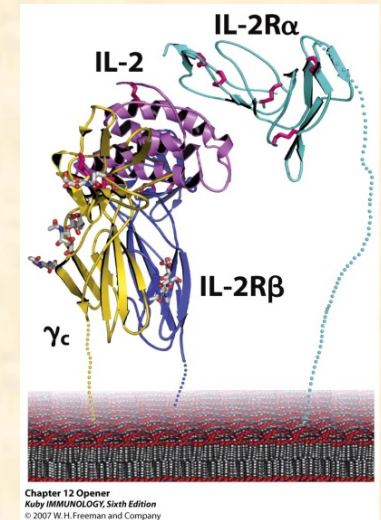


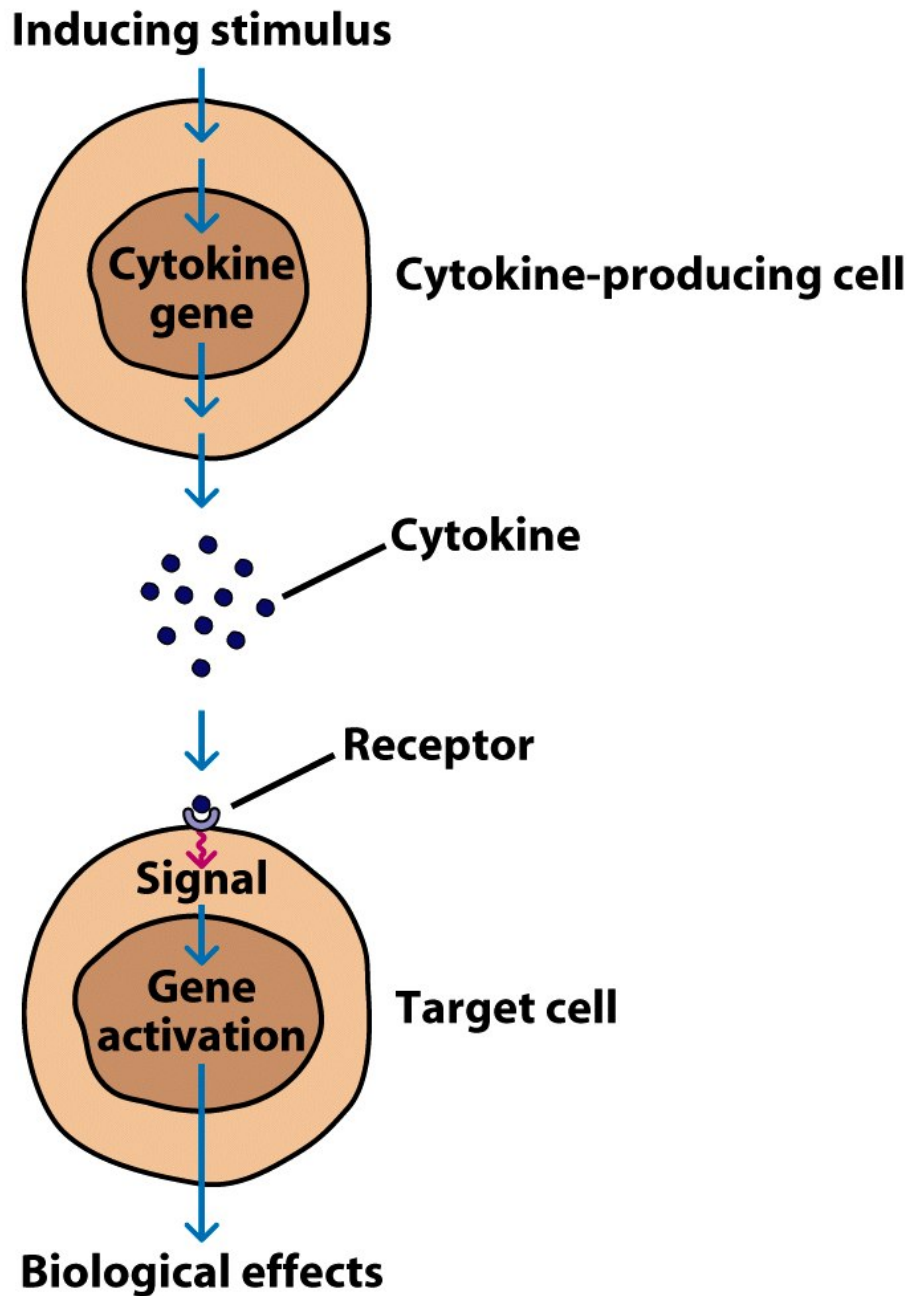
Kuby, Ch. 12
CYTOKINES

April 2013

Cytokines

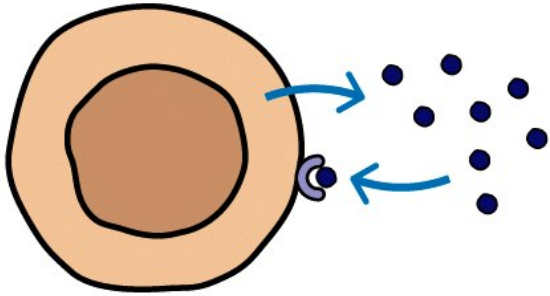
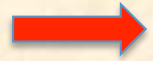
- Low-molecular weight regulatory proteins or glycoproteins
- Secreted by WBC and various other cells
- Assist in regulating development of immune effector cells
 - Some possess direct effector functions of their own
- Referred to as Interleukins
 - IL-1 through IL-29 have been described



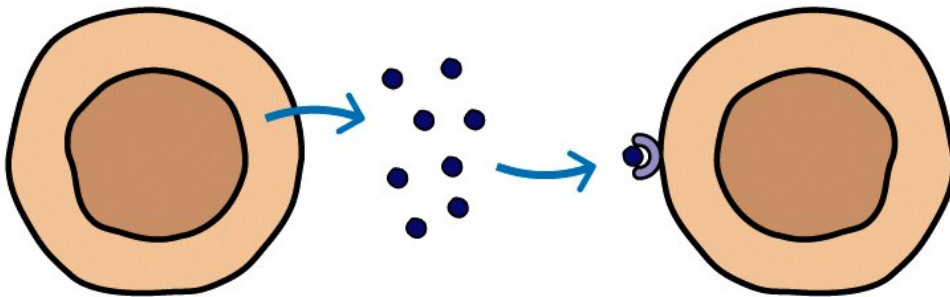
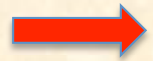


- ◎ Cytokines bind to specific receptors on **outside**
- ◎ **Inside**: Trigger signal transduction pathways that alter gene expression in target cells
- ◎ **HOW?**
- ◎ Exhibit pleiotropy, redundancy, synergy, antagonism, cascade induction

Figure 12-1a
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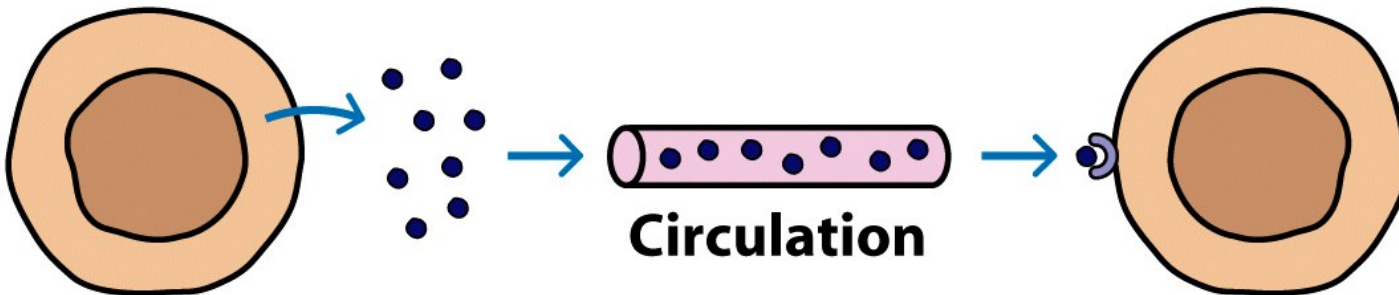


Autocrine action



Paracrine action

Nearby cell



Endocrine action

Circulation

Distant cell

Figure 12-1b
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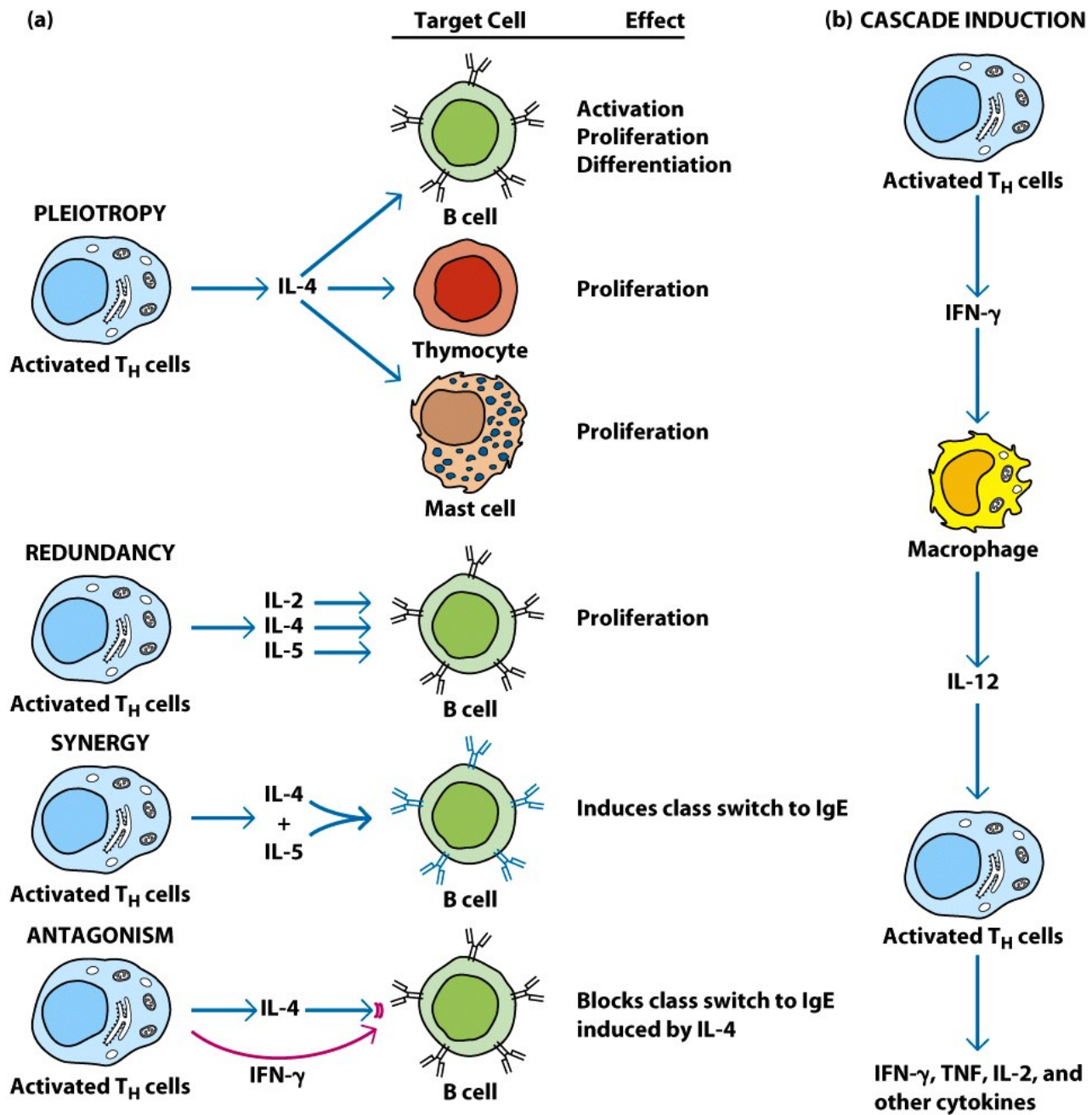


Figure 12-2
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- Activity was 1st recognized in 1960s
 - Supernatants from in vitro cultures of lymphocytes were found to contain soluble factors
 - Could regulate proliferation, differentiation, maturation of immune cells
 - Purification of these was hampered because of low concentration in culture
 - Gene-cloning techniques changed this – made it possible to produce pure cytokines

- ELISA using monoclonal antibodies

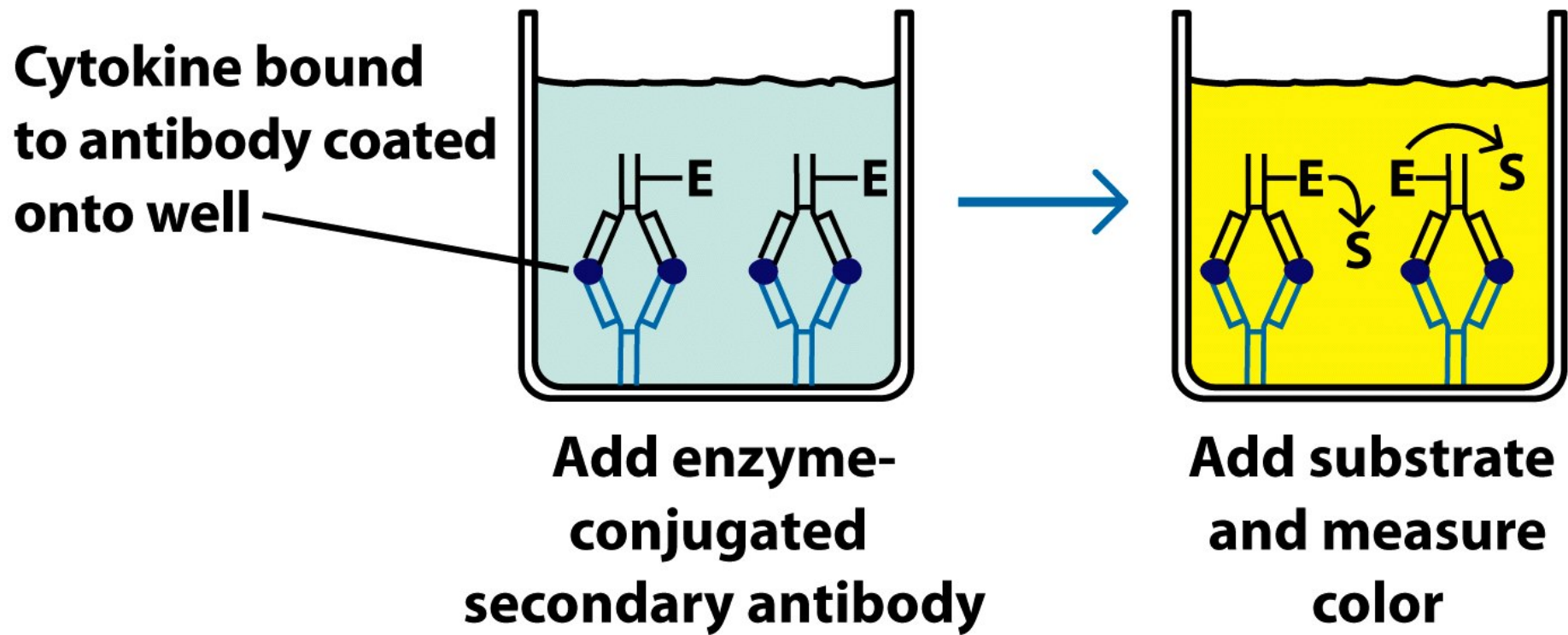
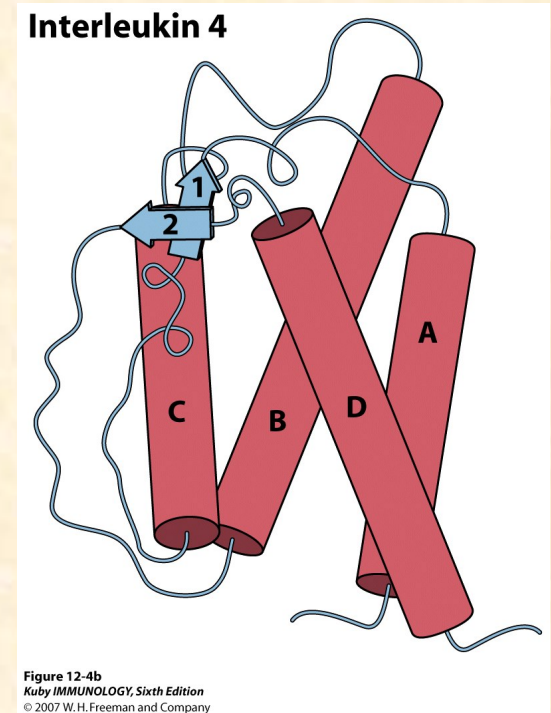
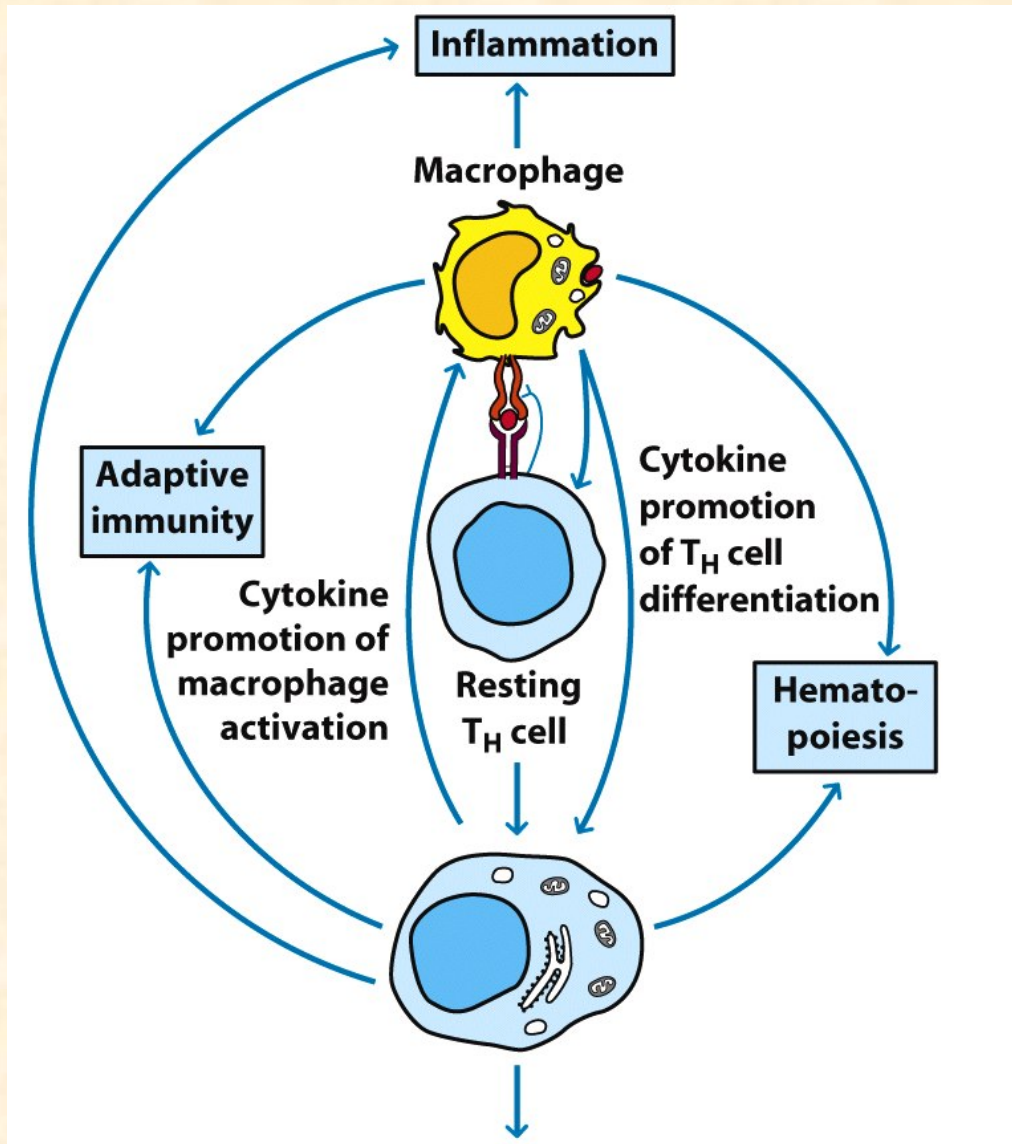


Figure 12-3a
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- Cytokines belong to 4 families
 - Hematopoietin family
 - Interferon family
 - Chemokine family
 - Tumor necrosis family



- Based on structural studies
- All have molecular mass less than ($<$) 30kDa
- All have similarities and few rarely act alone



T_H
 DC
 MΦ

Cytokine promotion of activation, differentiation, proliferation, or cell death of T cells, B cells, macrophages, dendritic cells, NK cells, and other leukocytes.

Figure 12-5
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PHYSIOLOGIC RESPONSES OF CYTOKINES

- Development humoral and cellular immune responses
- Induction of inflammatory response
- Regulation of hematopoiesis
- Control of cell proliferation and differentiation
- Healing of wounds

Their action is not antigen specific but the process that created them initially was antigen specific

SOME CYTOKINE ACTIVITIES

SPECIFIC OR NOT?

TABLE 12-1 Functional groups of selected cytokines*		
Cytokine†	Secreted by‡	Targets and effects
SOME CYTOKINES OF INNATE IMMUNITY		
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor necrosis factor- α (TNF- α)	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T _H 1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon α (IFN- α) (this is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN- β)	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
SOME CYTOKINES OF ADAPTIVE IMMUNITY		
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T _H 2 cells, mast cells	Promotes T _H 2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T _H 2 cells	Eosinophil activation and generation
Transforming growth factor β (TGF- β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgA; inhibits macrophages
Interferon γ (IFN- γ)	T _H 1 cells, CD8 ⁺ cells, NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation
<p>*Many cytokines play roles in more than one functional category. †Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine. ‡Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.</p>		

- Non-specificity of cytokines conflicts with established specificity of immune system
 - How does this work?
 - Careful expression of the receptors for those cytokines on specific cells
 - Receptors are often only expressed after exposure to antigen

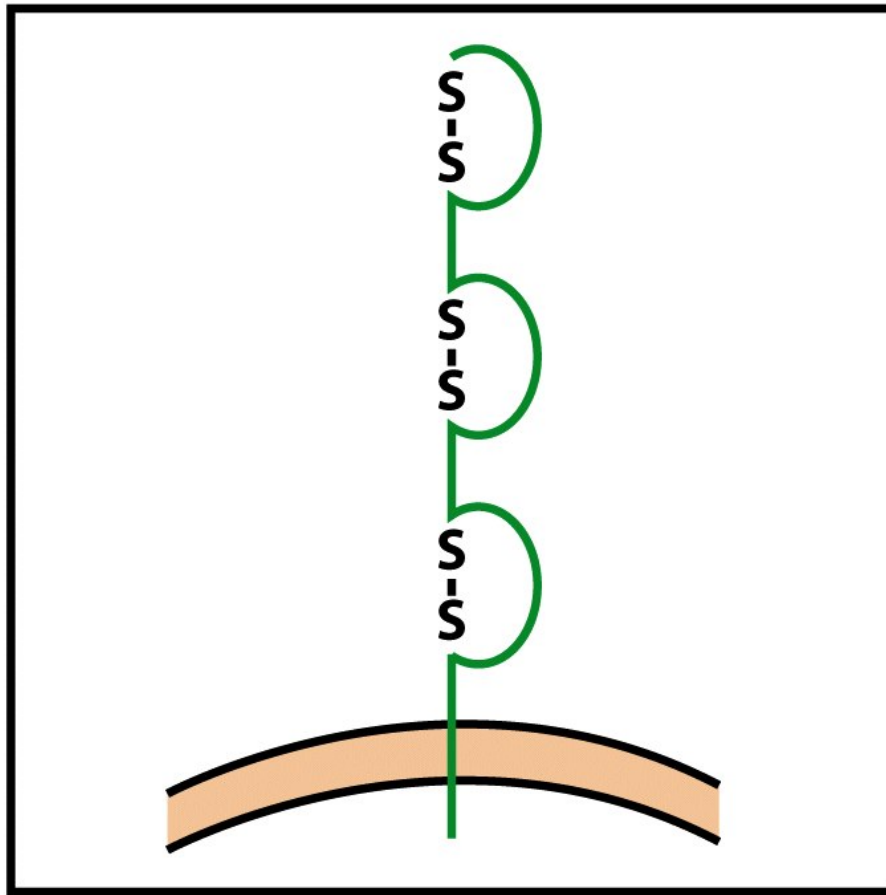
TOPIC CHANGE: Cytokine Receptors

- Cytokine receptors fall into 5 families
 - Immunoglobulin superfamily receptors
 - Class I cytokine receptor family (hematopoietin)
 - Class II cytokine receptor family (interferon)
 - TNF receptor family
 - Chemokine receptor family

RECEPTOR FAMILY

LIGANDS

Immunoglobulin superfamily receptors

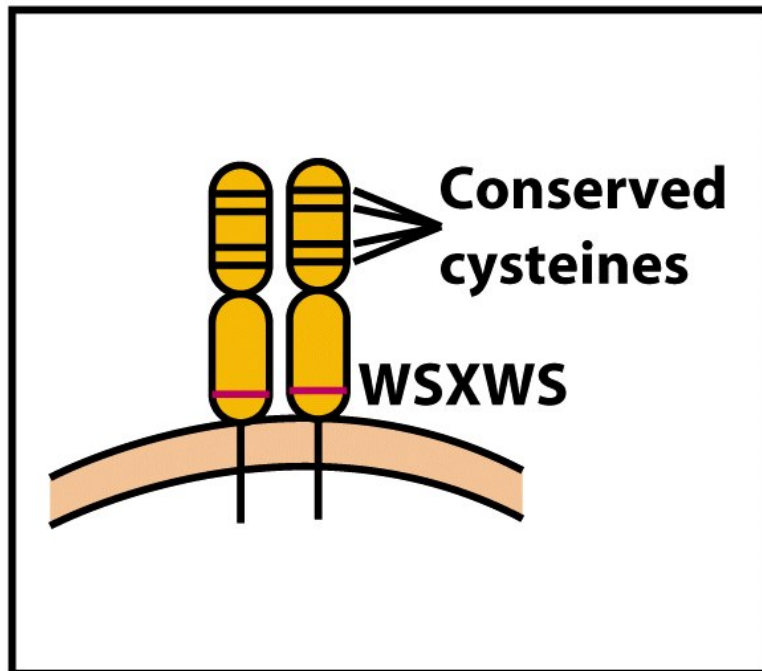


IL-1
M-CSF
C-Kit
IL-18

Figure 12-6a
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RECEPTOR FAMILY

Class I cytokine receptors (hematopoietin)



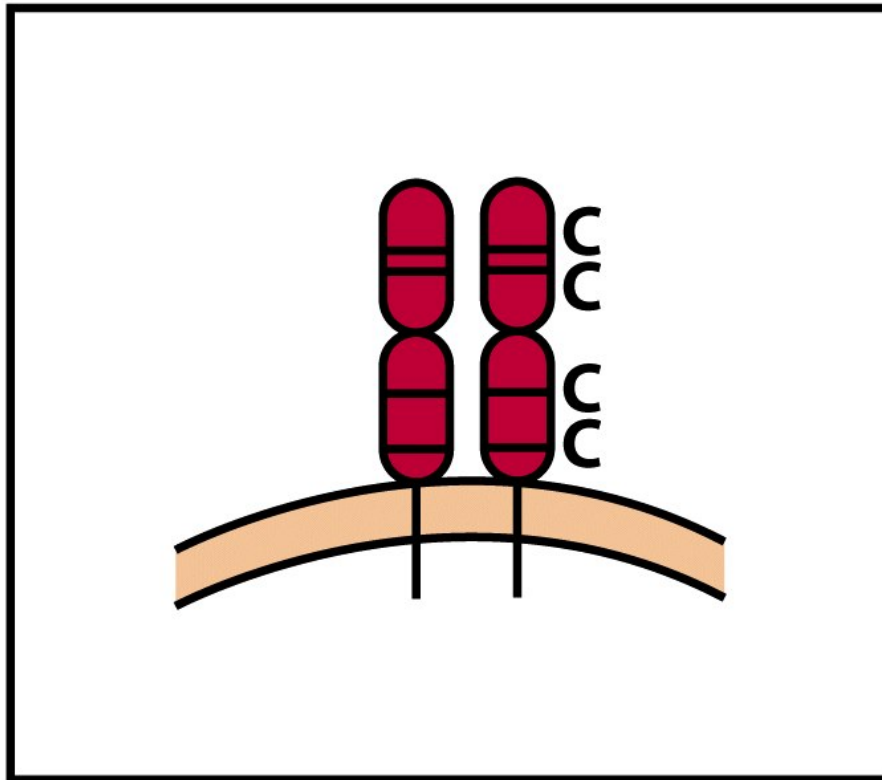
LIGANDS

IL-2	IL-21
IL-3	IL-23
IL-4	IL-27
IL-5	GM-CSF
IL-6	G-CSF
IL-7	OSM
IL-9	LIF
IL-11	CNTF
IL-12	Growth hormone
IL-13	Prolactin
IL-15	

Figure 12-6b
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RECEPTOR FAMILY

Class II cytokine receptors (interferon)



LIGANDS

IFN- α

IFN- β

IFN- γ

IL-10

IL-19

IL-20

IL-22

IL-24

IL-26

IL-28

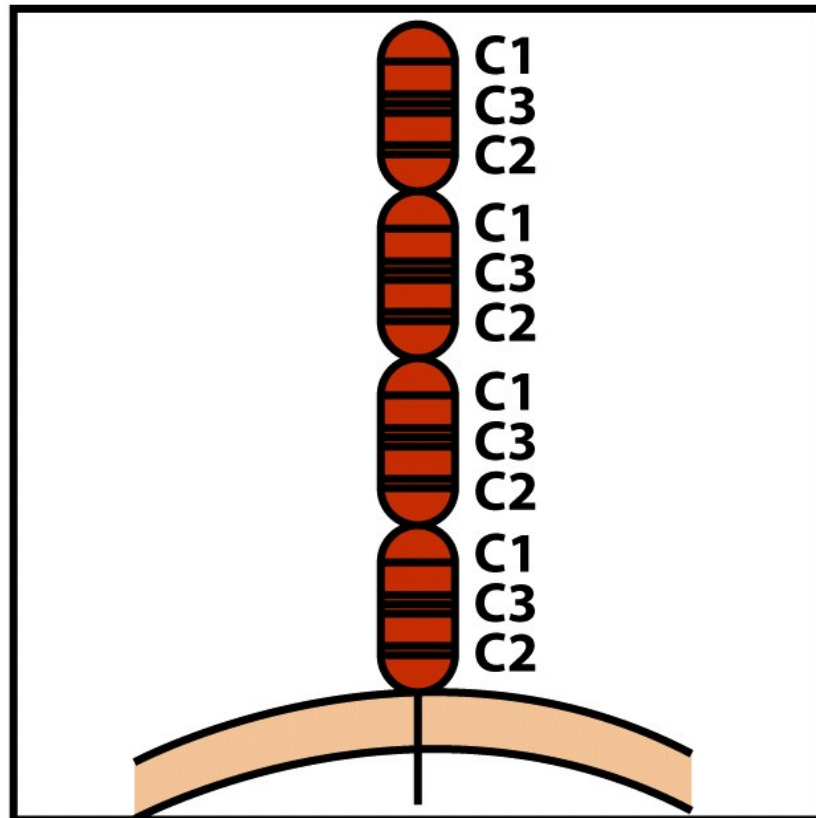
IL-29

Figure 12-6c
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RECEPTOR FAMILY

LIGANDS

TNF receptors



TNF- α

TNF- β

CD27L

CD30L

CD40L

Nerve growth factor (NGF)

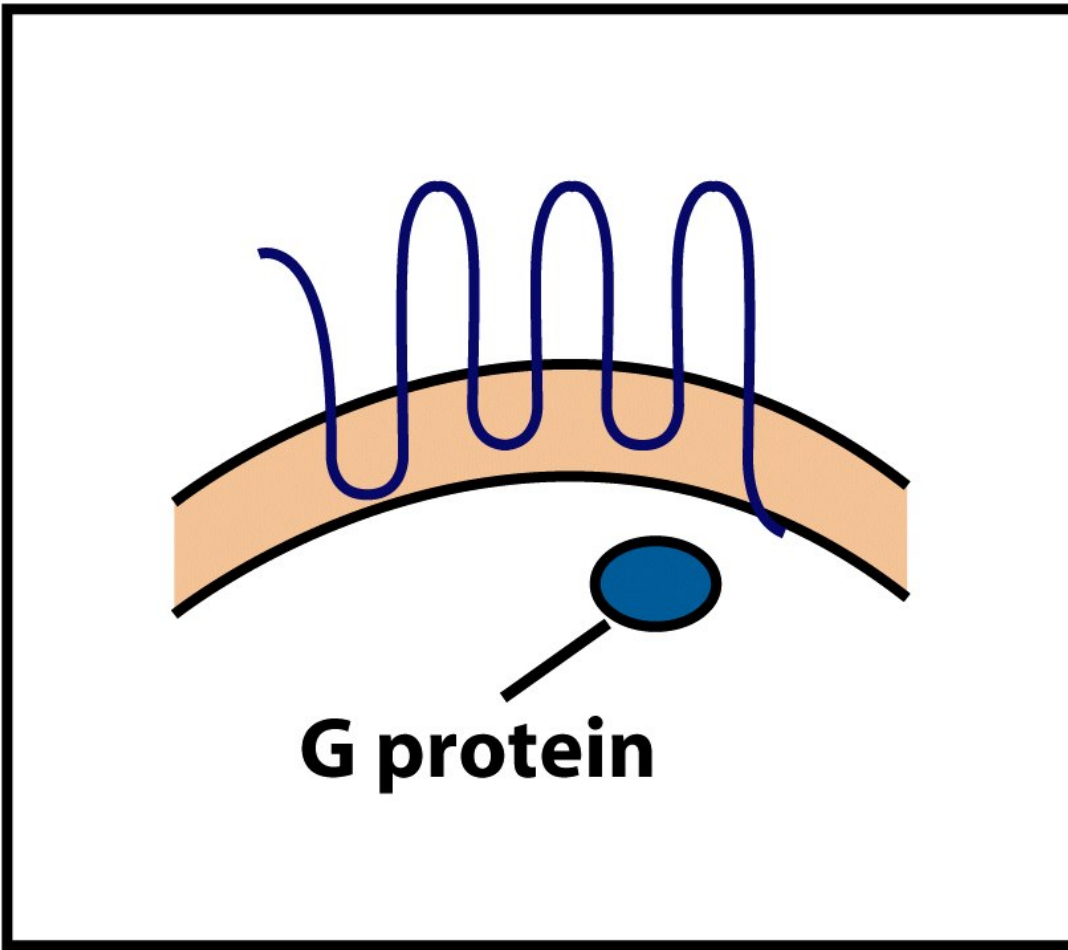
FAS

Figure 12-6d
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RECEPTOR FAMILY

LIGANDS

Chemokine receptors



- IL-8**
- RANTES**
- MIP-1**
- PF4**
- MCAF**
- NAP-2**

Figure 12-6e
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HOMework QUESTION 1

**WHAT IS THE DIFFERENCE BETWEEN CLASS 1
AND CLASS 2 CYTOKINE RECEPTORS**

and

**IMMUNOGLOBULIN, TNF, AND CHEMOKINE
RECEPTORS?**

Due Friday, beginning of class

RECEPTOR FAMILY

Class I cytokine receptors (hematopoietin)

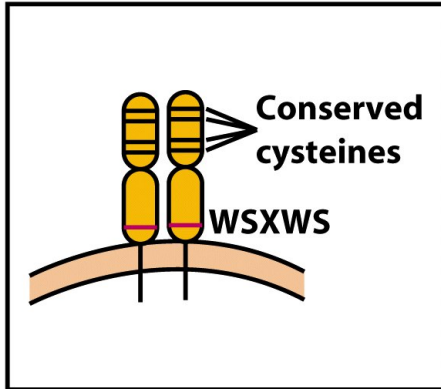


Figure 12-6b
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LIGANDS

IL-2 IL-21
 IL-3 IL-23
 IL-4 IL-27
 IL-5 GM-CSF
 IL-6 G-CSF
 IL-7 OSM
 IL-9 LIF
 IL-11 CNTF
 IL-12 Growth hormone
 IL-13 Prolactin
 IL-15

RECEPTOR FAMILY

Immunoglobulin superfamily receptors

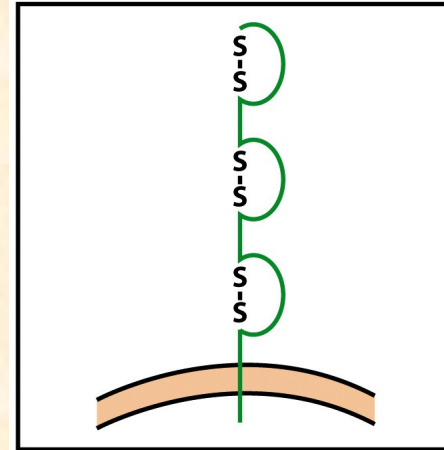


Figure 12-6a
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LIGANDS

IL-1
 M-CSF
 C-Kit
 IL-18

RECEPTOR FAMILY

Class II cytokine receptors (interferon)

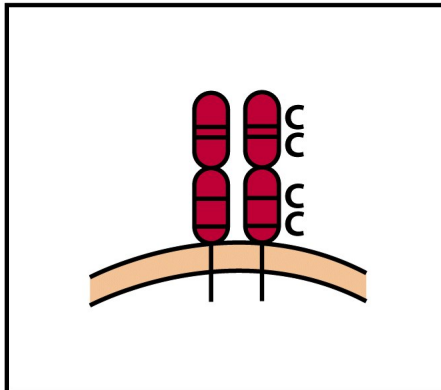


Figure 12-6c
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LIGANDS

IFN- α
 IFN- β
 IFN- γ
 IL-10
 IL-19
 IL-20
 IL-22
 IL-24
 IL-26
 IL-28
 IL-29

RECEPTOR FAMILY

TNF receptors

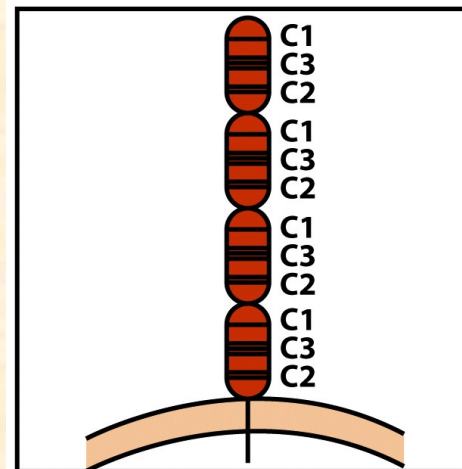
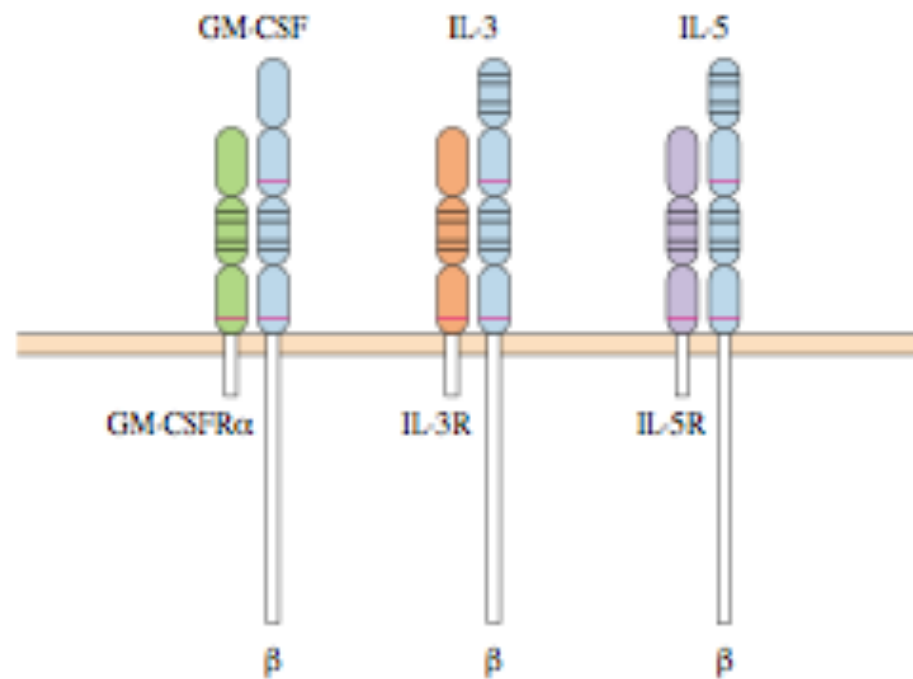


Figure 12-6d
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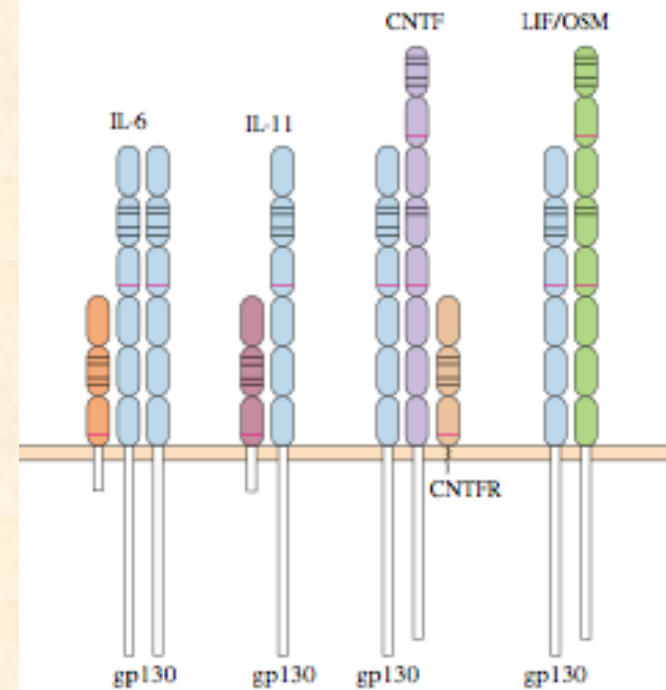
LIGANDS

TNF- α
 TNF- β
 CD27L
 CD30L
 CD40L
 Nerve growth factor (NGF)
 FAS

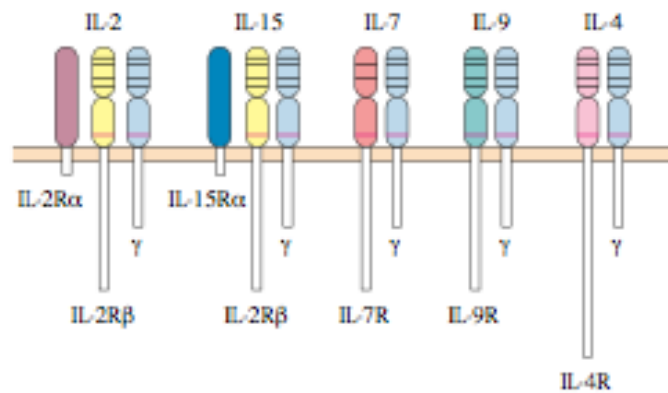
(a) GM-CSF receptor subfamily (common β subunit)



(b) IL-6 Receptor subfamily (common gp130 subunit)



(c) IL-2 receptor subfamily (common γ subunit)



- IL-2R is the most thoroughly studied cytokine receptor
 - Expressed by T cells for proliferation
 - Also called CD25, surface marker in mature T cells
 - Complete receptor has 3 subunits

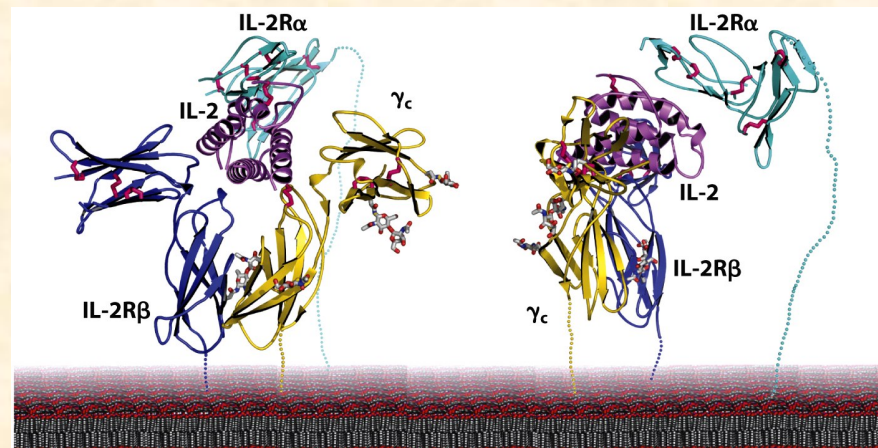
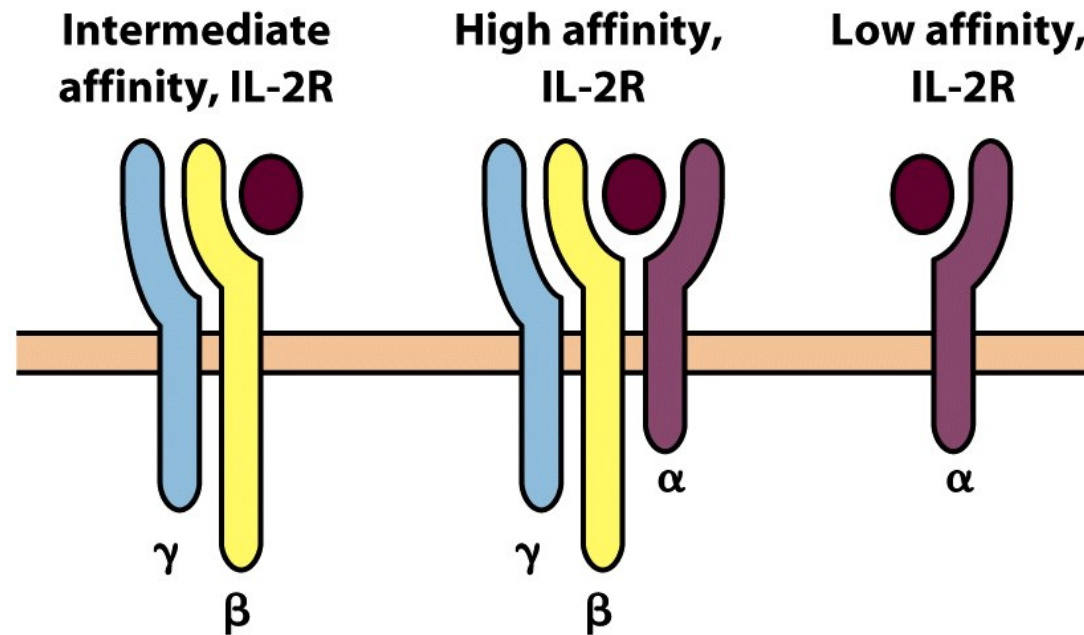


Figure 12-9b
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Subunit

composition:

IL-2R β
IL-2R γ

IL-2R α
IL-2R β
IL-2R γ

IL-2R α

Dissociation

constant (K_d):

10^{-9} M

10^{-11} M

10^{-8} M

Cells

expressed by:

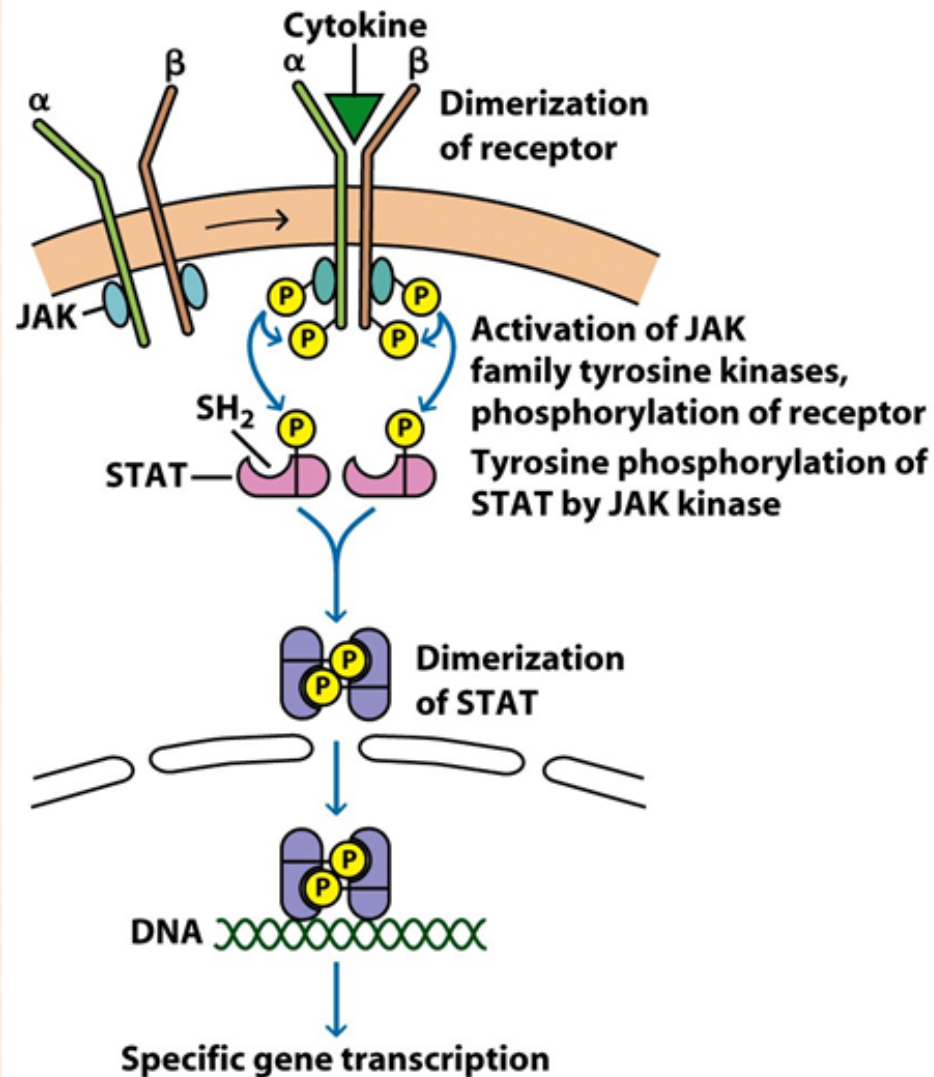
NK cells
Resting T cells
(low numbers)

Activated CD4⁺ and CD8⁺ T cells
Activated B cells
(low numbers)

Figure 12-9a
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- Majority of cytokine receptors are in Class I and Class II families
 - These cytokine receptors lack signaling motifs, this was puzzling to researchers
 - IFN- γ (Class II) was studied because of its ability to block viral replication like other interferons
 - » However, this IFN plays immunoregulatory role which leads to understanding the process of signal transduction through cytokine receptors
 - » Look on next slide

Signaling pathway of cytokine receptor



- 1. Cytokine receptor is composed of separate subunits
- 2. Different inactive protein tyrosine kinases associated with different subunits
 - Look at blue circles associated with receptor
- 3. Cytokine binding induces association of 2 separate receptor subunits (α and β) and activates receptor associated JAK
- 4. Activated JAKs create docking sites for the STAT transcription factors by phosphorylation
- 5. STAT transcription factors translocate from receptor docking sites at the membrane to the nucleus, activate transcription of genes

Figure 12-10
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Cytokine Antagonists

- Number of proteins can inhibit cytokine activity
 - Can bind to receptor OR
 - Can bind to cytokine, disabling it
 - Enzymatic cleavage of receptors and release of these can bind cytokines in the blood
 - Marker of chronic T cell activation (transplant rejection, AIDS)
- Viruses have developed strategies using their genomic information
 - Cytokine homologs
 - Cytokine binding proteins
 - Homologs of cytokine receptors
 - Interference with intracellular signaling
 - Interference with cytokine secretion

TABLE 12-3**Viral mimics of cytokines and cytokine receptors**

Virus	Products
Leporipoxvirus (a myxoma virus)	Soluble IFN-γ receptor
Several poxviruses	Soluble IFN-γ receptor
Vaccinia, smallpox virus	Soluble IL-1β receptor
Epstein-Barr	IL-10 homolog
Human herpesvirus-8	IL-6 homolog; also homologs of the chemokines MIP-I and MIP-II
Cytomegalovirus	Three different chemokine receptor homologs, one of which binds three different soluble chemokines (RANTES, MCP-1, and MIP-1α)

TWO T_H CELL SUBSETS

CD4+ T_H cells exert most of helper functions through secreted cytokines

- 2 populations based on secreted cytokines:
 - T_H1
 - Cell-mediated functions – delayed hypersensitivity, activation of T_C cells, production of opsonization-promoting IgG antibodies
 - Promote excessive inflammation and tissue injury
 - IFN- γ , TNF- β , IL-2
 - T_H2
 - Stimulates eosinophil activation, provides help to B cells, promotes production of large amounts of IgM, IgE, and non-complement activating IgG isotypes
 - Supports allergic reactions
 - IL-4, IL-5

TWO T_H CELL SUBSETS

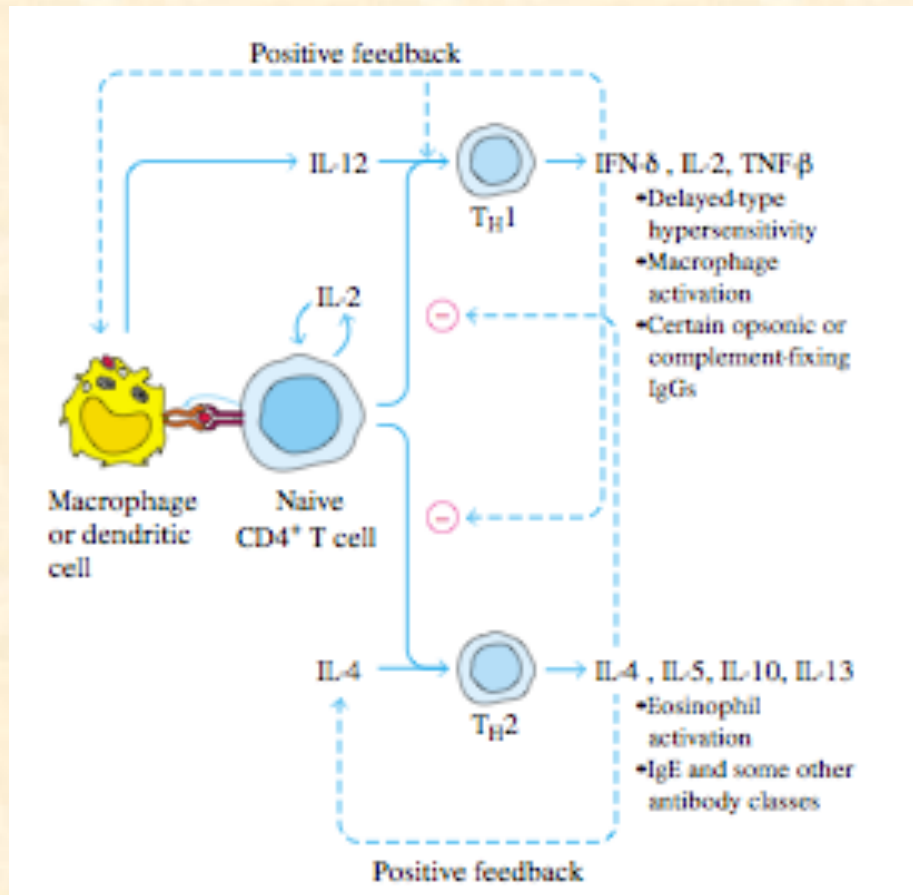
- Some T_H cells do not show T_H1 or T_H2 profiles
- Specific for type of antigenic challenge
- Both secrete IL-3 and GM-CSF

TABLE 12-4	Cytokine secretion and principal functions of mouse T _H 1 and T _H 2 subsets	
	T _H 1	T _H 2
CYTOKINE SECRETION		
IL-2	+	-
IFN-γ	++	-
TNF-β	++	-
GM-CSF	++	+
IL-3	++	++
IL-4	-	++
IL-5	-	++
IL-10	-	++
IL-13	-	++
FUNCTIONS		
Help for total antibody production	+	++
Help for IgE production	-	++
Help for IgG2a production	++	+
Eosinophil and mast-cell production	-	++
Macrophage activation	++	-
Delayed-type hypersensitivity	++	-
T _C -cell activation	++	-
SOURCE: Adapted from F. Powrie and R. L. Coffman, 1993, <i>Immunology Today</i> 14:270.		

Table 12-4
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- Cytokines produced by T_H1 and T_H2 subsets
 - Promote growth of subset that produces them
 - Inhibit development and activity of other subset
 - **T_H1 > intracellular pathogens**
 - **T_H2 > allergic diseases and helminth infections**
 - **Progression of some diseases depends on balance between T_H1 and T_H2 : leprosy, AIDS**
 - Intracellular pathogens use immune evasion to change the T_H1 - T_H2 balance

CROSS-REGULATION



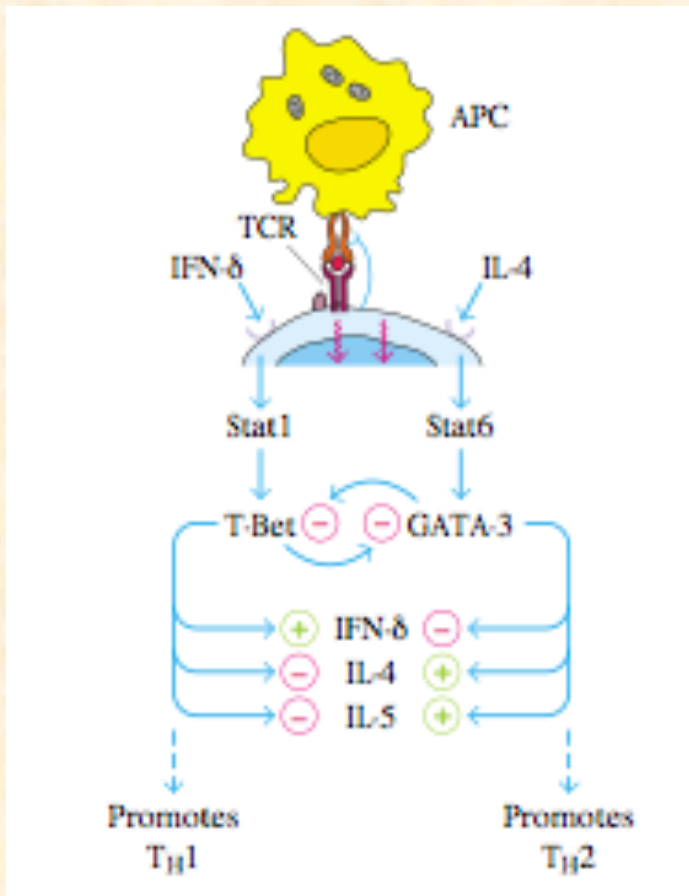
IDENTIFY POINTS OF POSITIVE AND NEGATIVE FEEDBACK

Gene Knock-outs:

Stat1: IL-12 <<<<

Stat6: IL-4 <<<<

CROSS-REGULATION



Stat1: T-bet

Stat6: GATA-3

T_{reg} : ANOTHER $CD4^+$ T CELL

- IL-4, IL-10, TGF- β

Cytokine Related Diseases

- SCID (Severe Combined Immunodeficiency Disease)
 - Genetic defects in cytokines, their receptors
- Defective receptor for IFN- γ
 - Susceptible to mycobacterial infections
- Over or under expression of cytokines or cytokine receptors

Cytokine Related Diseases

- Bacterial Septic Shock
 - Certain Gram- bacteria
 - Symptoms: drop in blood pressure, fever, diarrhea, blood clotting
 - Endotoxins bind TLRs on dendritic cells and macrophages
 - Over-produce IL-1 and TNF- α
 - Cytokine imbalance causes abnormal temp, abnormal respiration, capillary leakage, tissue injury, organ failure
 - Neutralization by monoclonal antibodies may help

Cytokine Related Diseases

- Bacterial Toxic Shock
 - Superantigens
 - Bind simultaneously to Class II MHC and T cell receptor, activating T cell despite antigenic specificity
 - Symptoms similar to sepsis

Cytokine Related Diseases!!

- Abnormalities in production of cytokines or receptors are associated with certain types of cancer
- Chagas Disease
 - Caused by *Trypanosoma cruzi*
 - Severe immunosuppression
 - Evidence that soluble factor produced by *T. cruzi* leads to reduction (90%) in T cell IL-2 (CD25) receptor

Cytokine-based Therapies

- Problems with cytokine therapies:
 - Effective dose levels
 - Short half-life
 - Potent biological response modifiers
 - Can cause unpredictable side effects

Suppression of T_H -cell proliferation and T_C -cell activation

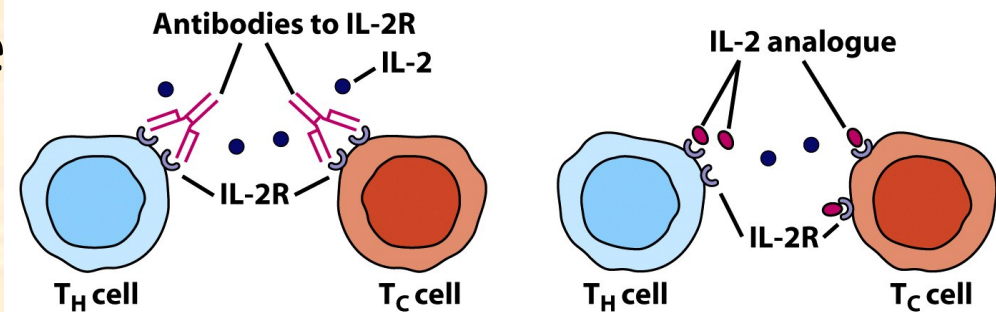


Figure 12-15a
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Destruction of activated T_H cells

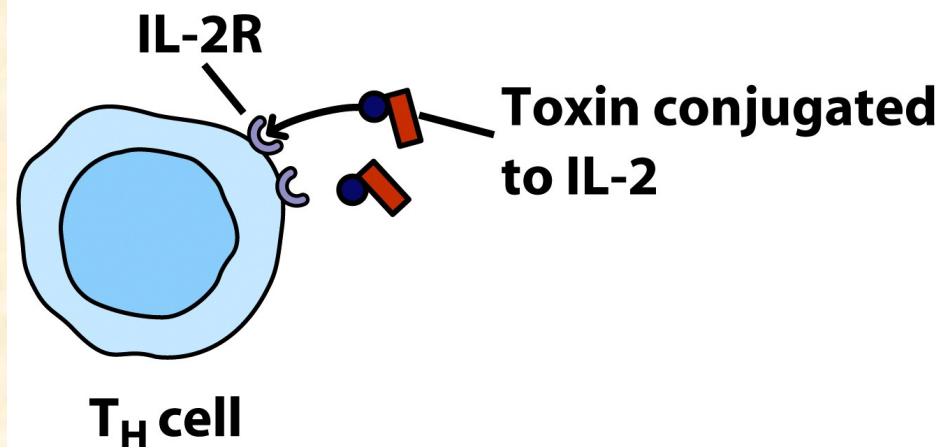


Figure 12-15b
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TABLE 12-5 Hematopoietic cytokines

Hematopoietic growth factor	Sites of production	Main functions
Erythropoietin	Kidney, liver	Erythrocyte production
G-CSF	Endothelial cells, fibroblasts, macrophages	Neutrophil production
Thrombopoietin	Liver, kidney	Platelet production
M-CSF	Fibroblasts, endothelial cells, macrophages	Macrophage and osteoclast production
SCF/ <i>c-kit</i> ligand	Bone marrow stromal cells, constitutively	Stem cell, progenitor cells survival/division; mast cell differentiation
Flt-3 ligand	Fibroblasts, endothelial cells	Early progenitor cell expansion; pre-B cells
GM-CSF	T cells (T _H 1 and T _H 2), macrophages, mast cells	Macrophage, granulocyte production; dendritic cell maturation and activation
IL-3	T cells (T _H 1 and T _H 2), macrophages	Stem cells and myeloid progenitor cell growth; mast cells
IL-5	Activated helper, T cells—T _H 2 response only	Eosinophil production; murine B-cell growth
IL-6	Activated T cells, monocytes, fibroblasts, endothelial cells	Progenitor-cell stimulation; platelet production; immunoglobulin production in B cells
IL-7	Bone marrow and lymphoid stromal cells	T-cell survival
IL-11	Bone marrow stromal cells and IL-1-stimulated fibroblasts	Growth factor for megakaryocytes

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor. Adapted from D. Thomas and A. Lopez, 2001, *Encyclopedia of Life Sciences: Haematopoietic growth factors*, Nature Publishing Group.

Table 12-5

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Cytokine-based therapies in clinical use		
Agent	Nature of agent	Clinical application
Enbrel	Chimeric TNF-receptor/IgG constant region	Rheumatoid arthritis
Remicade or Humira	Monoclonal antibody against TNF-α receptor	Rheumatoid arthritis Crohn's disease
Roferon	Interferon α-2a*	Hepatitis B Hairy-cell leukemia Kaposi's sarcoma
Intron A	Interferon α-2b	Hepatitis C[†] Melanoma
Betaseron	Interferon β-1b	Multiple sclerosis
Avonex	Interferon β-1a	Multiple sclerosis
Actimmune	Interferon γ-1β	Chronic granulomatous disease (CGD) Osteopetrosis
Neupogen	G-CSF (hematopoietic cytokine)	Stimulates production of neutrophils Reduction of infection in cancer patients treated with chemotherapy, AIDS patients
Leukine	GM-CSF (hematopoietic cytokine)	Stimulates production of myeloid cells after bone marrow transplantation
Neumega or Neulasta	Interleukin-11 (IL-11), a hematopoietic cytokine	Stimulates production of platelets
Epogen	Erythropoietin (hematopoietic cytokine)	Stimulates red-blood-cell production
<p>*Interferon α-2a is also licensed for veterinary use to combat feline leukemia.</p> <p>[†]Normally used in combination with an antiviral drug (ribavirin) for hepatitis C treatment.</p>		

- Cytokines play essential role in hematopoiesis

