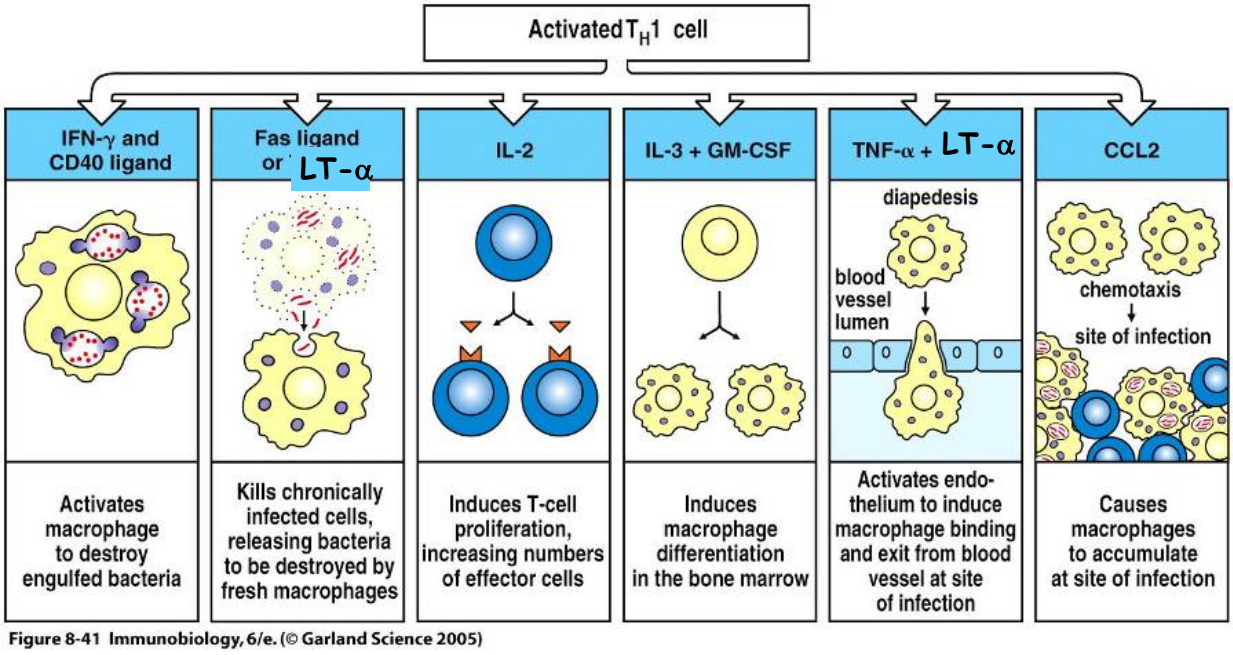


Figure 8-41 Immunobiology, 6/e. (© Garland Science 2005)

IFN-γ is the Th1 signature cytokine

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

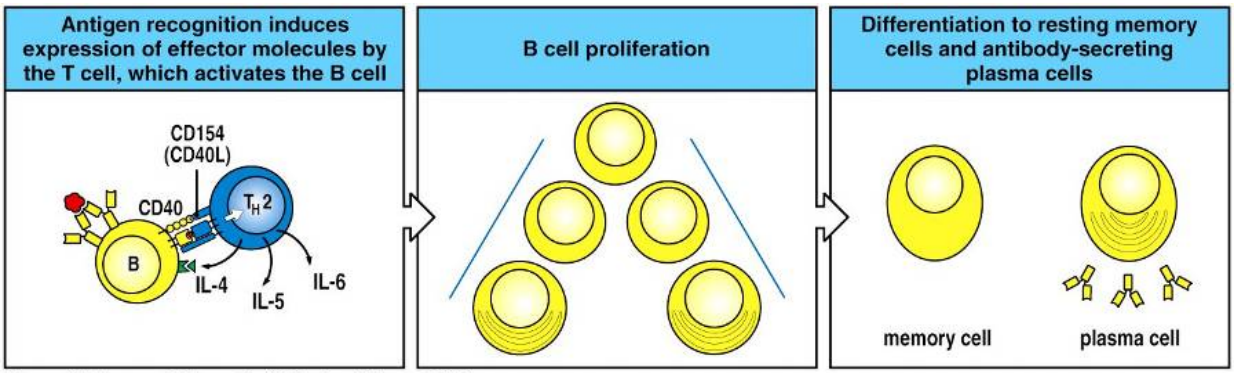
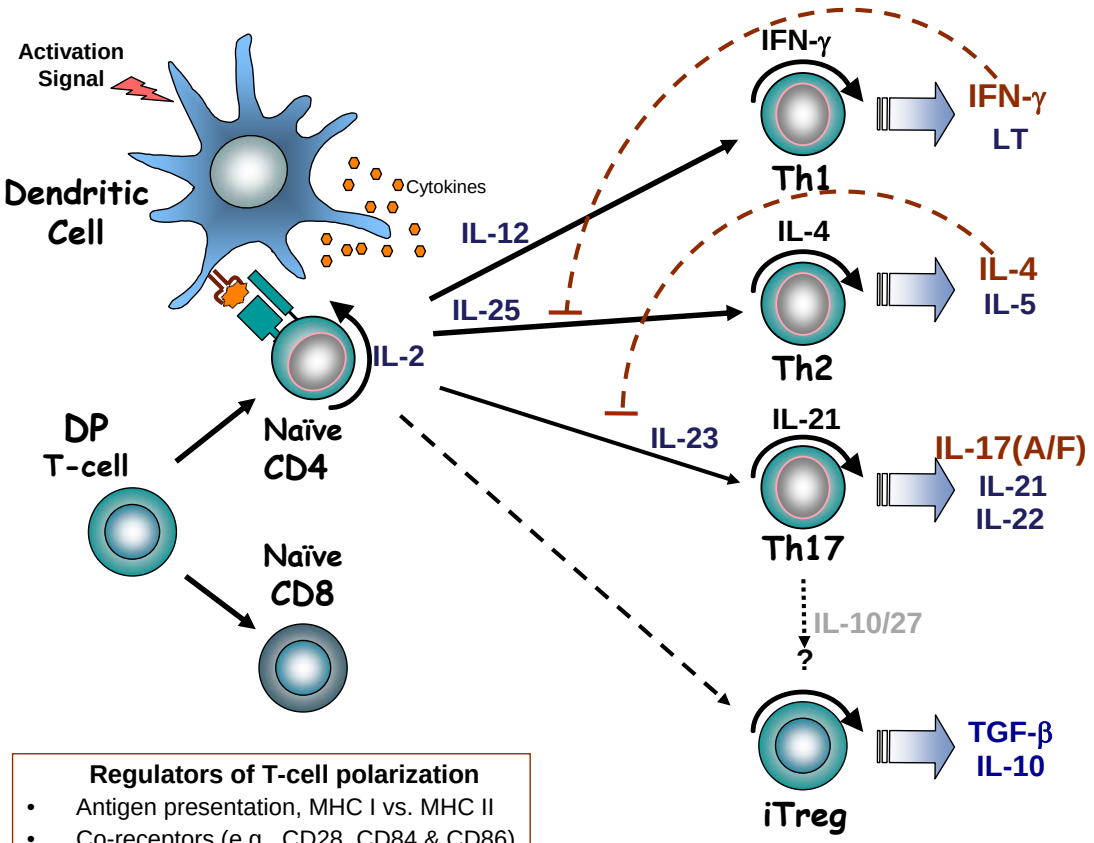


Figure 9-5 Immunobiology, 6/e. (© Garland Science 2005)

IL-4 the signatureTh2 effector cytokine

Negative Feedback Loops



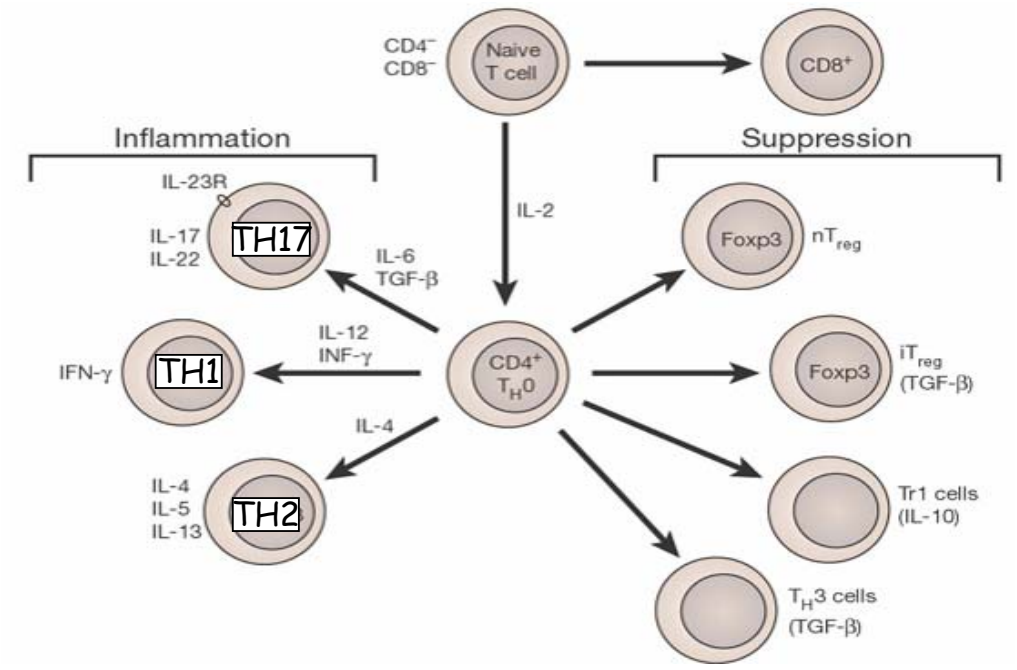
Regulators of T-cell polarization

- Antigen presentation, MHC I vs. MHC II
- Co-receptors (e.g., CD28, CD84 & CD86)
- Cytokines (and chemokines).

Were still learning about Th17 Cells

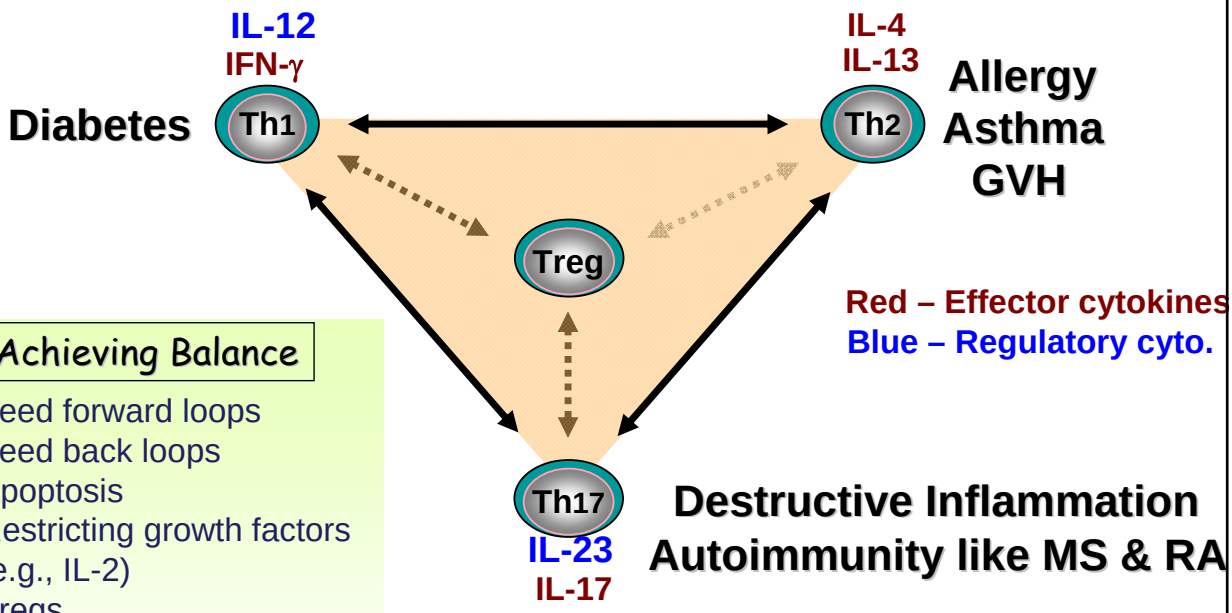
- An effector T-cell that arises from naïve CD4+ cells.
- Secretes IL-6, IL-22 and prodigious quantities of IL-17.
- Th17 cells evolved to combat pathogens not covered by Th1 (intracellular) or Th2 (helminths) cells.
- IL-17 deficient mice are highly susceptible to extra-cellular pathogens including Klebsiella, Borrelia and Citrobacter.
- IL-17 receptor is found on many cell types
  - IL-17 activates granulocytes (innate immunity)
  - IL-17 promotes cellular immunity by activating CD8 T-cells, NK cells and macrophages.
  - 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).
- Important in autoimmune disease like Multiple Sclerosis & Rheumatoid Arthritis.

The relative balance between effector T cells and regulatory T cells determines intestinal immunity vs tolerance



R. J. Xavier & D. K. Podolsky *Nature* 448, 427-434 (26 July 2007)

Failure to balance Th1, Th2 and Th17 cells



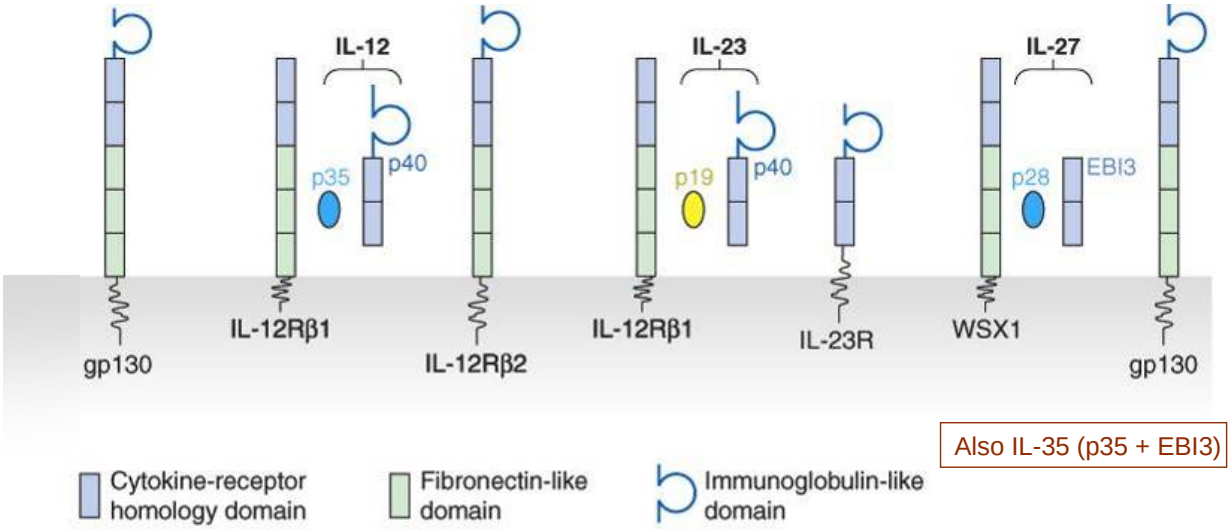
Red – Effector cytokines
Blue – Regulatory cyto.

Achieving Balance

- Feed forward loops
- Feed back loops
- Apoptosis
- Restricting growth factors (e.g., IL-2)
- Tregs
- Tolerogenic DCs & Macrophages
- Negative cytokines (IL-10, IL-27 & TGF-β)

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

IL-12, IL-23 and IL-27 ligand and receptors are structurally related & direct the development of distinct Th lineages



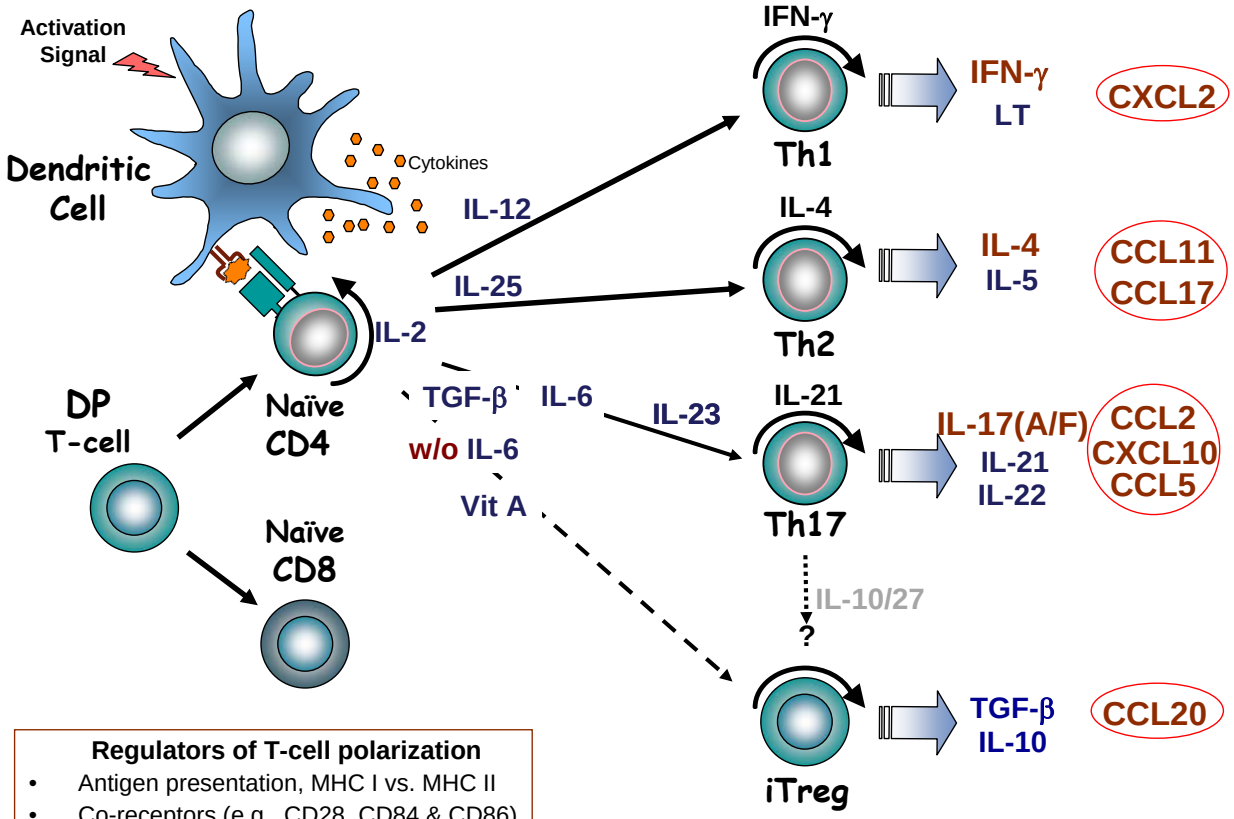
Also IL-35 (p35 + EBI3)

- Ligands and receptors are related to IL-6 which promotes both Th17 and Treg development
- IL-12 (secreted by APCs) promotes Th1 development.
- IL-23 (secreted by APCs) promotes the development of Th17 cells.
  - The presence of PGE_2 & purines favor the secretion of IL-23 over IL-12.
- IL-27 (secreted by some APCs) promotes the development of tolerogenic T-cells

Kastelein, RA, et al., Ann Rev. Imm., 2007

Chemokines

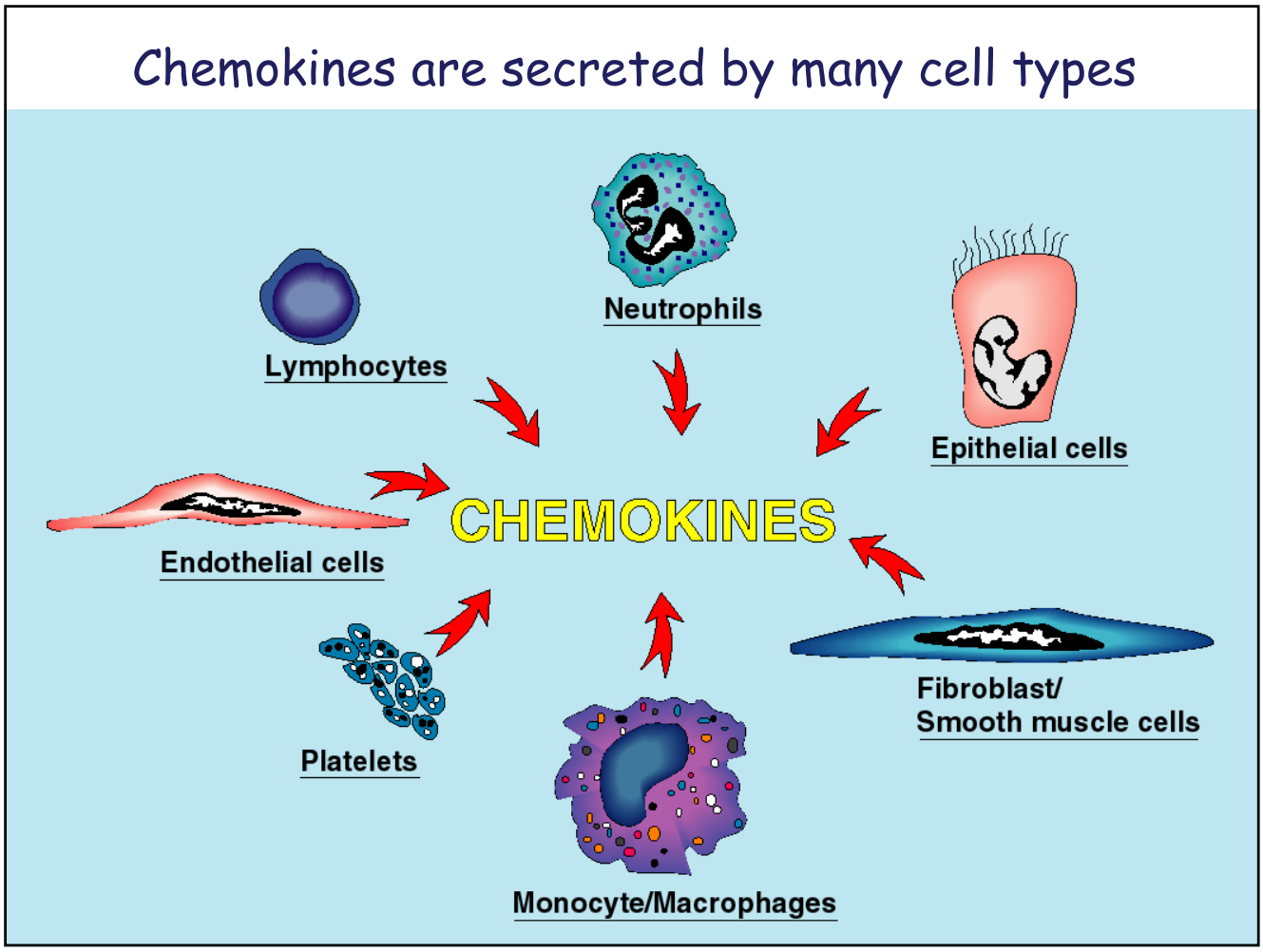
. . . and in the effector Th-cell paradigm . . . . . .



Regulators of T-cell polarization

- Antigen presentation, MHC I vs. MHC II
- Co-receptors (e.g., CD28, CD84 & CD86)
- Cytokines (and chemokines).

Chemokines are secreted by many cell types



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

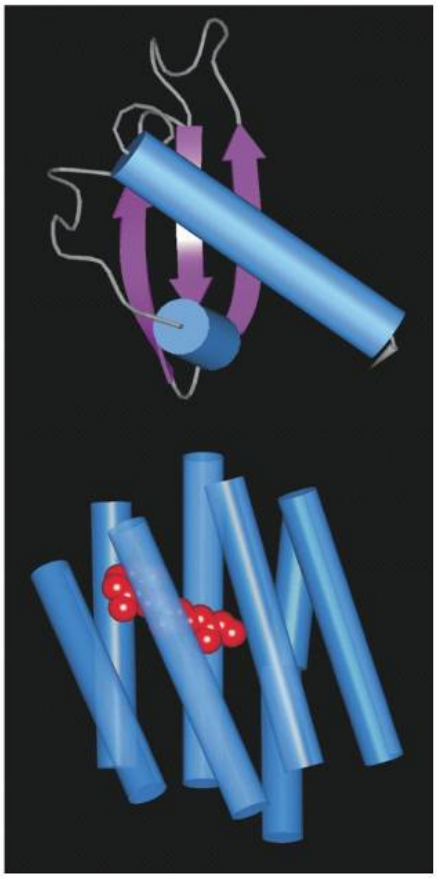
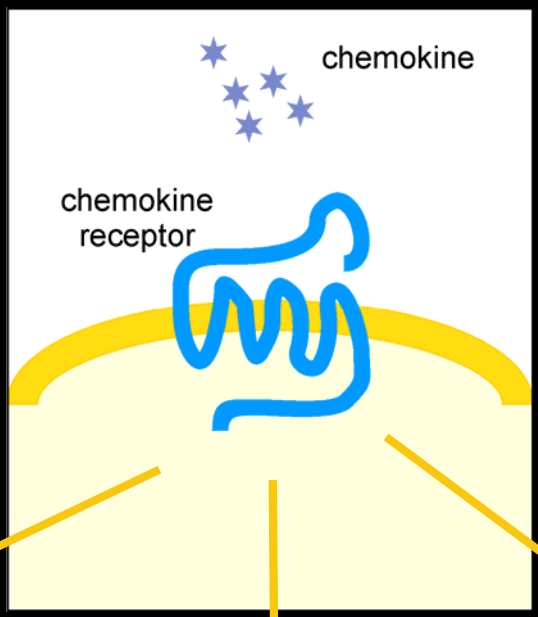


Figure 2-40 Immunobiology, 6/e. (© Garland Science 2005)

Chemokines are more than chemo-attractants

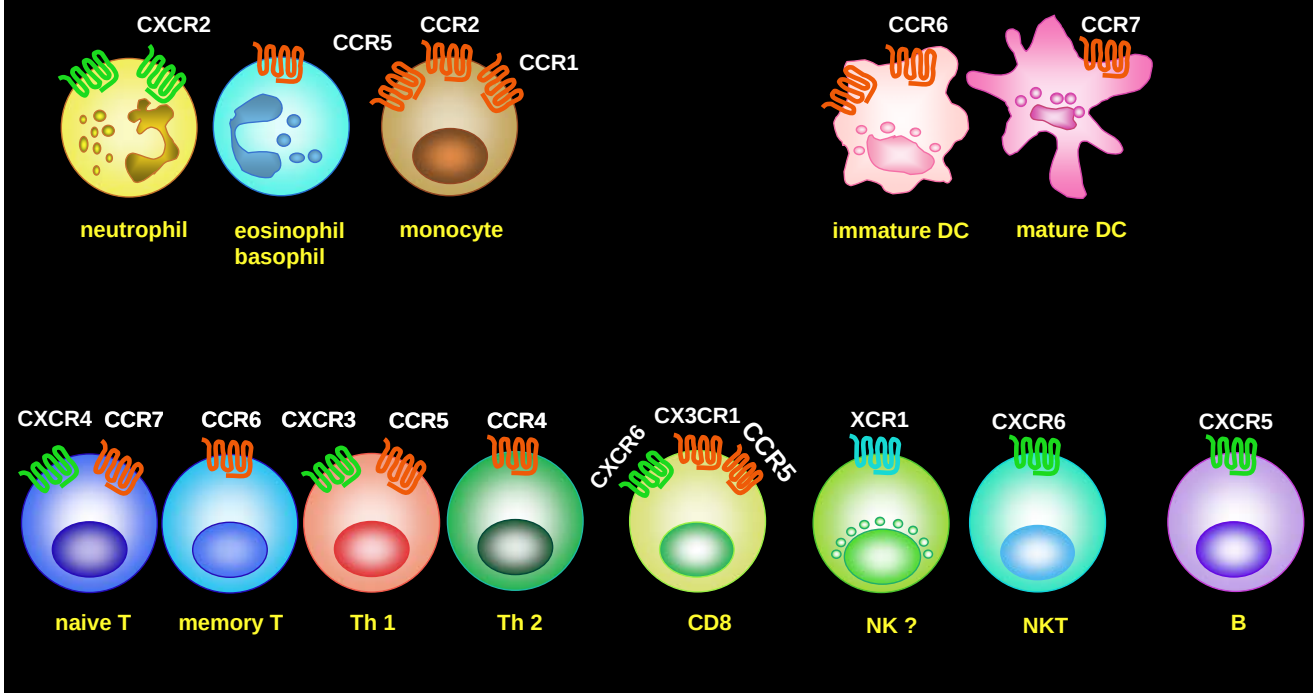


leukocyte trafficking

organogenesis

angiogenesis angiostasis

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

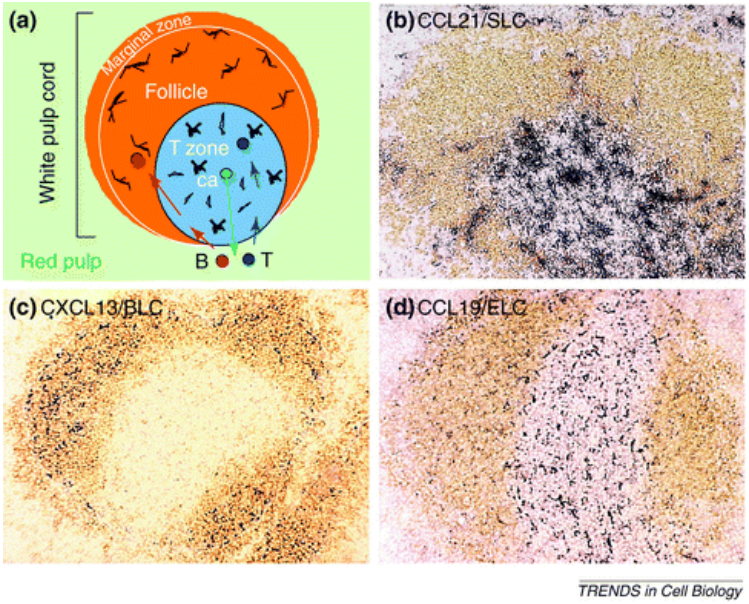


Functional Classification of Chemokines

- **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”

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

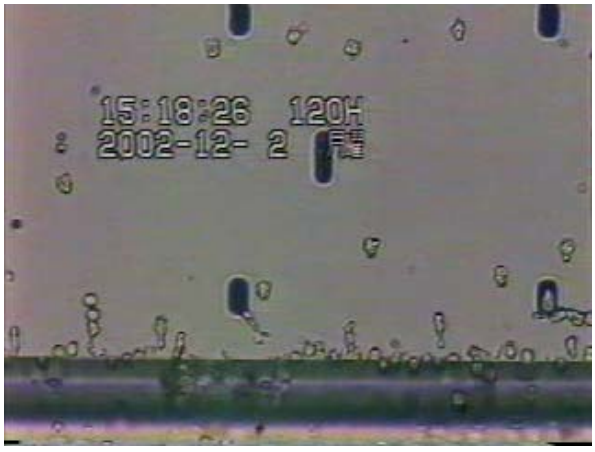
e.g. lymphocytes and APCs to spleen or LN



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



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) , LTB_4, PGD_2
  - Peptide-based, e.g., fMLP (bacterial-derived), C3a, C5a

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

Summary

1. Naïve CD4+ T-cells mature into several distinct T cell subsets in a process that is driven by antigen and cytokines. These subsets include Th1, Th2, Th17 and Treg cells. Critical cytokine dependent feed forward and feedback loops drive/regulate this process.

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 - secrete IL-4, which along with CD40, BAFF and MHC:Ag, potently activate B-cells. IL-4 also stimulates immunoglobulin class-switch IgG and IgE. 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. Tregs – negatively regulate T-cells through secretion of IL-10 and TGF-β (More on 9-19-08)

6. Chemokines are small proteins that activate G-protein-coupled receptors and are essential for homeostatic and inflammatory leukocyte trafficking. They also regulate other important activities in target cells. GPCRs make great drug targets.

Cytokines important in immune response

Type I & II Cytokine Receptors (Hematopoietin R.)

- IL-2 (**IL-2**, **IL-7**, IL-9, **IL-15** & IL-21)
- IL-4 (**IL-4** & IL-13)
- IL-6 (**IL-6**, IL-11, IL-31, LIF, OSM & **G-CSF**, etc.)
- IL-10 (**IL-10**, IL-19, IL-20, IL-22, IL-24 & IL-26)
- IL-12 (**IL-12**, **IL-23** & IL-27)
- **IFN-γ**
- IFN-I (**IFN-α**s, **IFN-β**, IFN-ω & IFN-λs)

Toll (TLR) /IL-1 Receptors

- **IL-1**
- **IL-18**

TNF Related Receptors

- LT-α
- **TNF**
- **CD40L**
- **FasL**
- BAFF

TGF-β Receptors

- **TGF-β** (very large family)

Chemokine Receptors

- Chemokines (*see above*)
  - Inflammatory
  - Non-inflammatory