Lecture 10

T-cell Effector Mechanisms-II: T-cell Polarization and 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 and chemokines?

- Small (10-30 kDa), usually secreted and usually glycosylated peptides.
- They bind specific, high affinity (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 functionally related Families

- Cytokines can be divided into 6 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 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-β, BAFF)
- TGF-β Family (e.g., TGF-β)
- Chemokines (e.g., CC and CXC families)
- Type I & II Cytokines (a.k.a., Hematopoietins or 4 Helix Bundle Cytokines; e.g., IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, IFN-γ, IFN-α/β)
- Also steroid hormones and prostaglandins

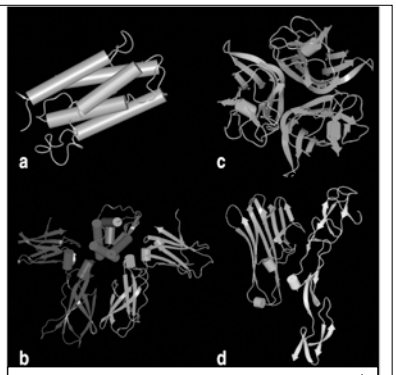
*Underlined cytokines are of particular importance

Cytokine Receptor Classes

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: TNR-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 Gα · GTP from Gβγ, Gα · GTP activates various cellular enzymes

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



IL4/IL4 Receptor

TNF-α Monomer/ Receptor Monomer

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

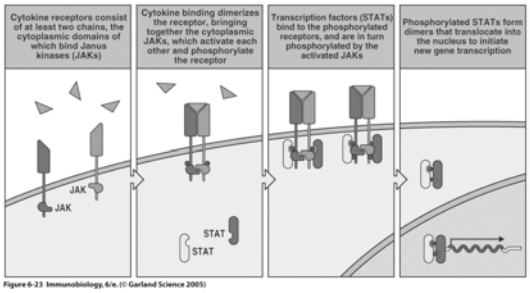


Figure 6-23 Immunobiology, 6/e. (© Garland Science 2005)

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")

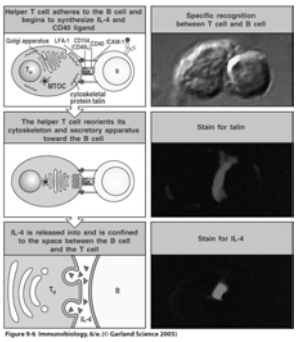
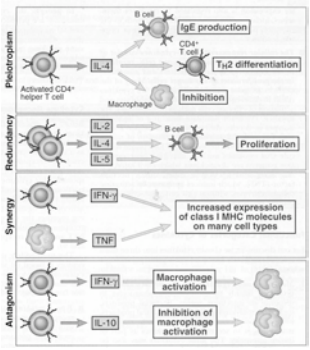


Figure 9-6 Immunobiology, 6/e. (© Garland Science 2005)

Important general properties of Cytokines and Chemokines

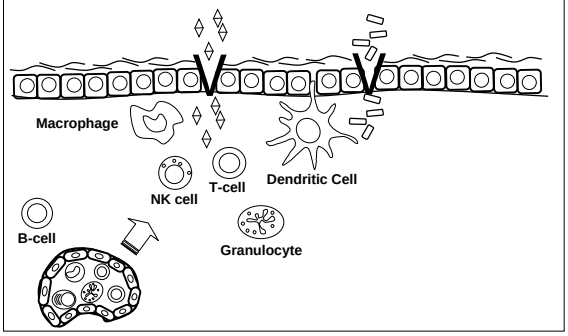
- Usually stimulate transient responses.
- Function at three ranges:
  - Autocrine - "self"
  - Paracrine - adjacent cells
  - Endocrine - through circulatory system
- **Pleitropism** - one ligand activate numerous types of responses (e.g., differentiation, growth & activation).
- **Redundancy** - two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

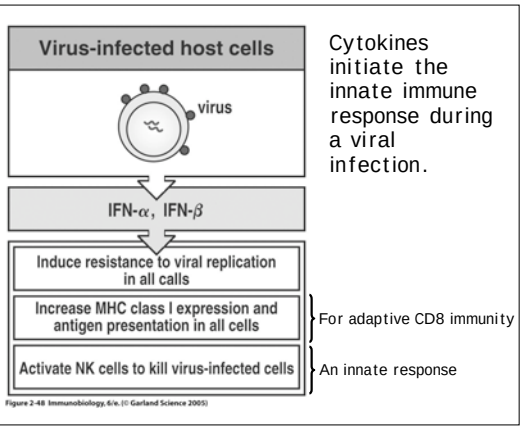
Properties of Cytokines



Some Biology . . . . . .

How do we protect ourselves from microbes?
The antiviral response





Cytokines initiate the innate immune response during a viral infection.

For adaptive CD8 immunity

An innate response

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Innate response to Virus

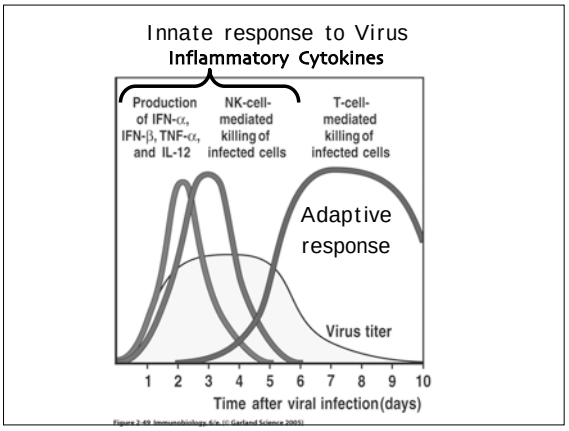


Figure 2-49 Immunobiology, 6/e. (© Garland Science 2005)

Cytokines and the evolving Th1-Th2 paradigm

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

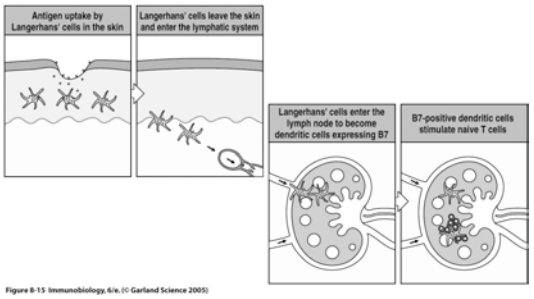
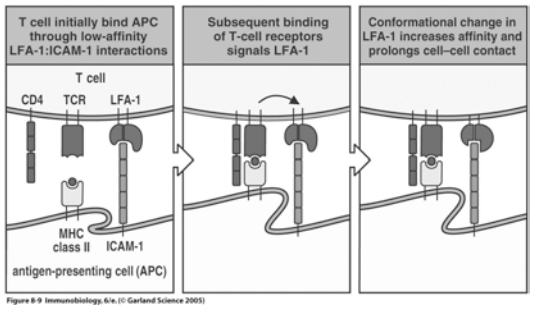
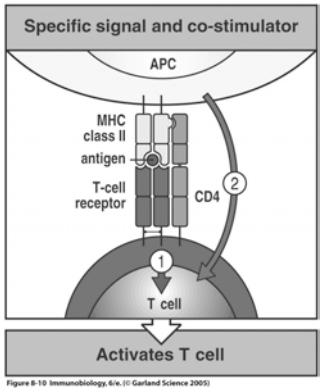


Figure 8-15 Immunobiology, 6/e. (© Garland Science 2005)

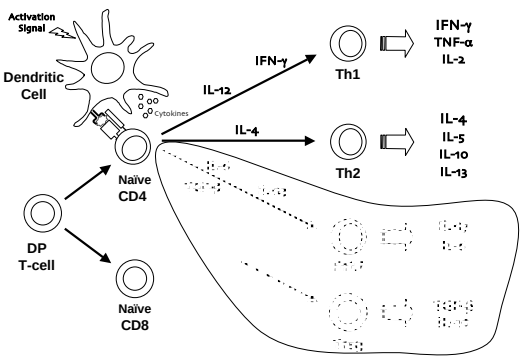
Naïve CD4 cells kiss APCs to sample them for right antigen ($1<10^4-10^6$)



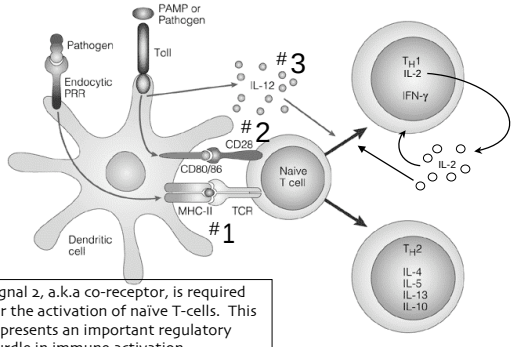
Signal #1 (TCR) and Signal #2 (coreceptors) direct activation of naïve T-cells.



Cytokines and T-cell polarization

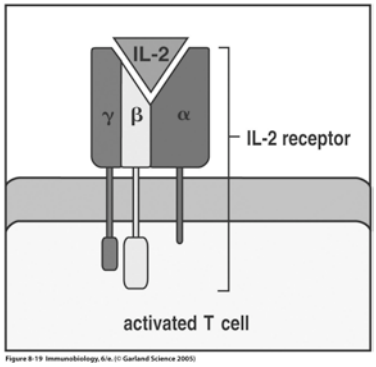


Cytokines direct Th1-Th2 polarization



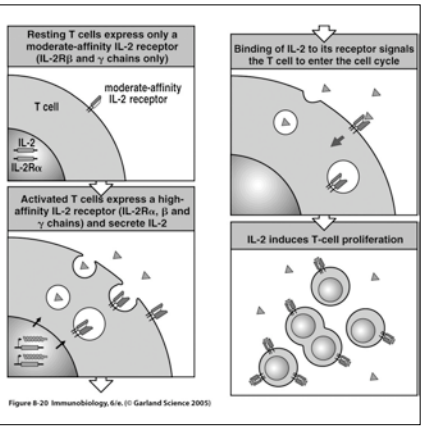
Signal 2, a.k.a co-receptor, is required for the activation of naïve T-cells. This represents an important regulatory hurdle in immune activation.

The important IL-2 "autocrine loop"

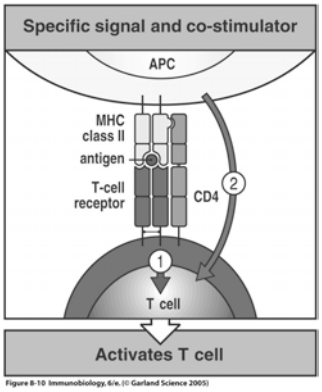


The IL-2 autocrine loop

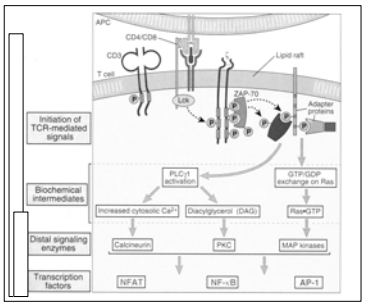
TCR stimulation leads to induction of IL-2 and IL-2 receptor α-chain to generate high affinity receptor, culminating in potent T-cell proliferation.



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

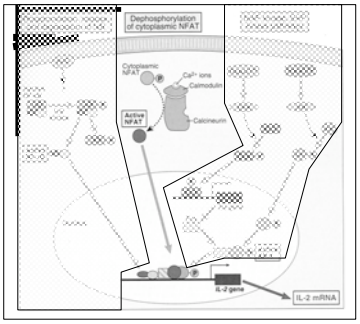


TCR-mediated Signal Transduction: A Tyrosine Kinase Cascade

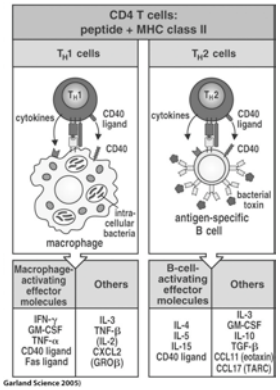


NFAT & TCR-mediated signal transduction culminate in cytokine production

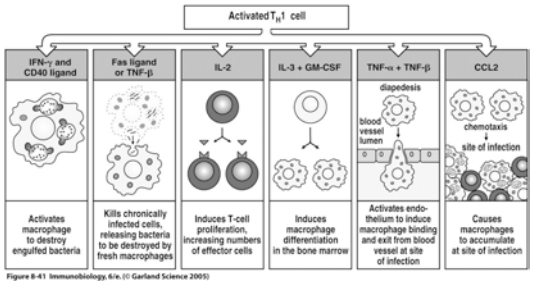
Cyclosporin A (CyA) & Tacrolimus (FK506) are two important drugs that block calcineurin and therefore the activation NFAT and the subsequent expression of **IL-2** production! Thus, they are potent immuno-suppressive drugs.



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

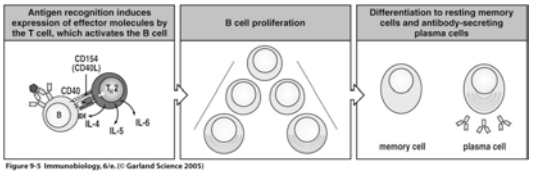


Important Th1 effector cytokines

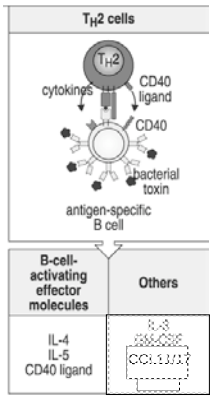


IFN-γ is the Th1 signature cytokine

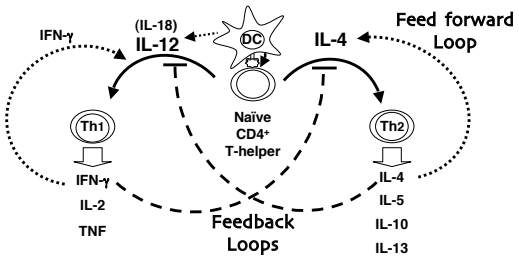
Important Th2 effector cytokines IL-4, IL-5 & IL-6 promote humoral immunity



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

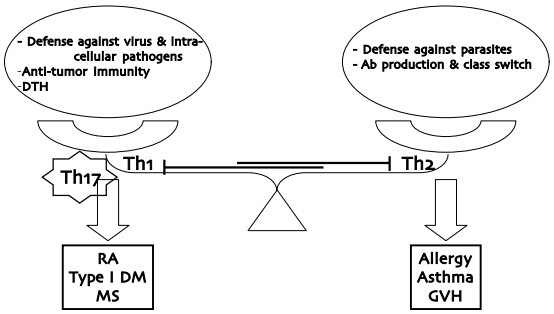


Polarization of CD4+ T-cells into functional Th1 and Th2 subsets



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

Failure to balance Th1 and Th2



RA: Rheumatoid arthritis
DM: Dibetes mellitus
MS: Multiple sclerosis
GVH: Graft-vs-Host disease
DTH: Delayed-type hypersensitivity

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

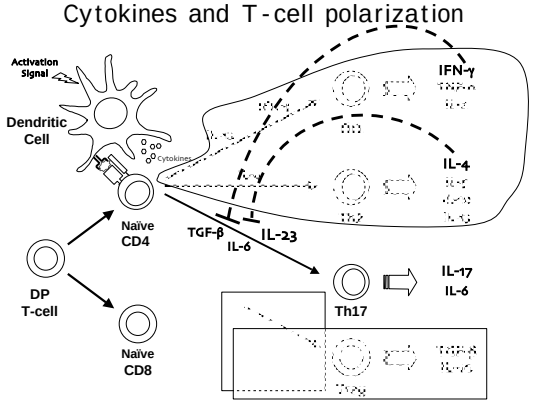
The Th17 Cell

- 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).

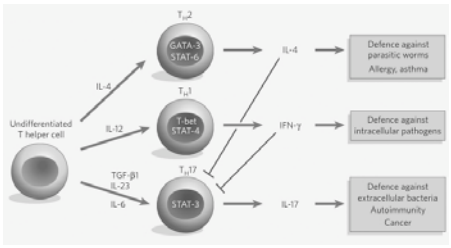
Th17 Cell Maturation

- Antigen plus TGF-β and IL-6 direct CD4 T-cells into the Th17 lineage.
- IL-23 is also essential for Th17 maturation /activty.
- IL-23 is 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



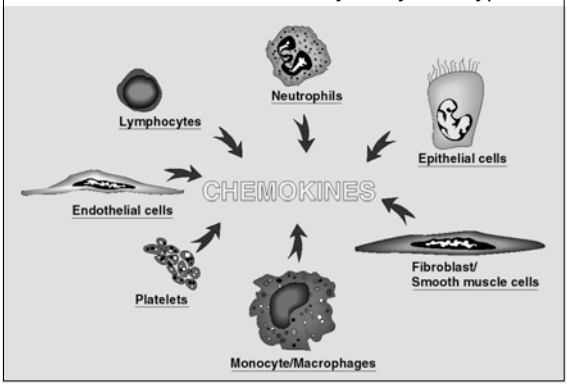
IL-17 Plays Important Roles in Autoimmunity and Cancer



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

Chemokines

Chemokines are secreted by many cell types



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

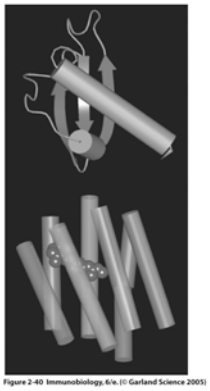
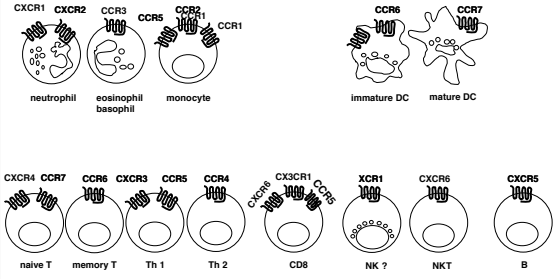
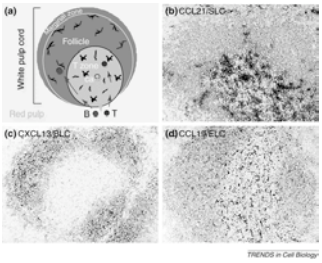


Figure 2-40 Immunobiology, 6/e. (© Garland Science 2005)

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

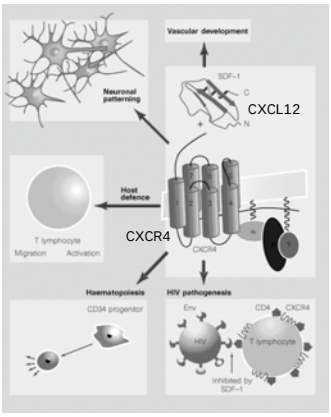


Homeostatic targeting of lymphocytes and APCs in the spleen



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

Chemokines are much more than just chemo-attractants



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, but be familiar with the broad biological properties described

Summary

1. Naive T-cells differentiate into distinct T cell subsets. Among the most important of Th1, Th2, and Th17 cells.
2. Th1 cells secrete IFN-γ and IL-2. IFN-γ is the predominant cytokine that activates macrophages to produce pro-inflammatory cytokines. IFN-γ often synergizes with innate immune stimuli (e.g., LPS). Th1 cells play important roles in acute bacterial and viral infections and are essential effectors of "Delayed Type Hypersensitivity," or DTH, which is characterized by the presence of IFN-γ-activated macrophages. IL-2 is required for proliferation of T-cells. Pathways leading to IL-2 production, especially those that activate NF-AT, are attractive drug targets (e.g., cyclosporin and FK506).
3. B-cells, via CD40 and MHC-peptide, activate T cells to release cytokines that activate B cells (e.g., IL-4). IL-4, in concert with CD40L on activated T-cells, stimulate B-cells to undergo class-switching to IgG and IgE. IL-4, the prototypical Th2 cytokine, is important to immunity against parasites (e.g., helminths)
4. During T-cell polarization, negative feedback loops regulate T-cell differentiation: IL-4 antagonizes the outgrowth of Th1 cells and IFN-γ antagonizes the outgrowth of Th2 cells.
5. Th17 cells stimulate neutrophils during acute bacterial infections and many other cells during chronic inflammation (e.g., in autoimmunity).
6. Chemokines are small proteins that activate G protein-coupled receptors and are essential for leukocyte trafficking. Collectively, they have multiple roles in many cell types besides directing traffic.