

# **CYTOKINES**

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“Last week’s EMT group was at full strength, although they were now tossing around phrases like *cytokines* and *pyruvic acid*.”

“A Few Good Men”

Richard A. Lovett

ANALOG

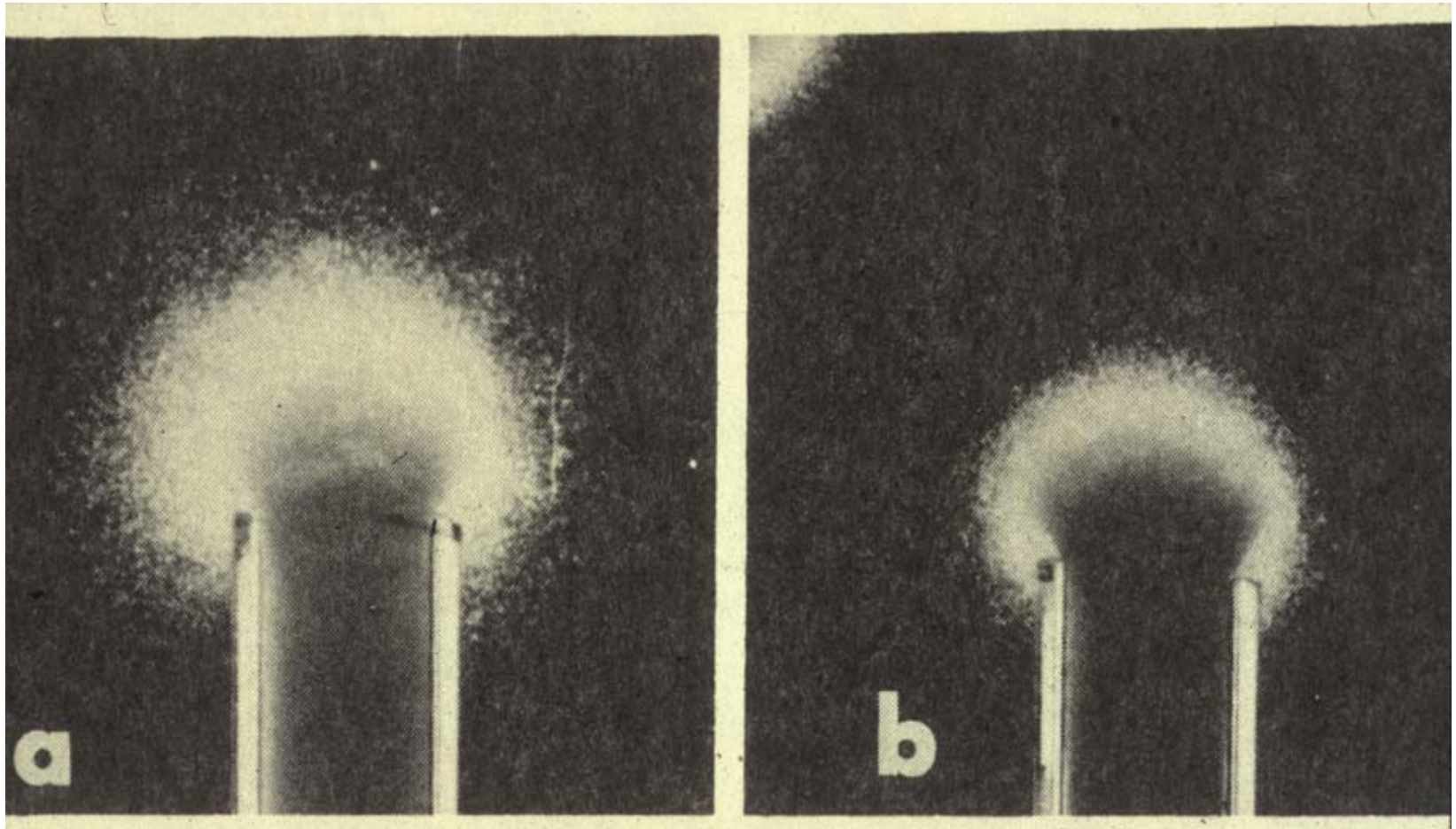
January/February 2005

# What are cytokines?

Regulatory peptides that can be produced by every nucleated cell type in the body.

# Some history!

- **1926** - Zinsser & Tamiya found permeability factors in supernatants of tuberculin-sensitized guinea pig cells exposed to tuberculo-protein.
- **1932** - Rich & Lewis showed migration of neutrophils & macrophages in cultures of tuberculin-sensitized tissues was inhibited by antigen.
- **1957** - Isaacs and Lindenmann - Type I interferon.
- **1966** - David, Bloom & Bennett used the capillary tube technique to show that antigens could stimulate sensitized lymphocytes in cultures to produce MIF.



# History continued!

- **1968** - Ruddle & Waksman identified LT.
- **1969** - Dumonde suggested the word “lymphokine” for these mediators.
- “Monokines” were defined when monocytes and mØ were cultured.
- **1974** - Based on the existence of lymphokines, monokines, as well as mediators derived from virally infected non-lymphoid cells, Cohen et al proposed that all these mediators should therefore be called “cytokines”.

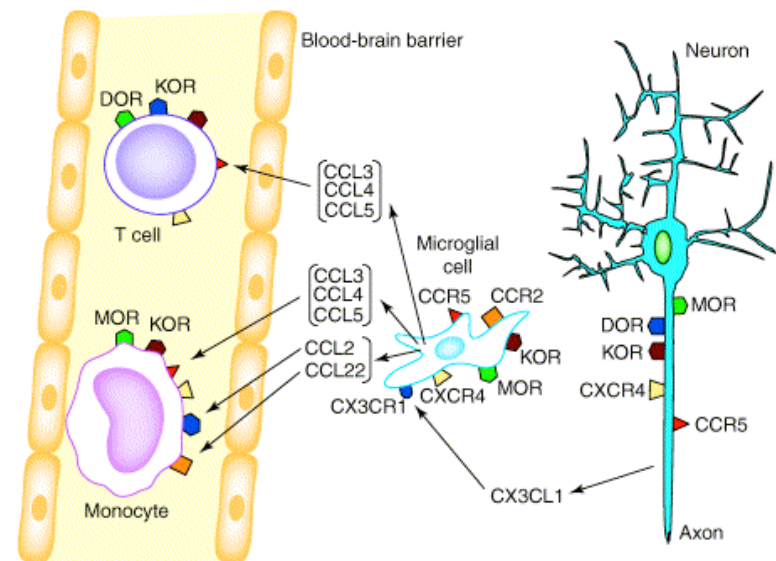
# 1st description of cytokines

“Lymphokine production represents a general biologic phenomenon which may play a role in various aspects of host defense. Thus such mediator substances should more properly be called ‘**cytokines**’. Lymphokines represent a restricted set of cytokines made by one class of cells (lymphocytes).”

Cohen, S., Bigazzi, P.E., and Yoshida, T. Cell. Immunol. 12:150-159, 1974

“Although opium-induced inhibition of chemotaxis of leukocytes was described over a century ago...only recently has an understanding emerged...whereby opioids affect not only pain but regulate all the signs of inflammation...opioids share many of the properties of cytokines and chemokines, and...had they been discovered first by immunologists rather than pharmacologists, opioids would be considered a class of messenger molecules (cytokines) of the immune system.”

Rogers, T. J. and Peterson, P.K.  
Trends in Immunology, 24:116-121, 2003



“Cytokine: more than a new word, a new concept proposed by Stanley Cohen thirty years ago.”

*Cytokine 28 (2004) 242-247.*

Horst Ibelgauft's web site: **[www.copewithcytokines.de](http://www.copewithcytokines.de)**

# Interleukin terminology

By **1978**, approximately 100 different activities had been described.

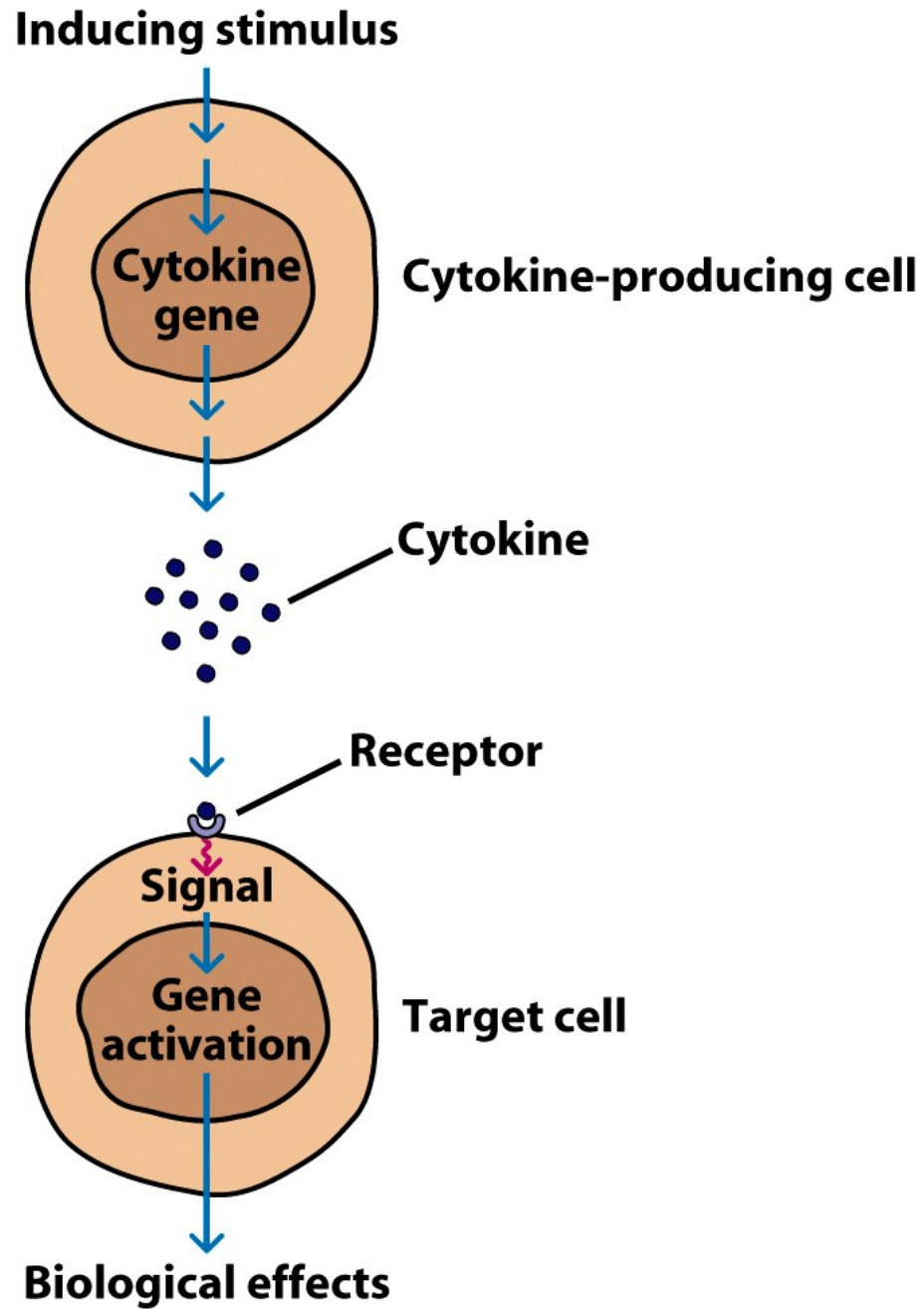
2nd International Lymphokine Workshop (Ermatingen, Switzerland): Interleukin-1 (IL-1) and Interleukin-2 (IL-2).

35 interleukins have been identified (OVID search - 2/3/09).

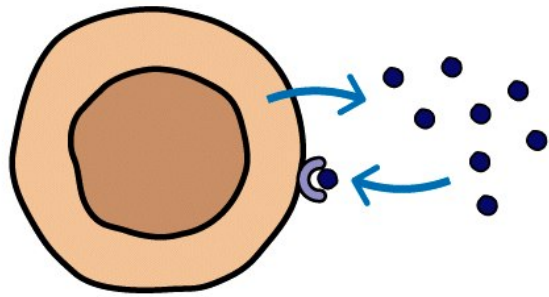
# FEATURES OF CYTOKINES

Adapted from J. Vilcek in *The Cytokine Handbook* (4th ed.), edited by Thomson & Lotze (2003)

1. Simple polypeptides or glycoproteins  
molecular weight of 30 kDa or less  
homodimers or homotrimers.  
exception: IL-12 (heterodimer).
2. Constitutive production  
low or absent  
production is regulated by inducing  
stimuli at the level of  
transcription or translation.
3. Action results from cytokine binding to  
specific high-affinity surface receptors.

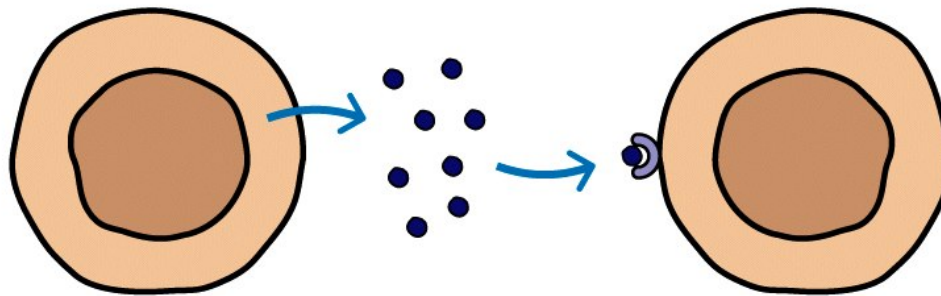


**Figure 12-1a**  
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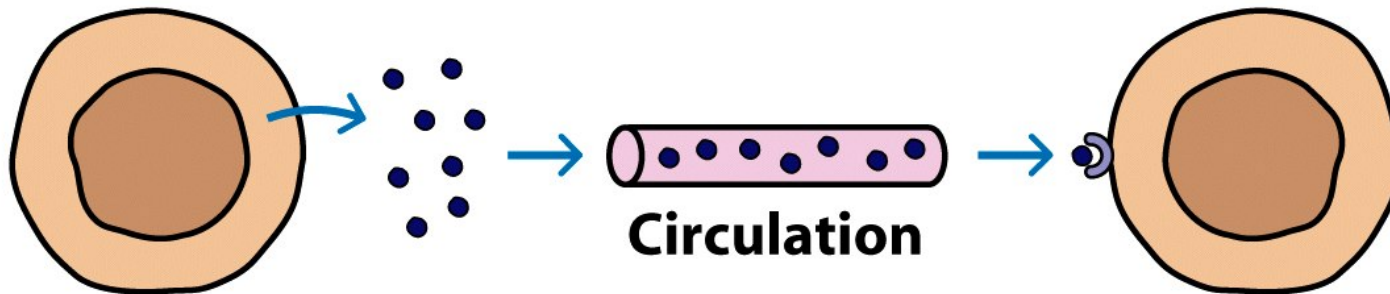
**Autocrine action**

Production is transient. Action radius is short (autocrine or paracrine not endocrine).



**Paracrine action**

**Nearby cell**



**Endocrine action**

**Distant cell**

# FEATURES OF CYTOKINES

Adapted from J. Vilcek in *The Cytokine Handbook* (4th ed.), edited by Thomson & Lotze (2003)

Continued-

Cytokine actions result in an altered pattern of gene expression in the target cells. These can be:

- increase or decrease in the rate of cell proliferation.

- change in the state of cell differentiation.

- change in the expression of some differentiated functions.

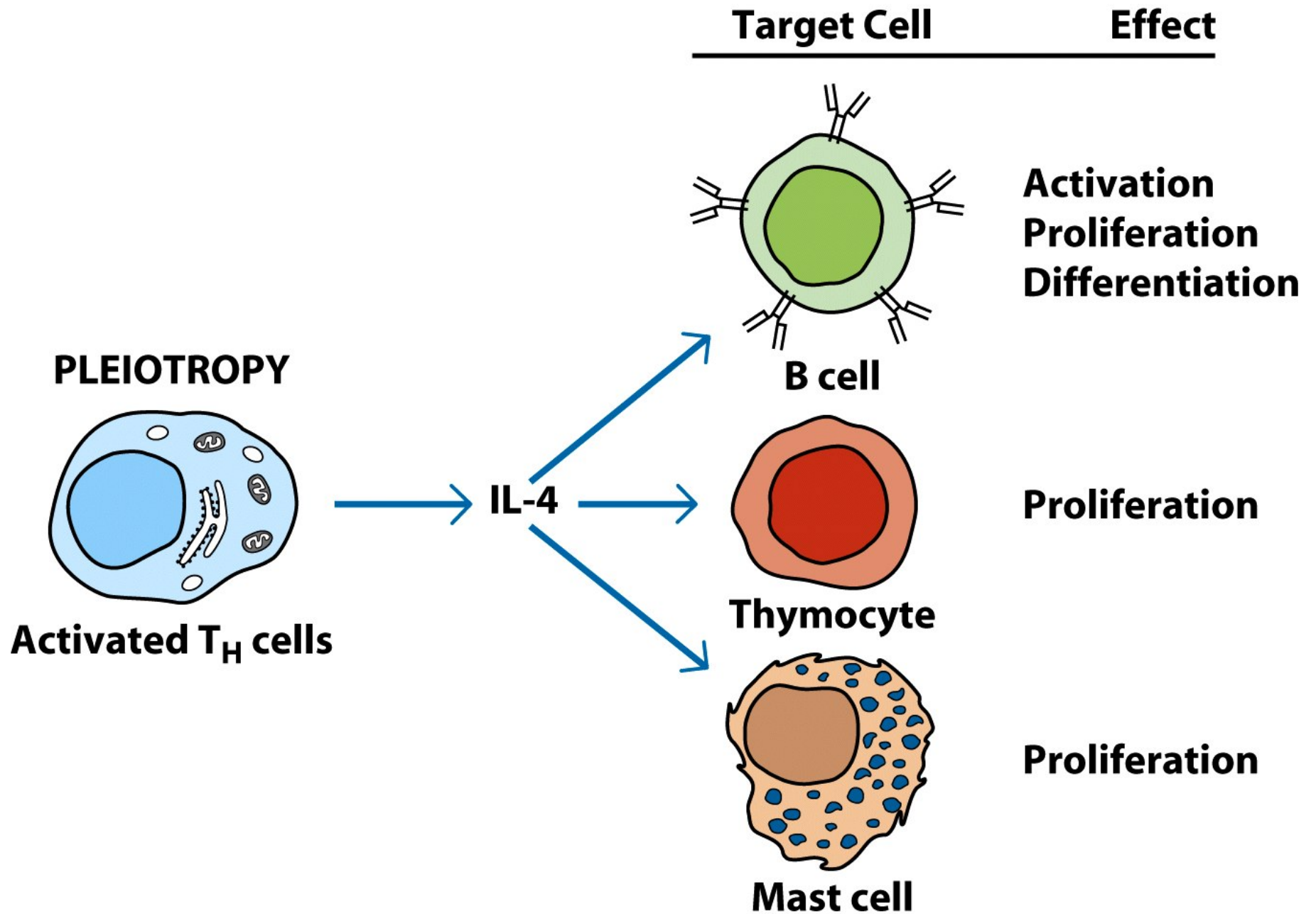


Figure 12-2a part 1  
 Kuby IMMUNOLOGY, Sixth Edition  
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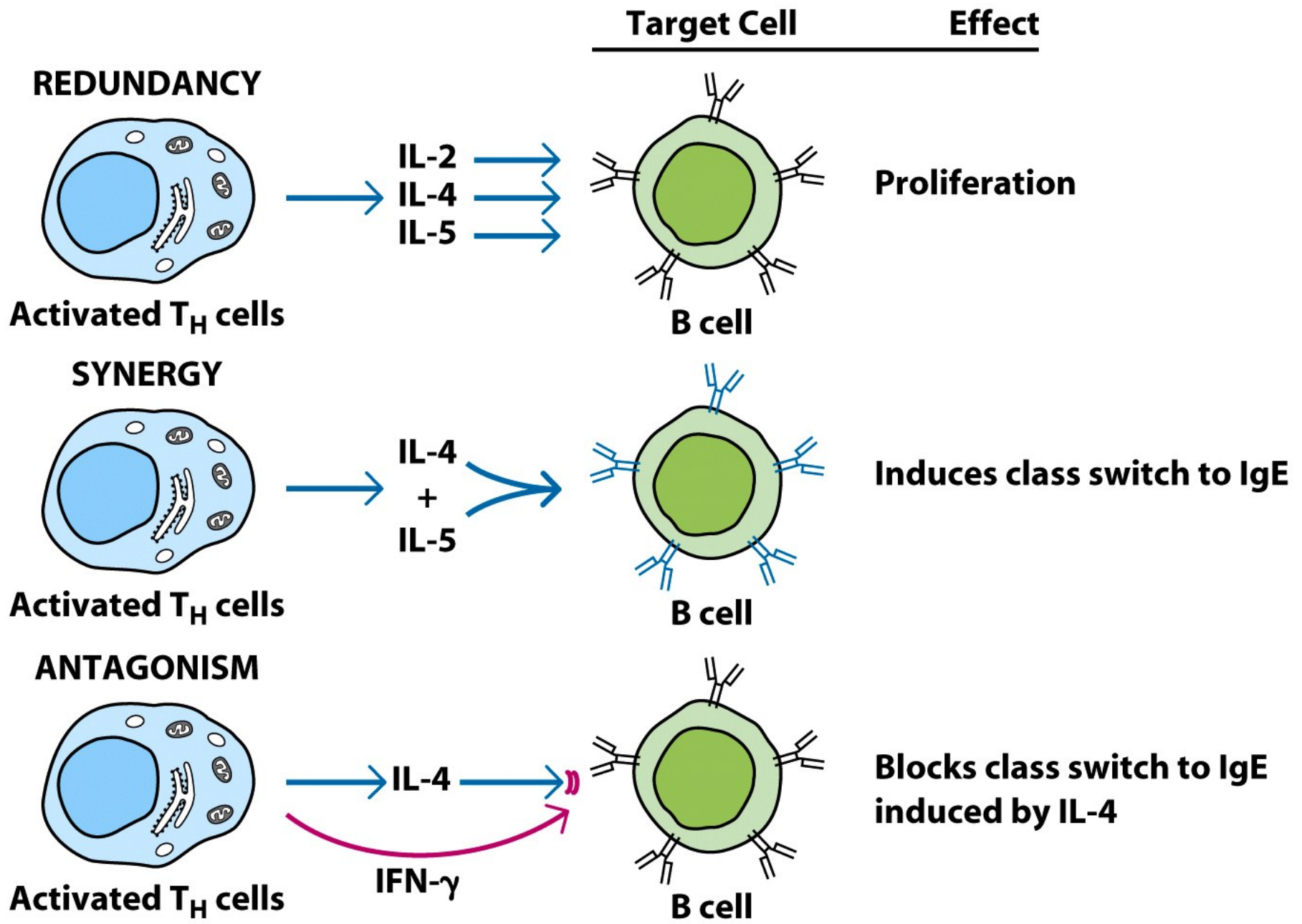
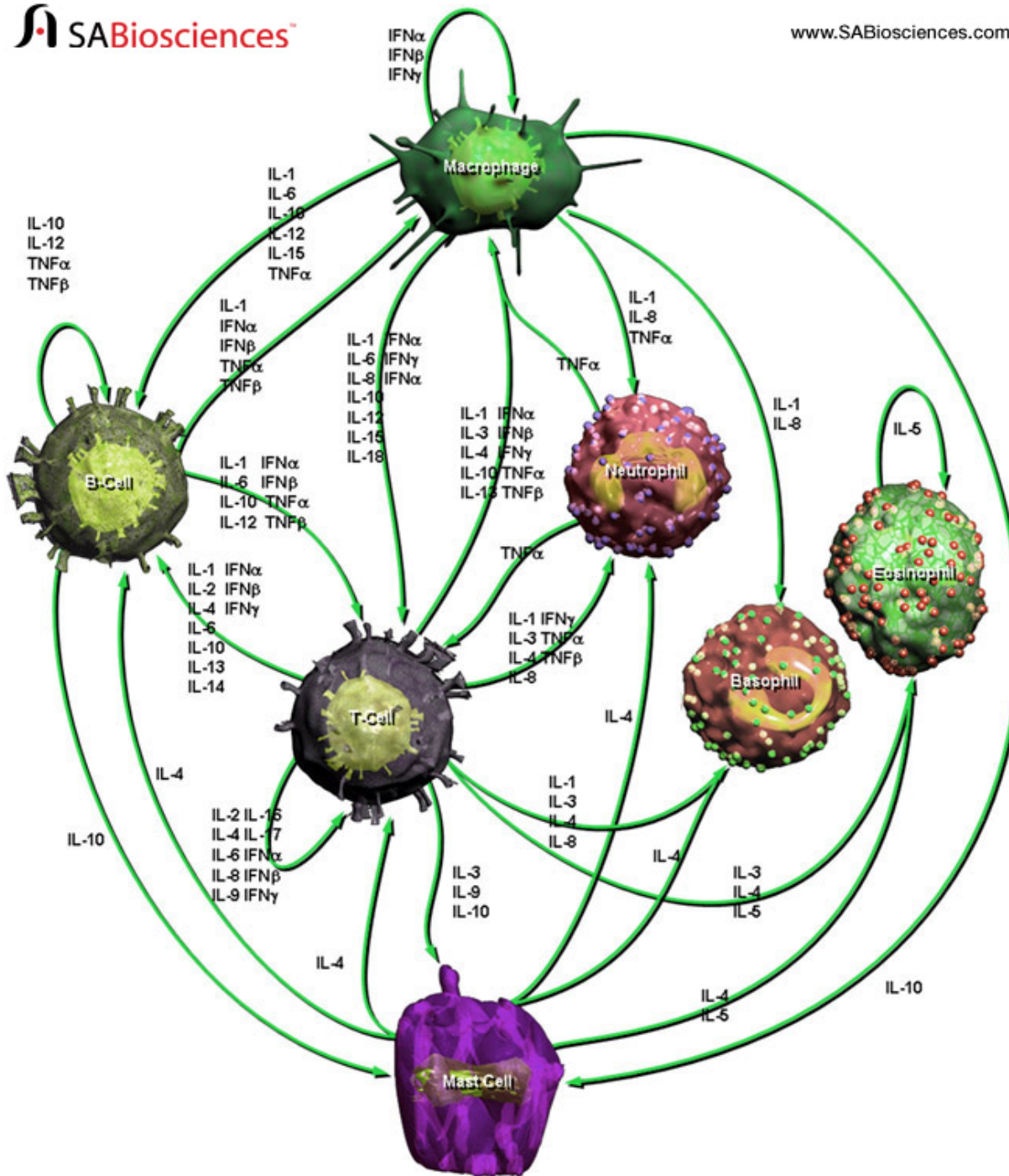


Figure 12-2a part 2  
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# CATEGORIES OF CYTOKINES

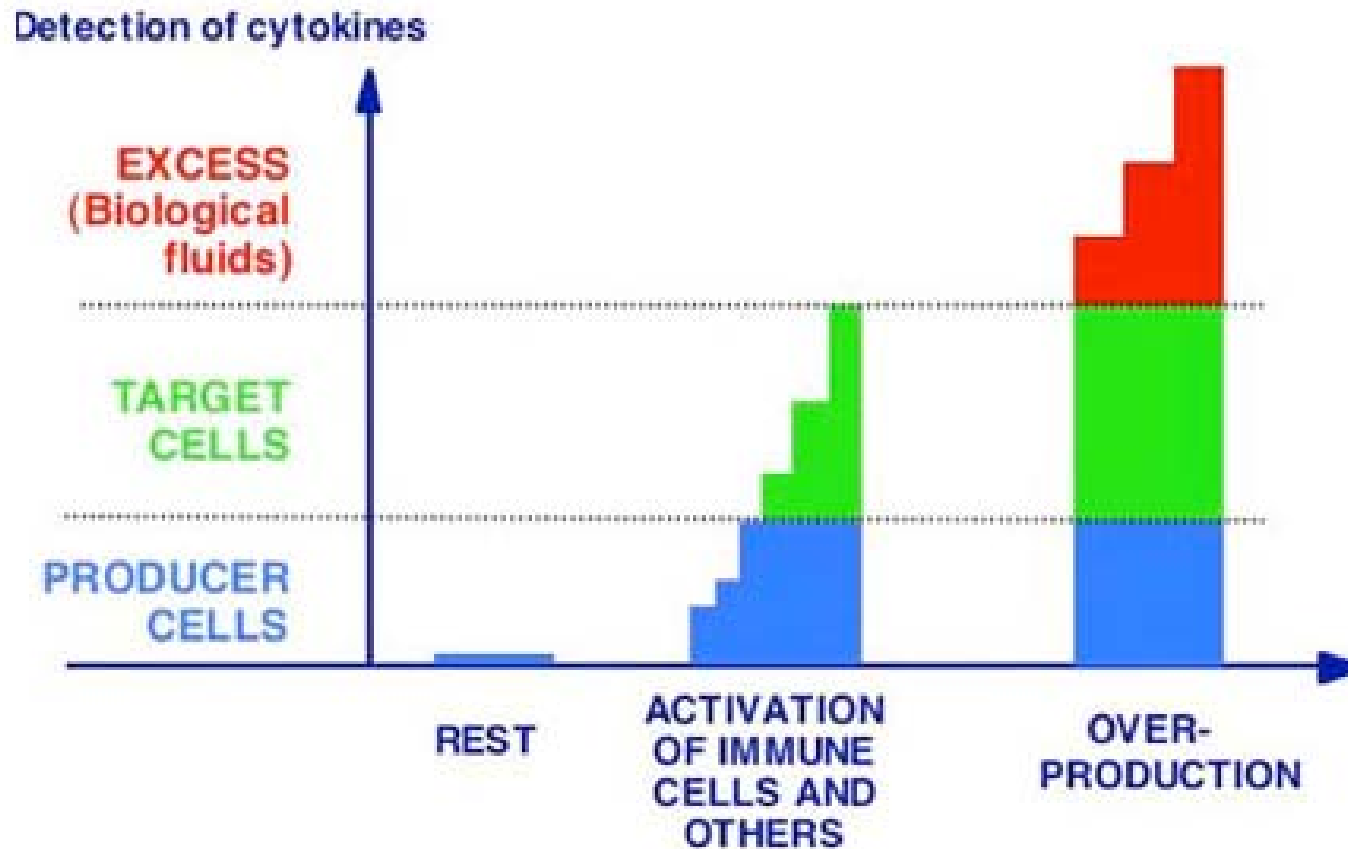
- Mediators and regulators of innate immunity  
IL-1, IL-8, IL-10, IL-12, TNF- $\alpha$ , RANTES,  
IFN- $\alpha$ , $\beta$ , IFN- $\gamma$
- Mediators and regulators of adaptive immunity  
IL-2, IL-4, IL-5, TGF- $\beta$ , IFN- $\gamma$
- Mediators of hematopoiesis  
IL-3, IL-7, colony stimulating factors

# Mediators of innate immunity

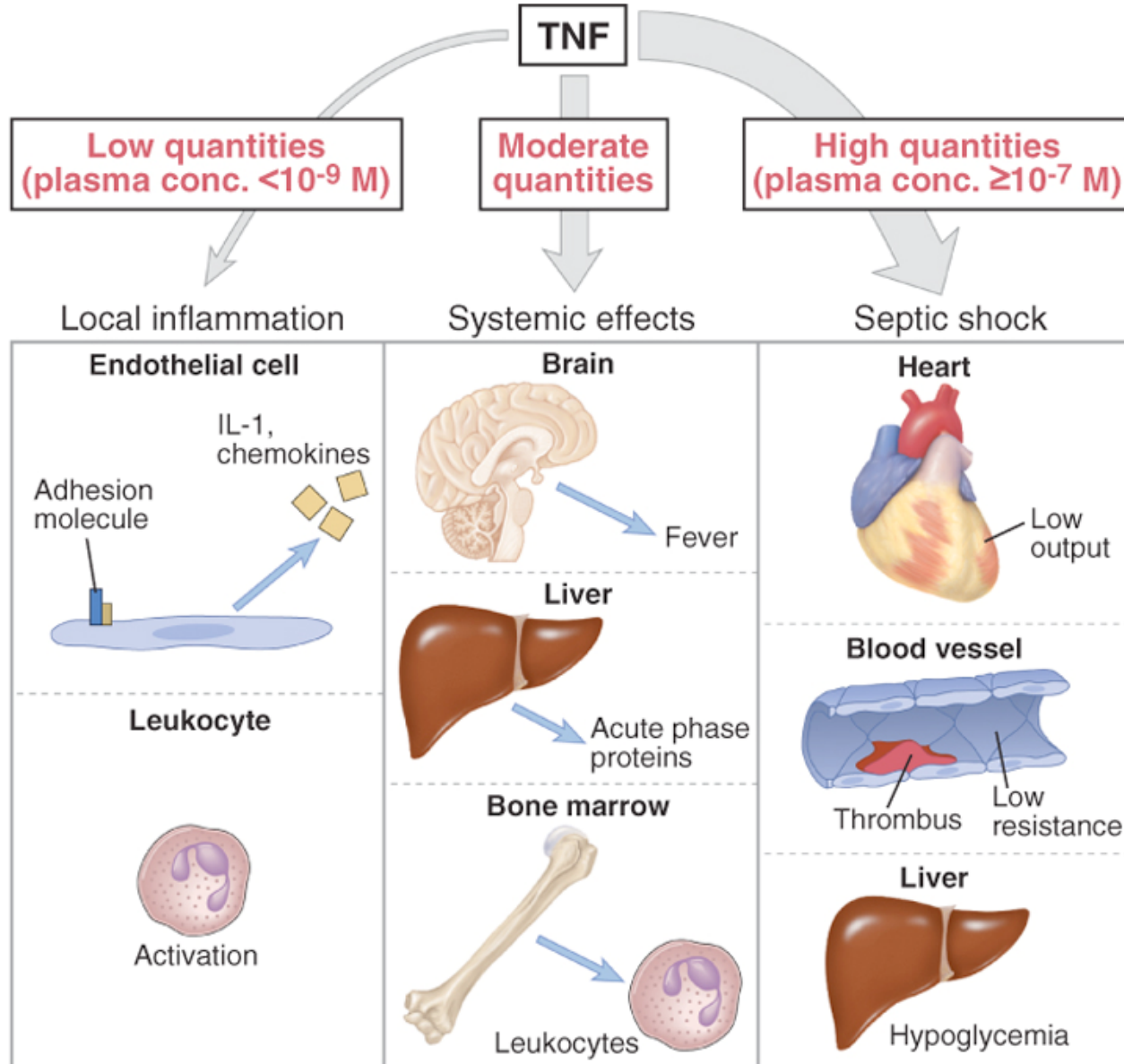
- Chemokines
- TNF- $\alpha$
- Type I interferons
- IFN- $\gamma$
- IL-1
- IL-6
- IL-12
- IL-15
- IL-18

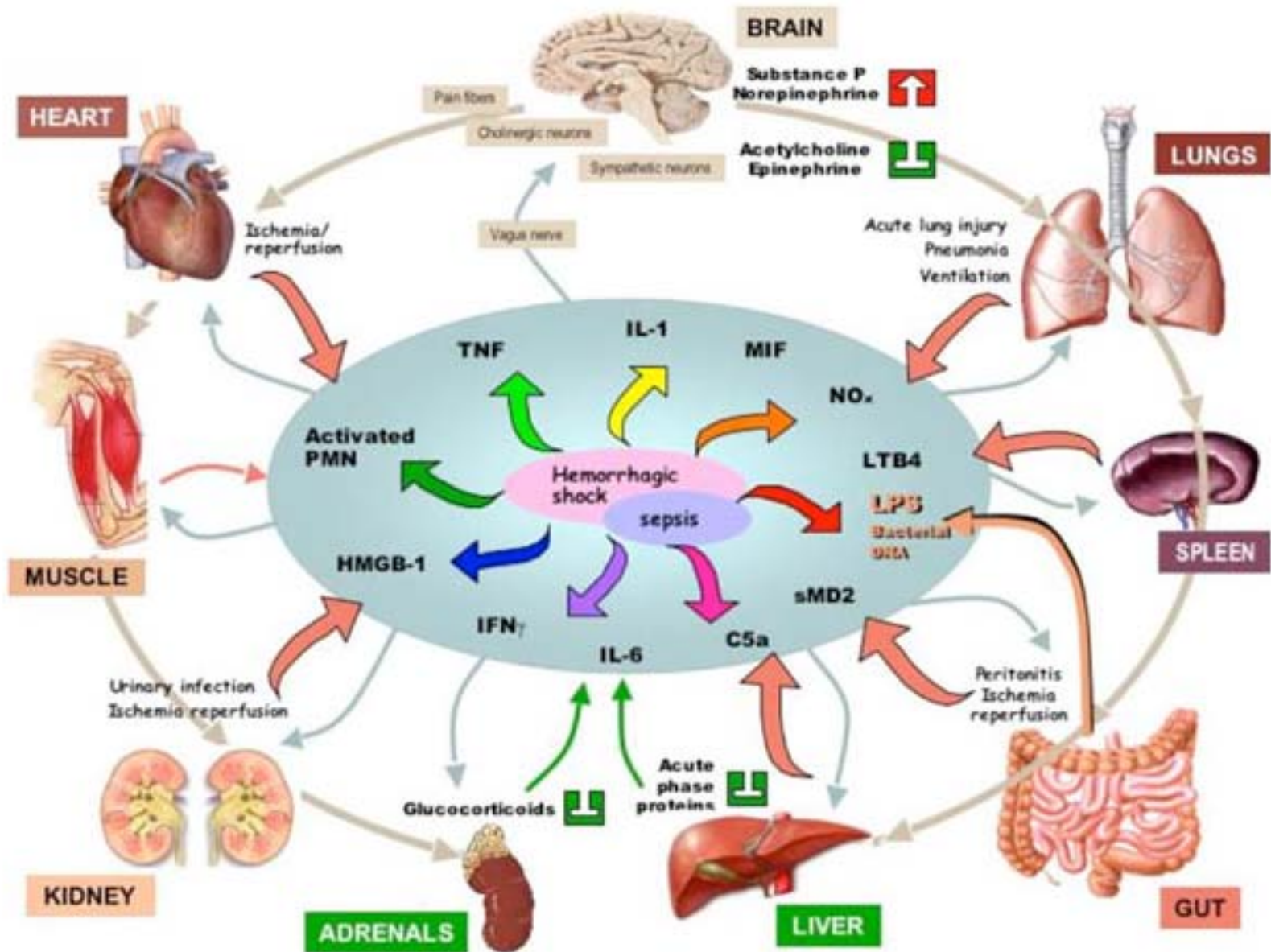
## Circulating cytokines : the tip of the iceberg ?

*Cavaillon et al. Circ. Shock 1992, 38, 145*



# Biologic actions of TNF

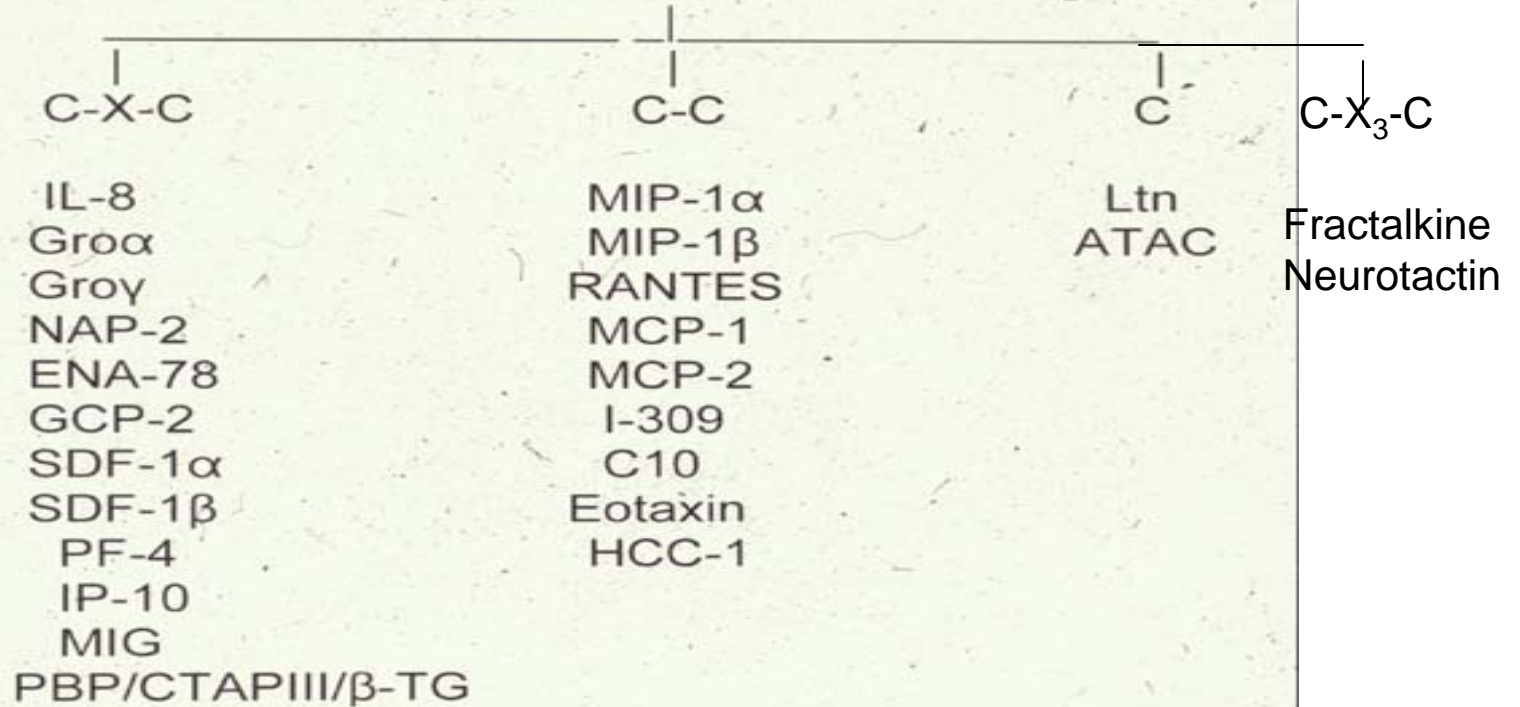




# What is a chemokine?

1. “Chemotactic cytokines” - subfamily of cytokines.
2. 8-12 kD polypeptides with two internal disulfide loops
3. Classified on the basis of amino acid sequence relatedness and not by function.
4. Subclassed based on the number and positioning of the highly conserved cysteines.
5. Released during the early phase of cell response to injury, allergens, antigens, and invading microorganisms.
6. Selectively attract leukocytes to inflammatory foci, inducing both cell migration and activation.
7. Also play a role in the development of many nonlymphoid organs.

# Chemokine Superfamily

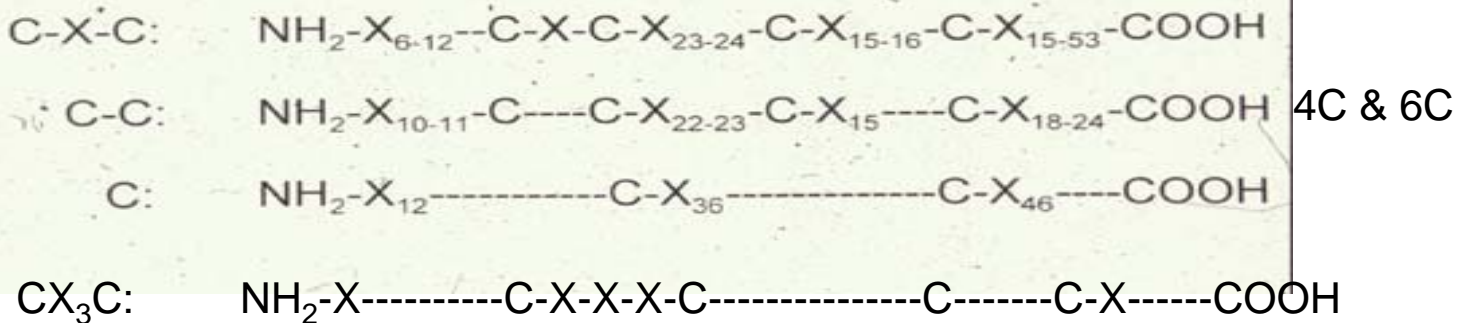


ELR = glu-leu-arg

ELR+ (PMNs)

ELR-

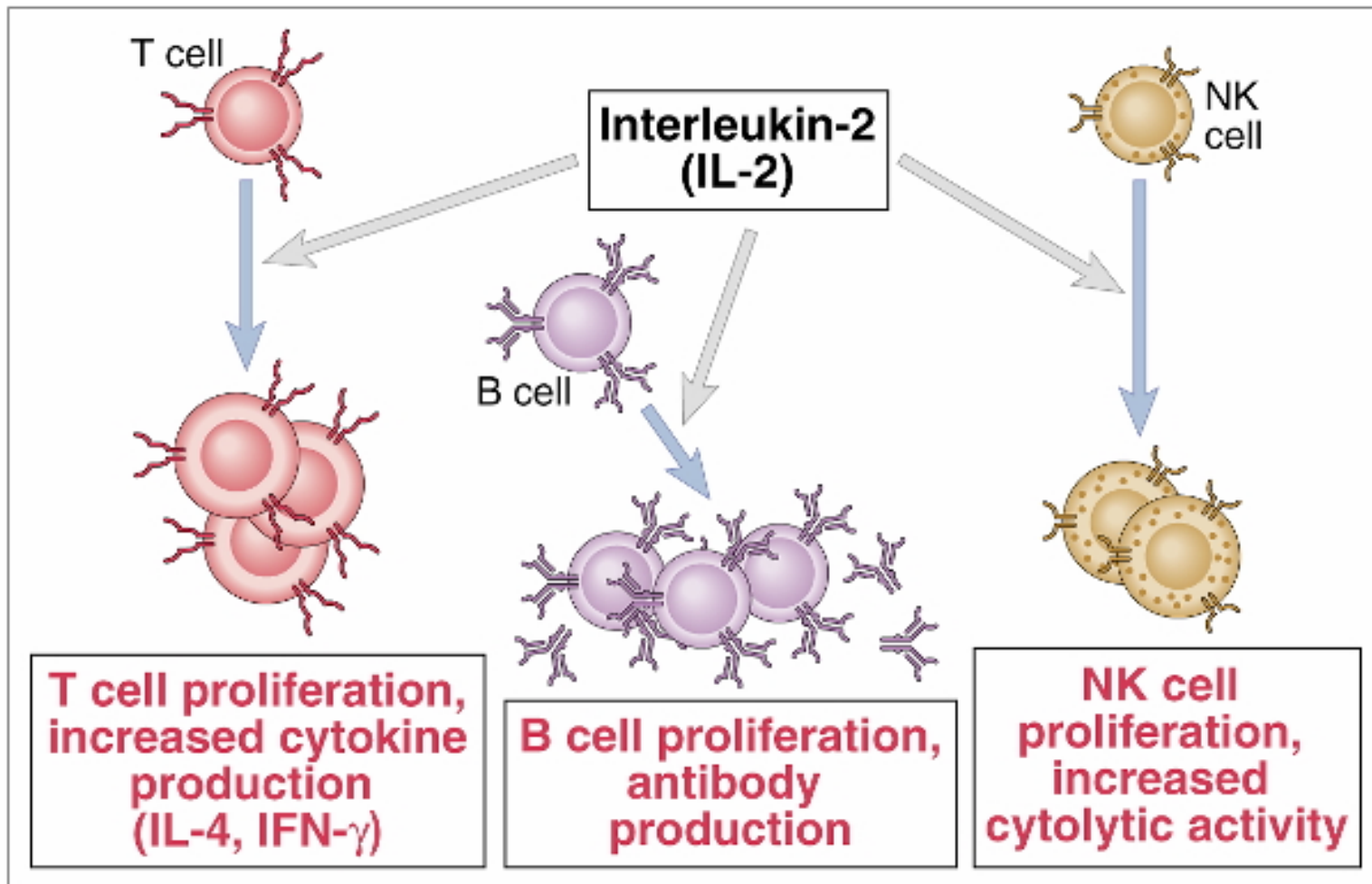
## Amino Acid Structure



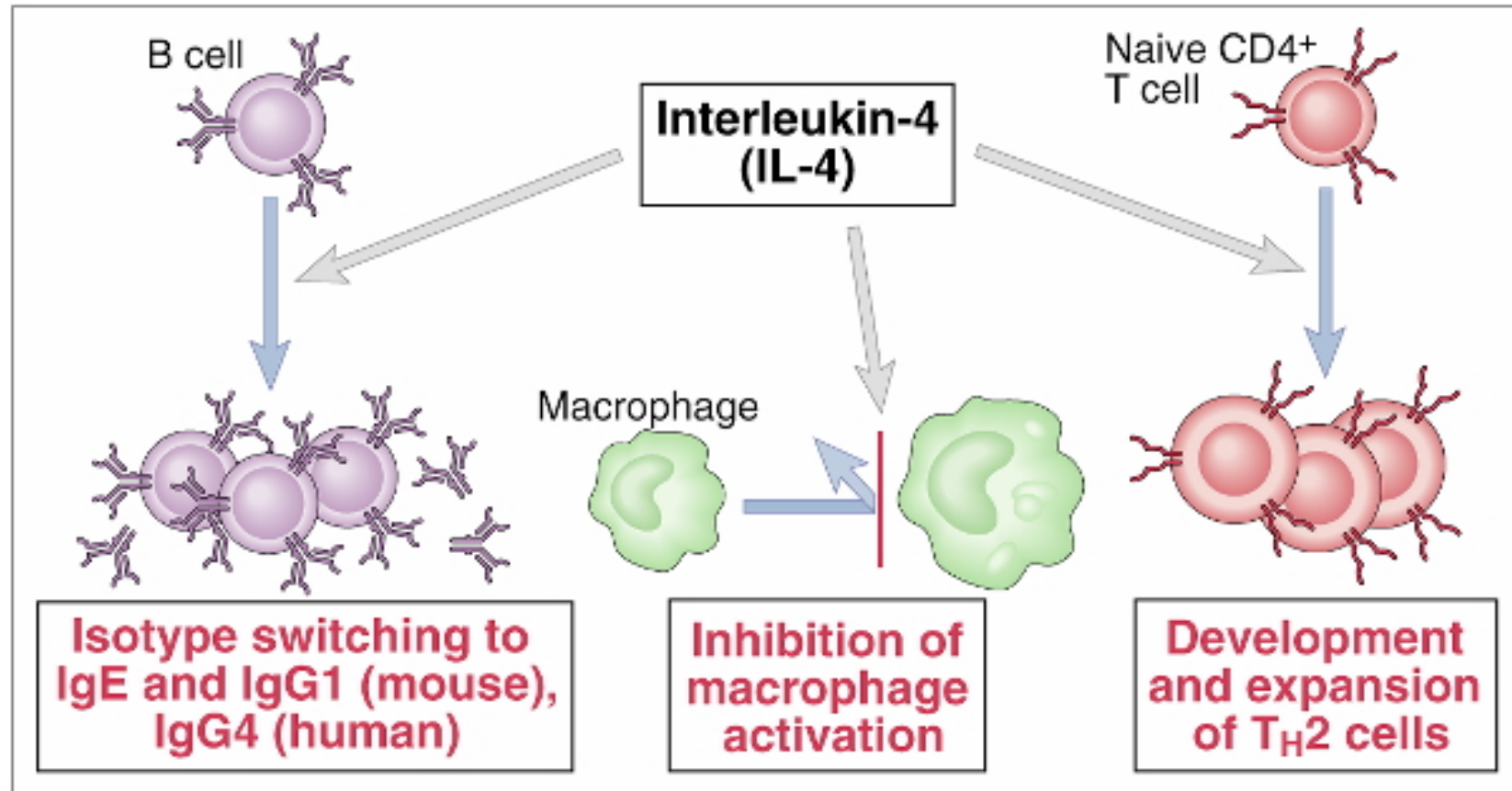
# Cytokines of adaptive immunity

- IL-2
- IL-4
- IL-5
- IFN- $\gamma$
- TGF- $\beta$
- LT (aka TNF- $\beta$ )

# Biologic activities of IL-2

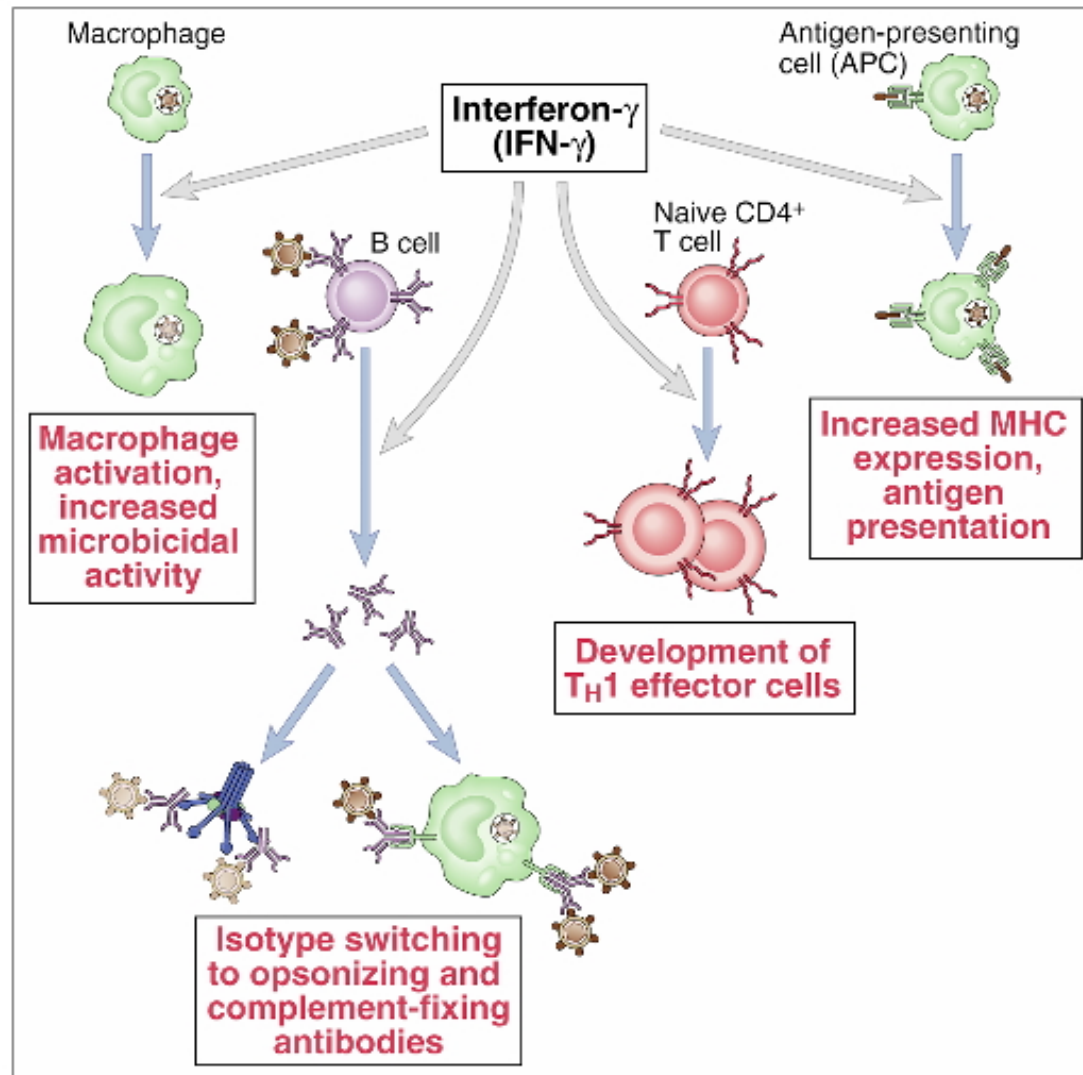


# Biologic activities of IL-4



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# Biologic activities of IFN- $\gamma$



**TABLE 12-4**Cytokine secretion and principal functions of mouse T<sub>H</sub>1 and T<sub>H</sub>2 subsets

Cytokine/function	T <sub>H</sub> 1	T <sub>H</sub> 2
CYTOKINE SECRETION		
IL-2	+	-
IFN- $\gamma$	++	-
TNF- $\beta$	++	-
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 <sub>C</sub> -cell activation	++	-

SOURCE: Adapted from F. Powrie and R. L. Coffman, 1993, *Immunol. Today* 14:270.

**TABLE 12-5****Hematopoietic cytokines**

<b>Hematopoietic growth factor</b>	<b>Sites of production</b>	<b>Main functions</b>
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 <sub>H</sub> 1 and T <sub>H</sub> 2), macrophages, mast cells	Macrophage, granulocyte production; dendritic cell maturation and activation
IL-3	T cells (T <sub>H</sub> 1 and T <sub>H</sub> 2), macrophages	Stem cells and myeloid progenitor cell growth; mast cells
IL-5	Activated helper, T cells—T <sub>H</sub> 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

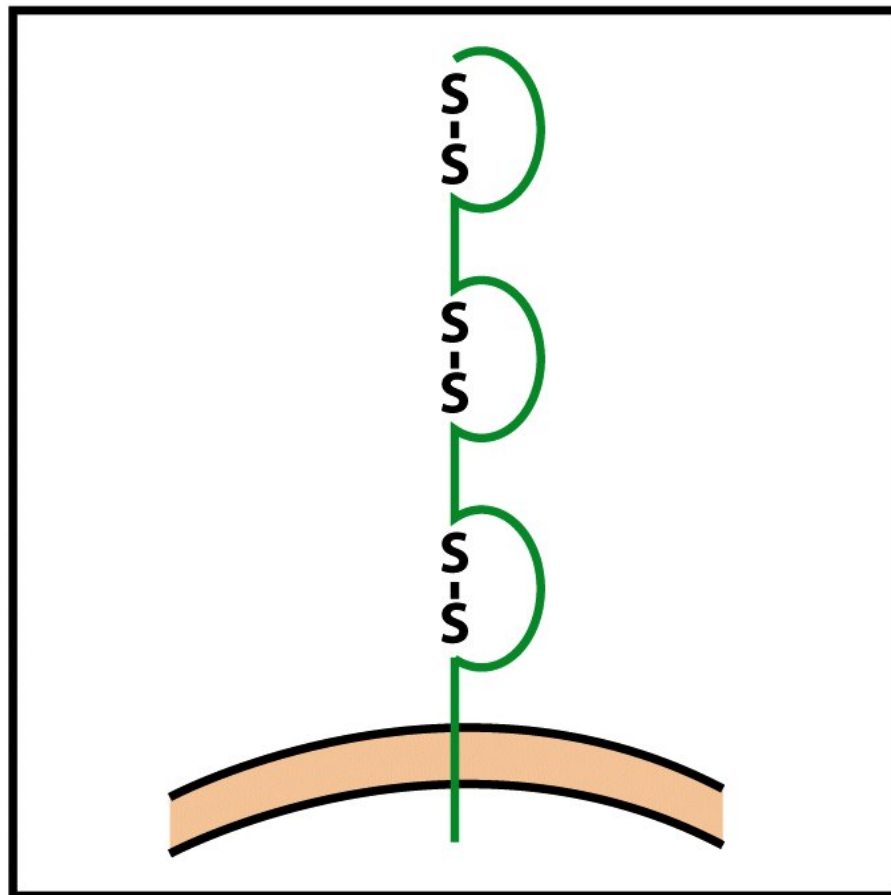
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# RECEPTOR FAMILY

# LIGANDS

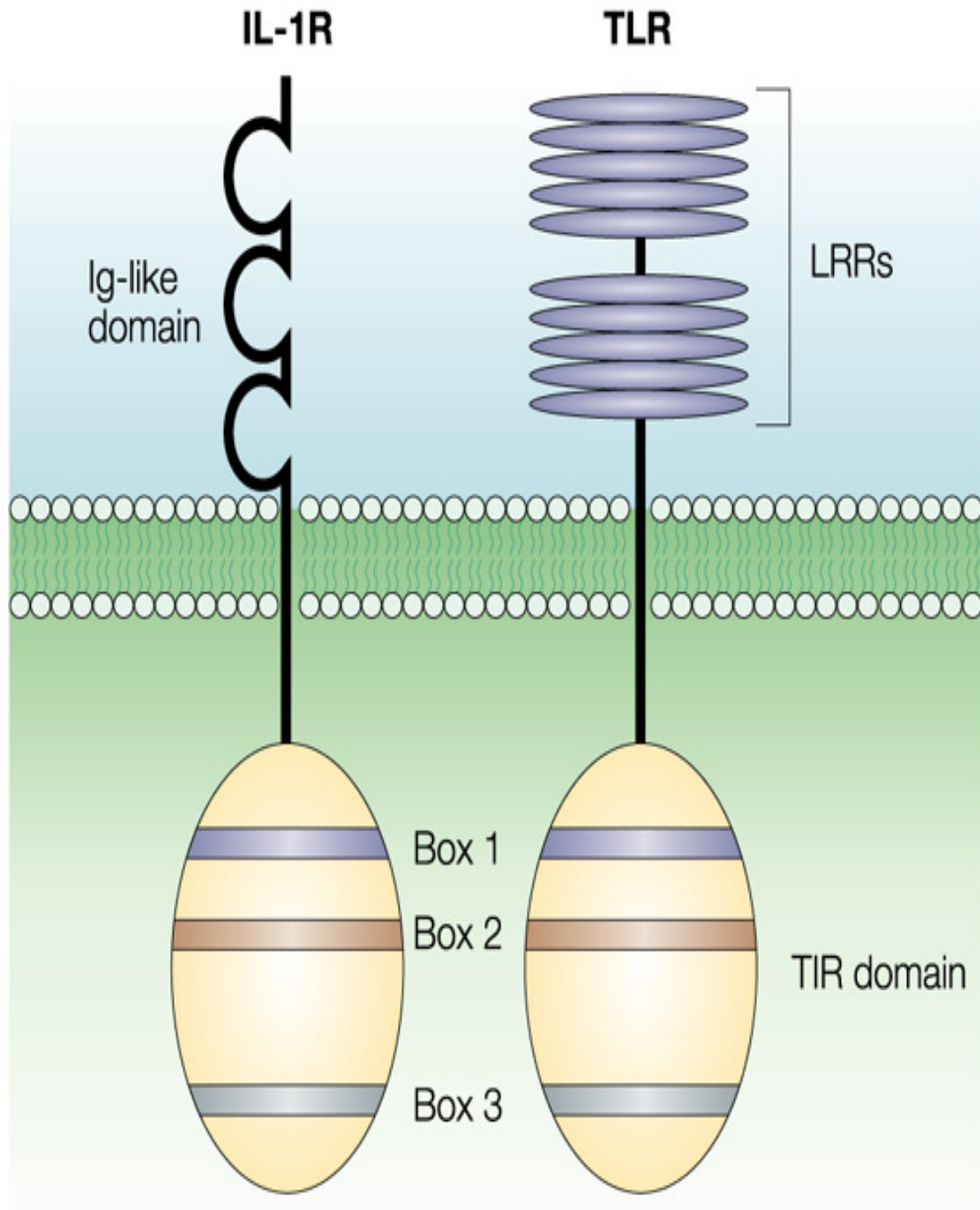
## Immunoglobulin superfamily receptors



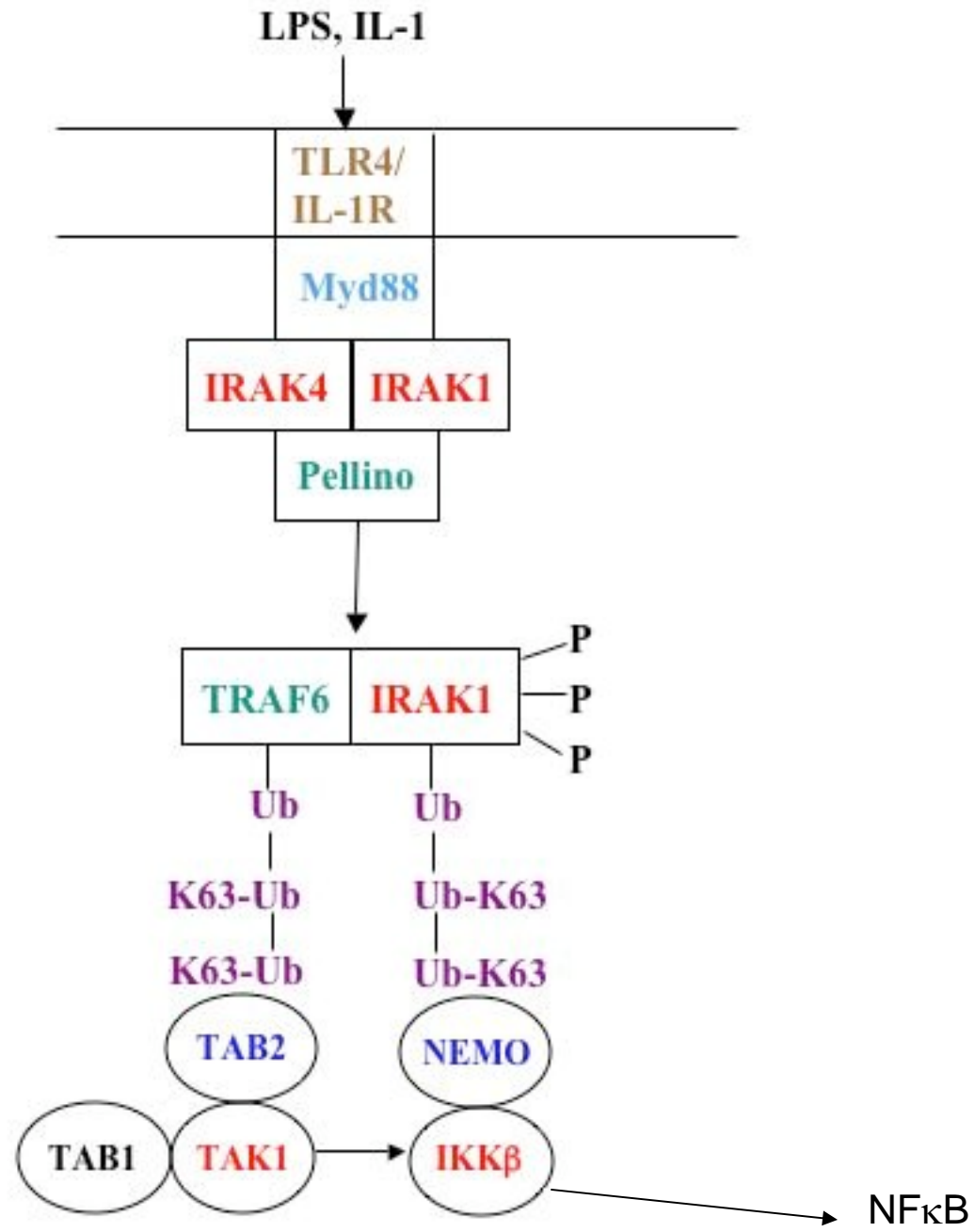
- IL-1
- M-CSF
- C-Kit
- IL-18
- IL-33

Figure 12-6a  
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**a**

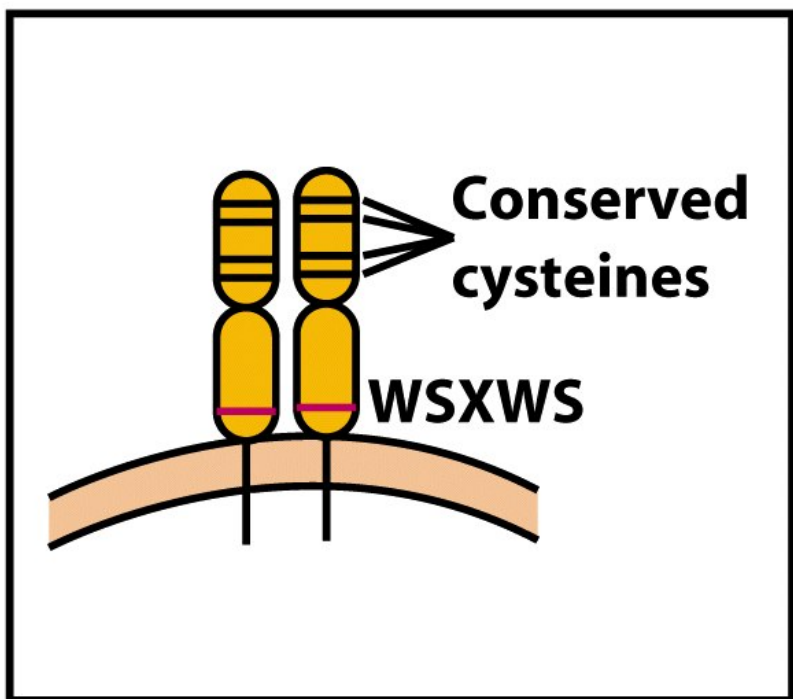


Akira, S. & Takeda, K.  
Nat. Rev. Immunol. 4, 599-  
511, 2004



# 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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WSXWS=try-ser-X-try-ser

# GM-CSF receptor subfamily (common $\beta$ subunit)

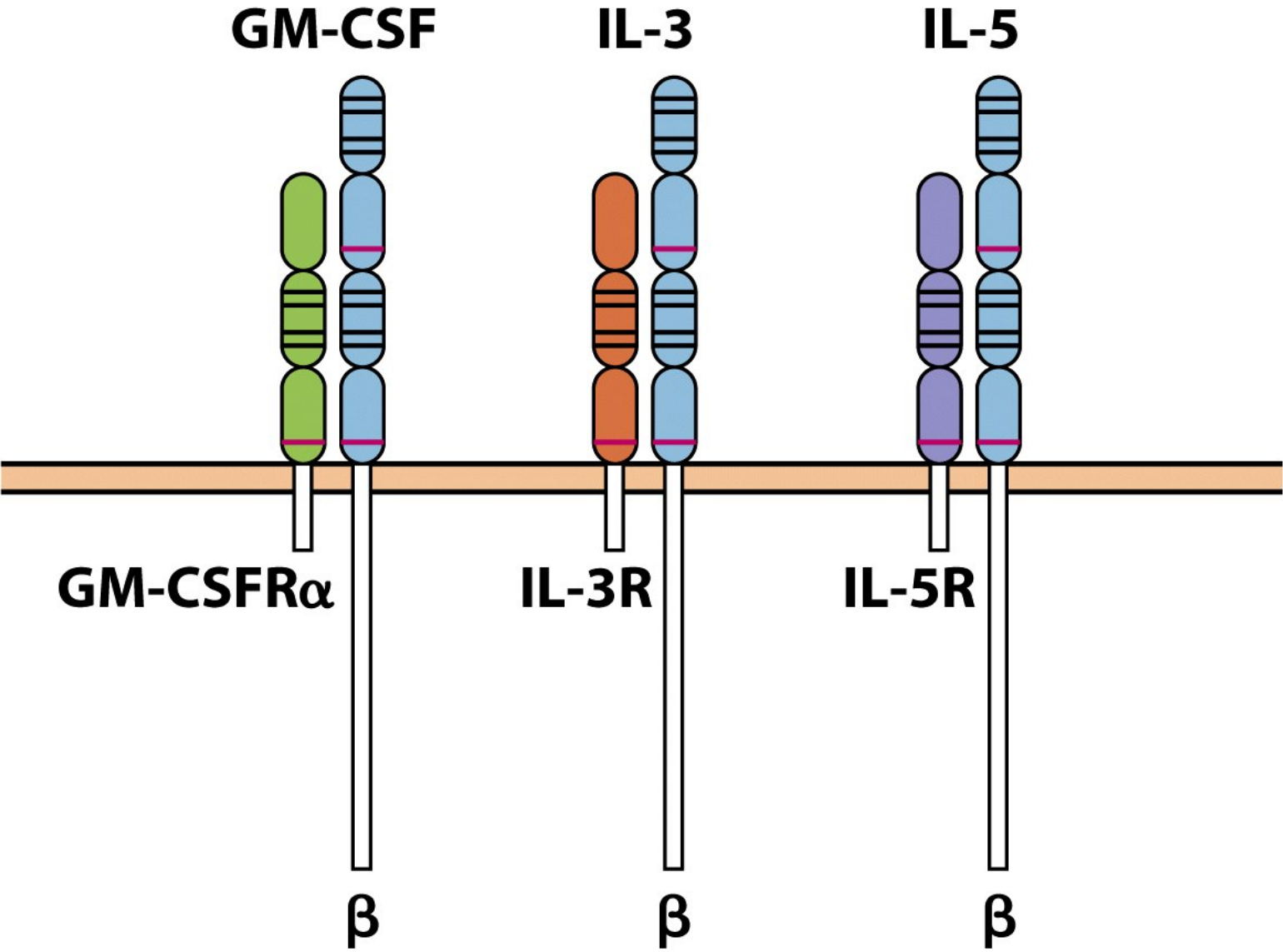


Figure 12-7a  
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# IL-6 Receptor subfamily (common gp130 subunit)

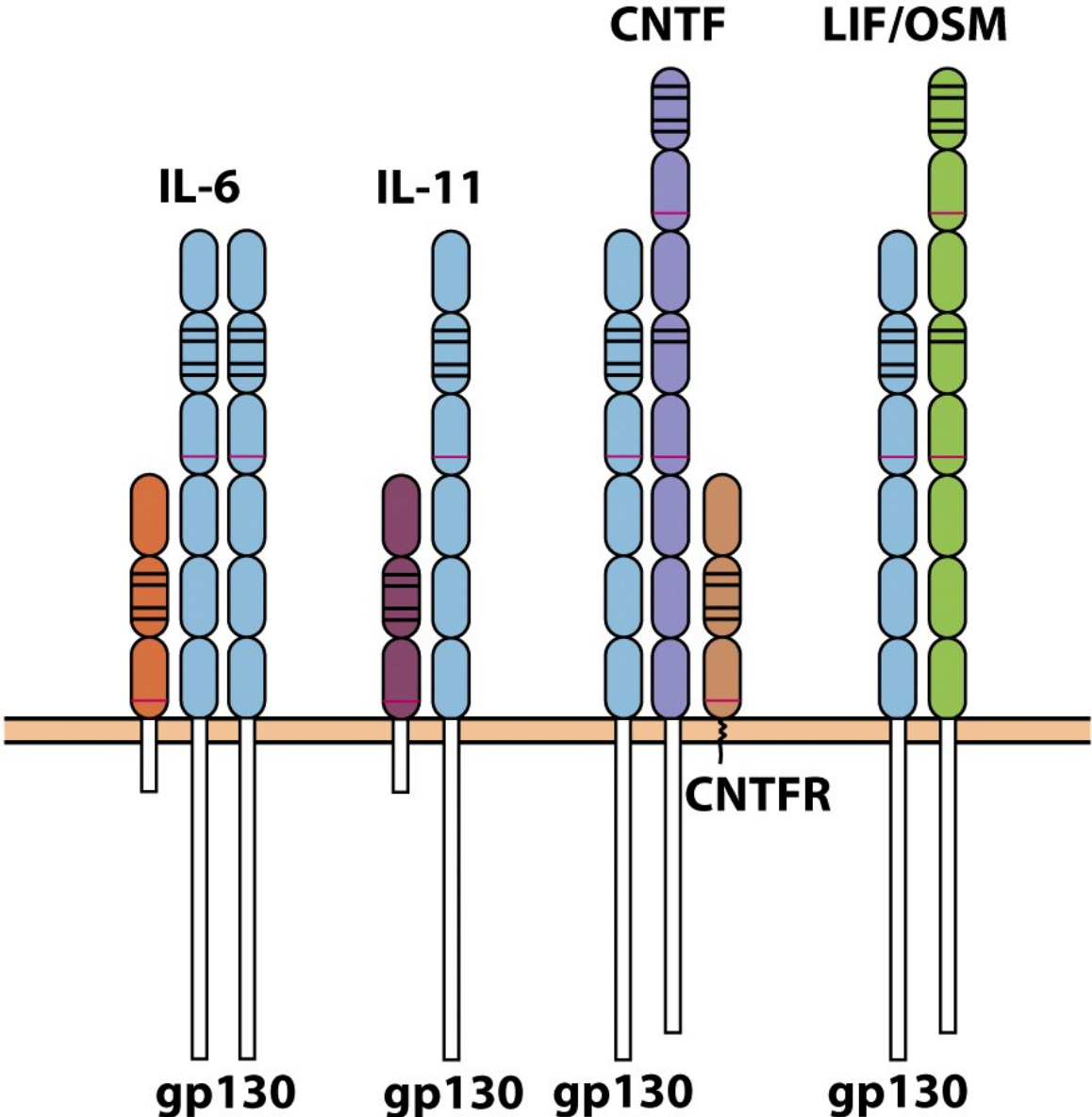


Figure 12-7b  
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# IL-2 receptor subfamily (common $\gamma$ subunit)

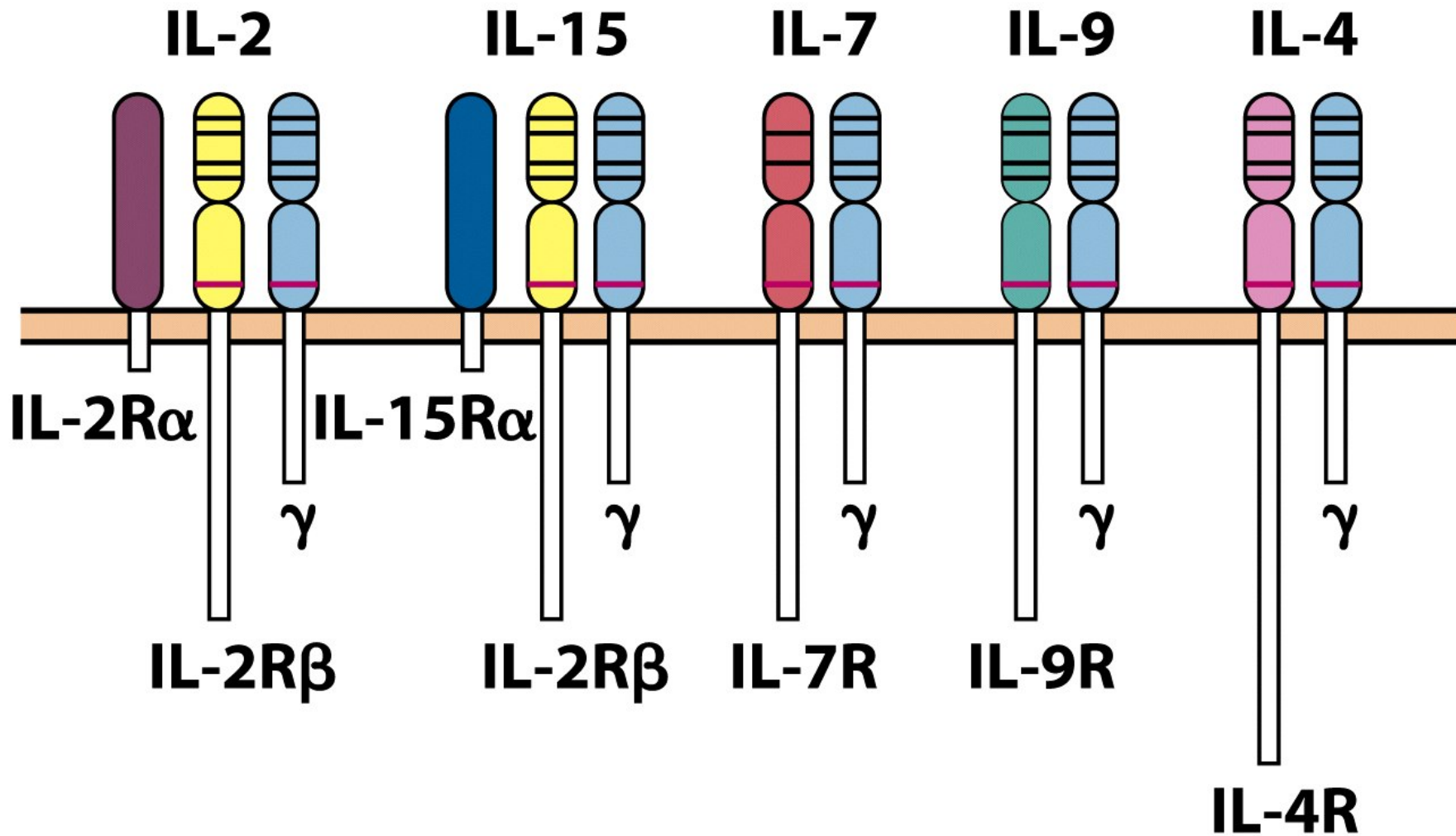
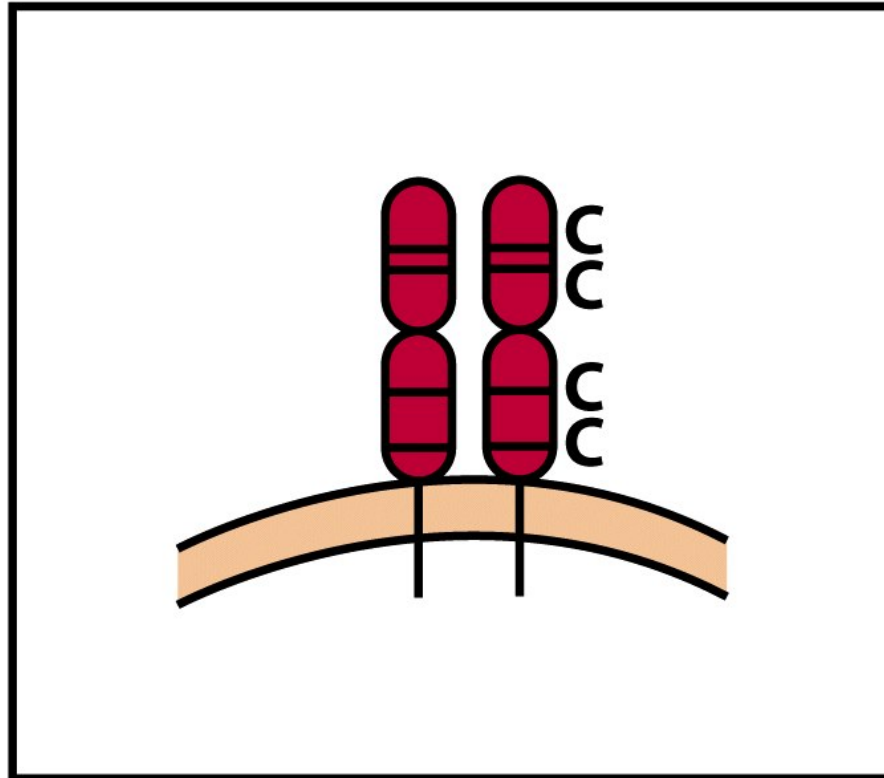


Figure 12-7c  
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# RECEPTOR FAMILY

# LIGANDS

## Class II cytokine receptors (interferon)



IFN- $\alpha$

IFN- $\beta$

IFN- $\gamma$

IL-10

IL-19

IL-20

IL-22

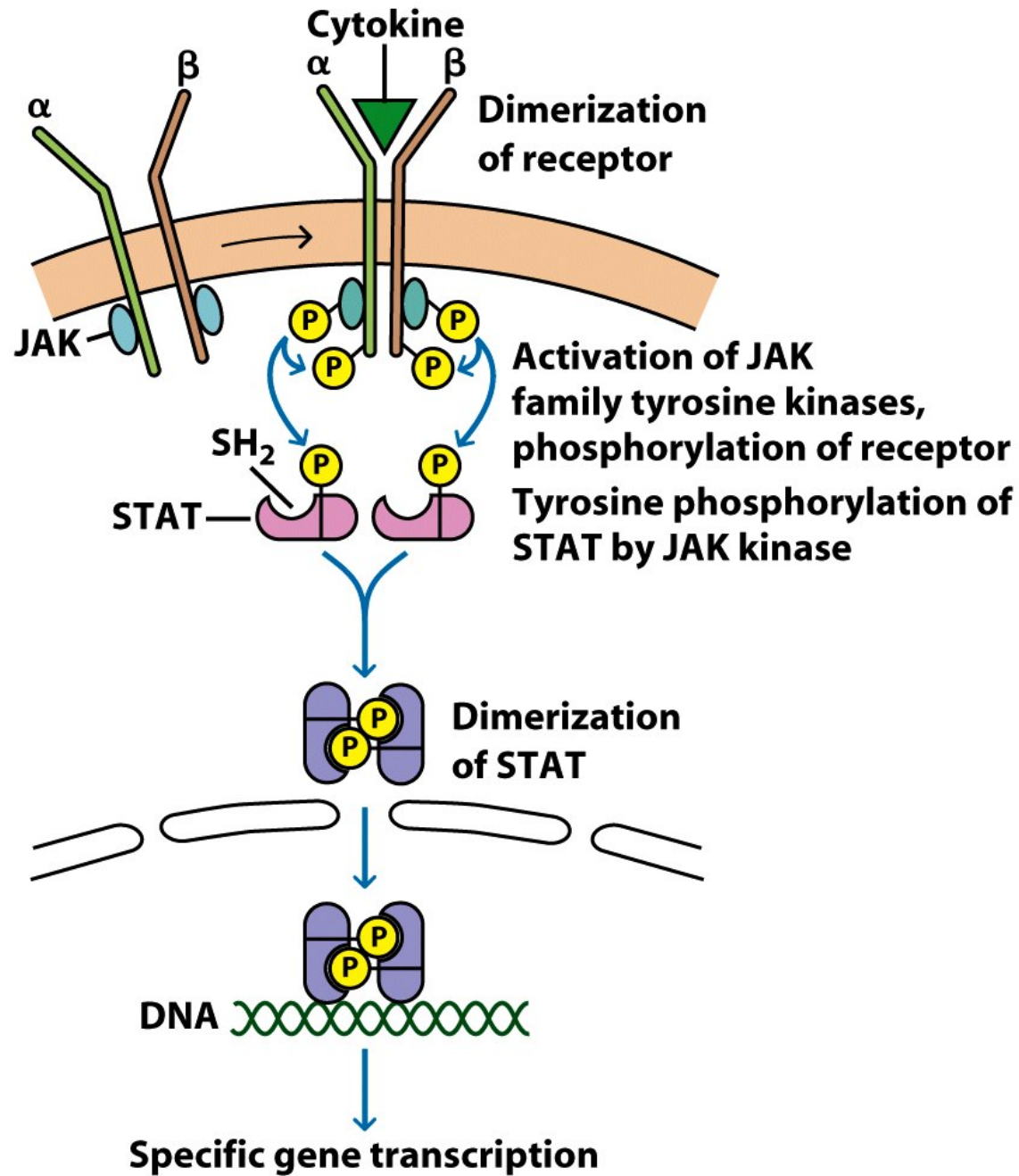
IL-24

IL-26

IL-28

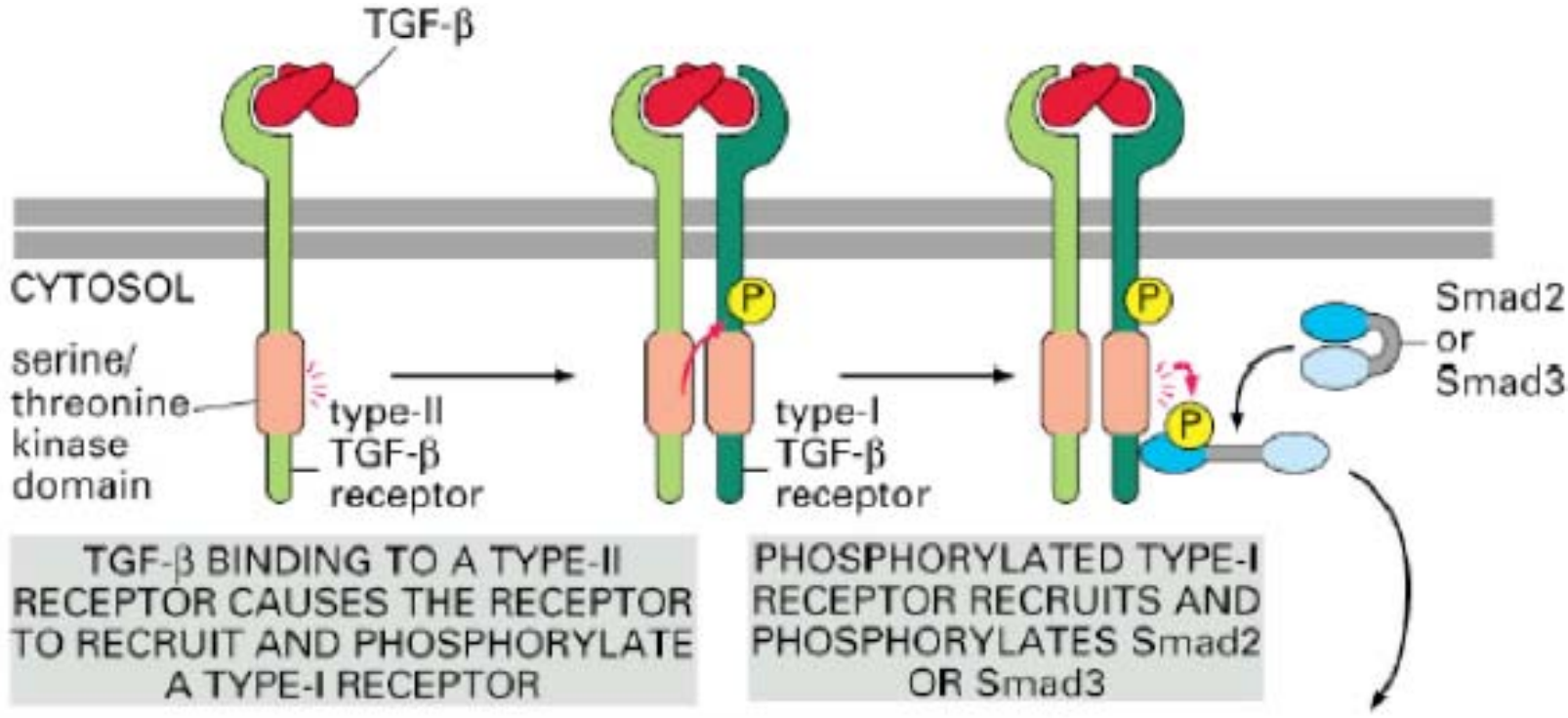
IL-29

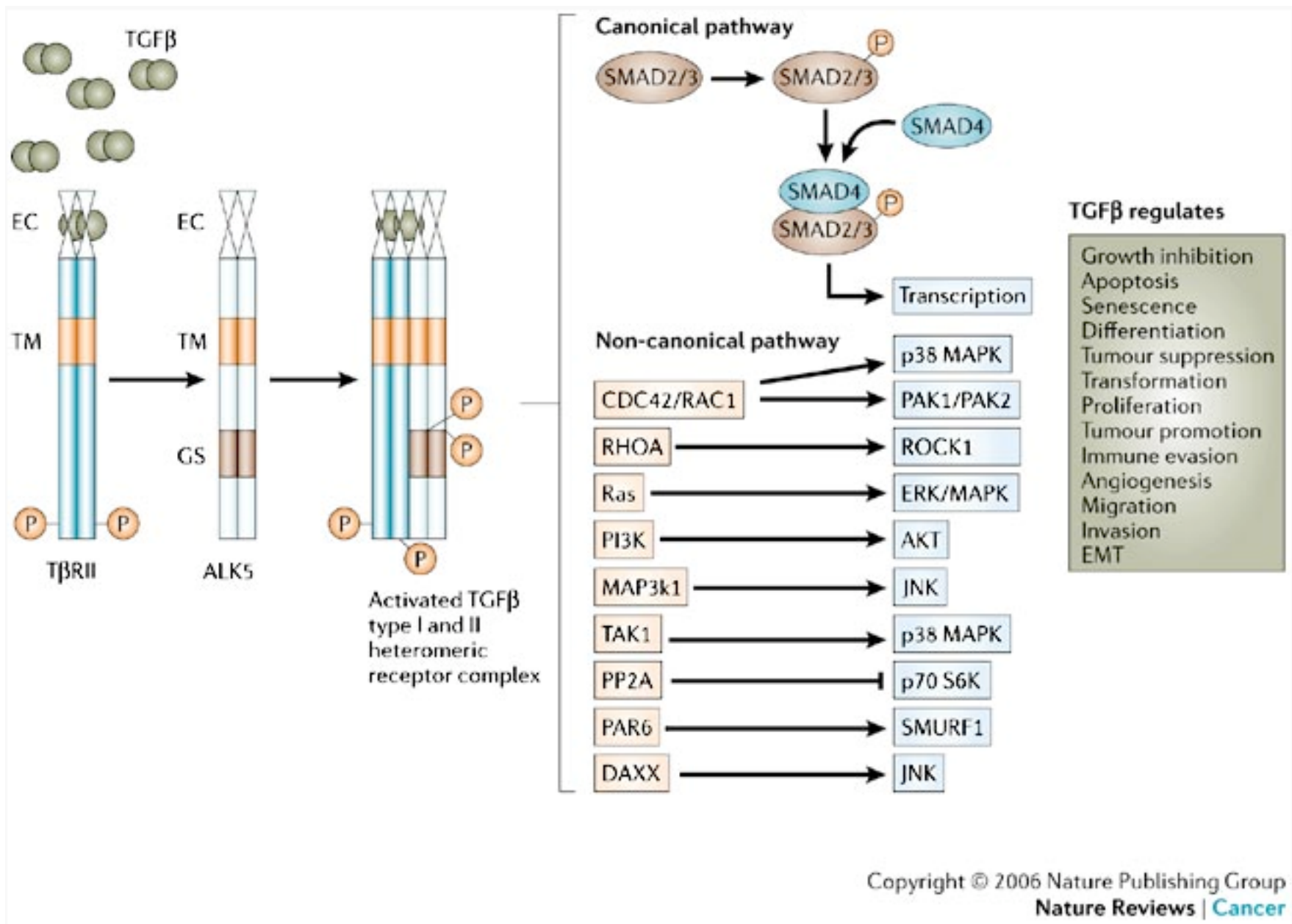
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**Figure 12-10**  
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# TGF-β Receptors



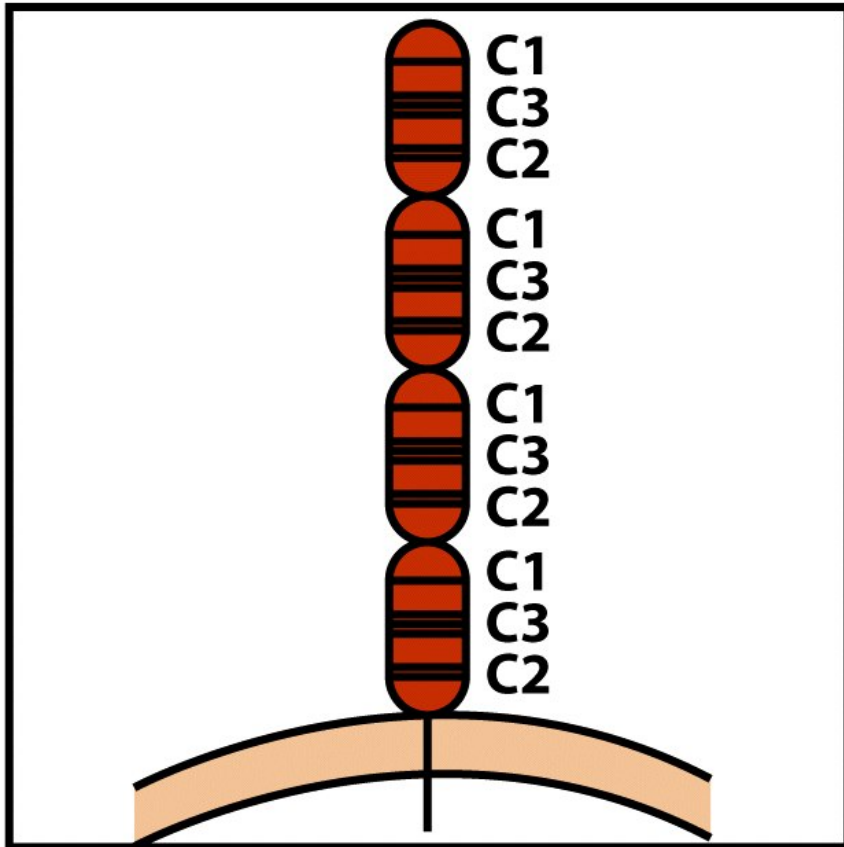


Bierie & Moses. Nat. Rev. Cancer 6:506-520, 2006

# RECEPTOR FAMILY

# LIGANDS

## TNF receptors



**TNF- $\alpha$**

**TNF- $\beta$**

**CD27L**

**CD30L**

**CD40L**

**Nerve growth factor (NGF)**

**FAS**

Figure 12-6d

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# Signal transduction by TNF receptors

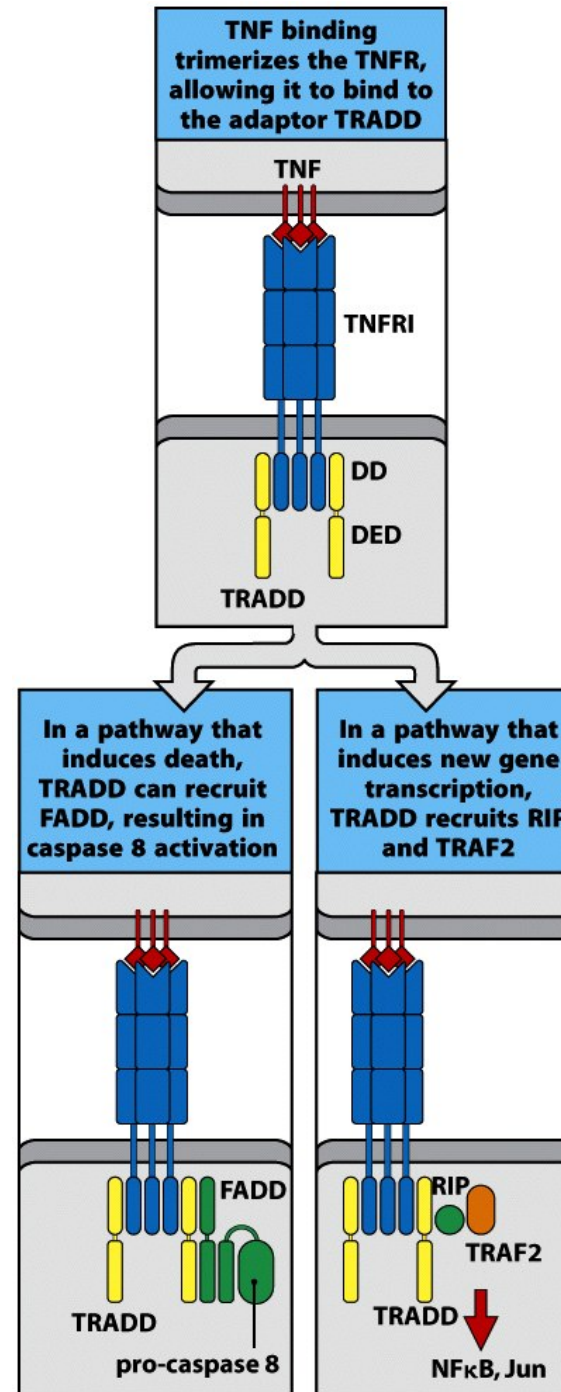
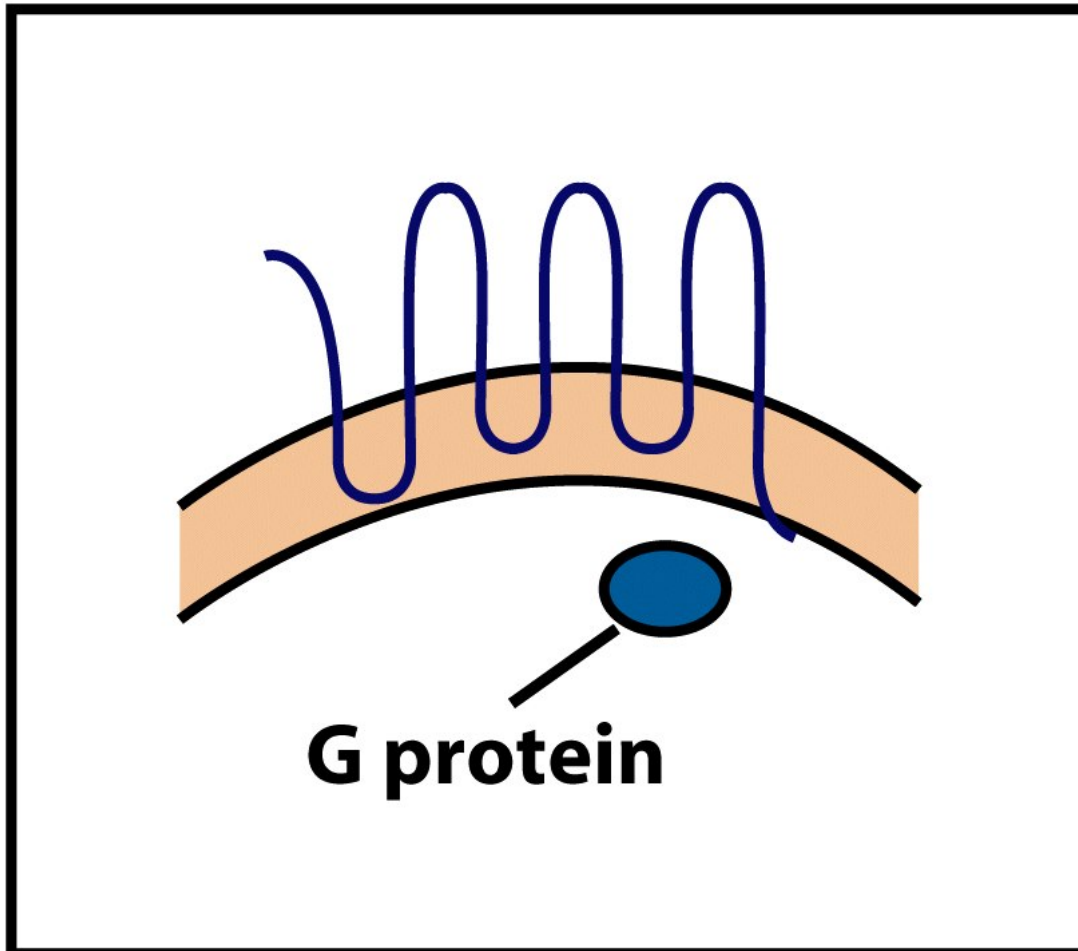


Figure 6-32 Immunobiology, 7ed. (© Garland Science 2008)

# RECEPTOR FAMILY

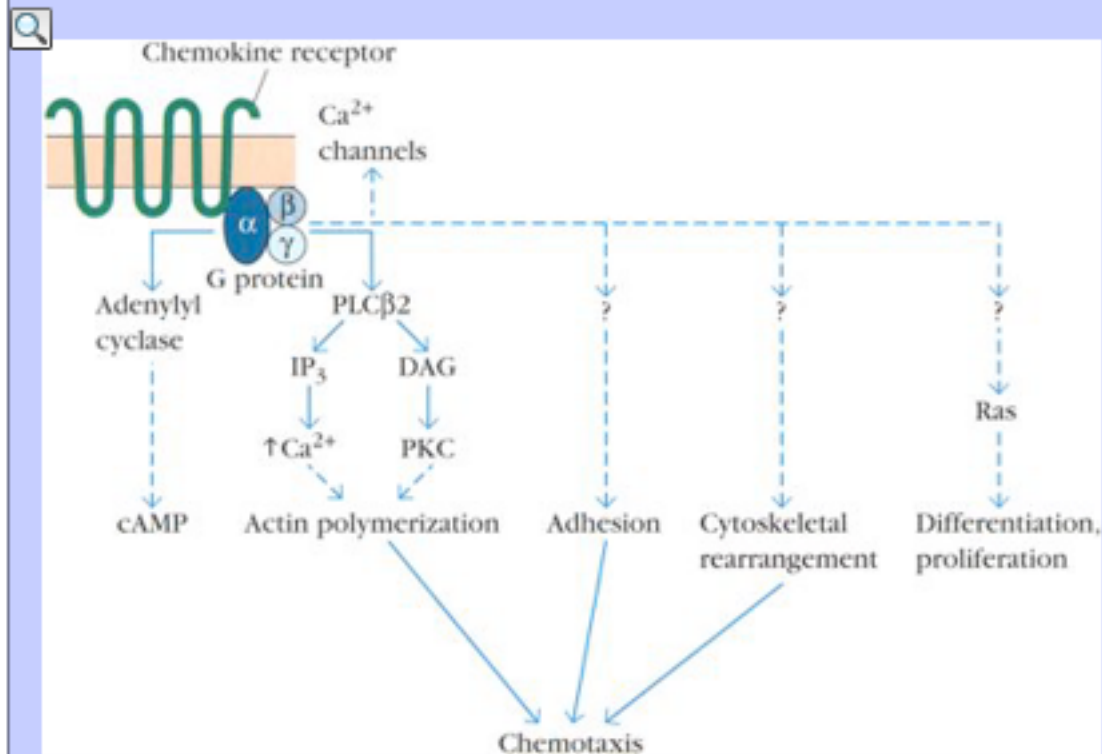
# LIGANDS

## Chemokine receptors



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

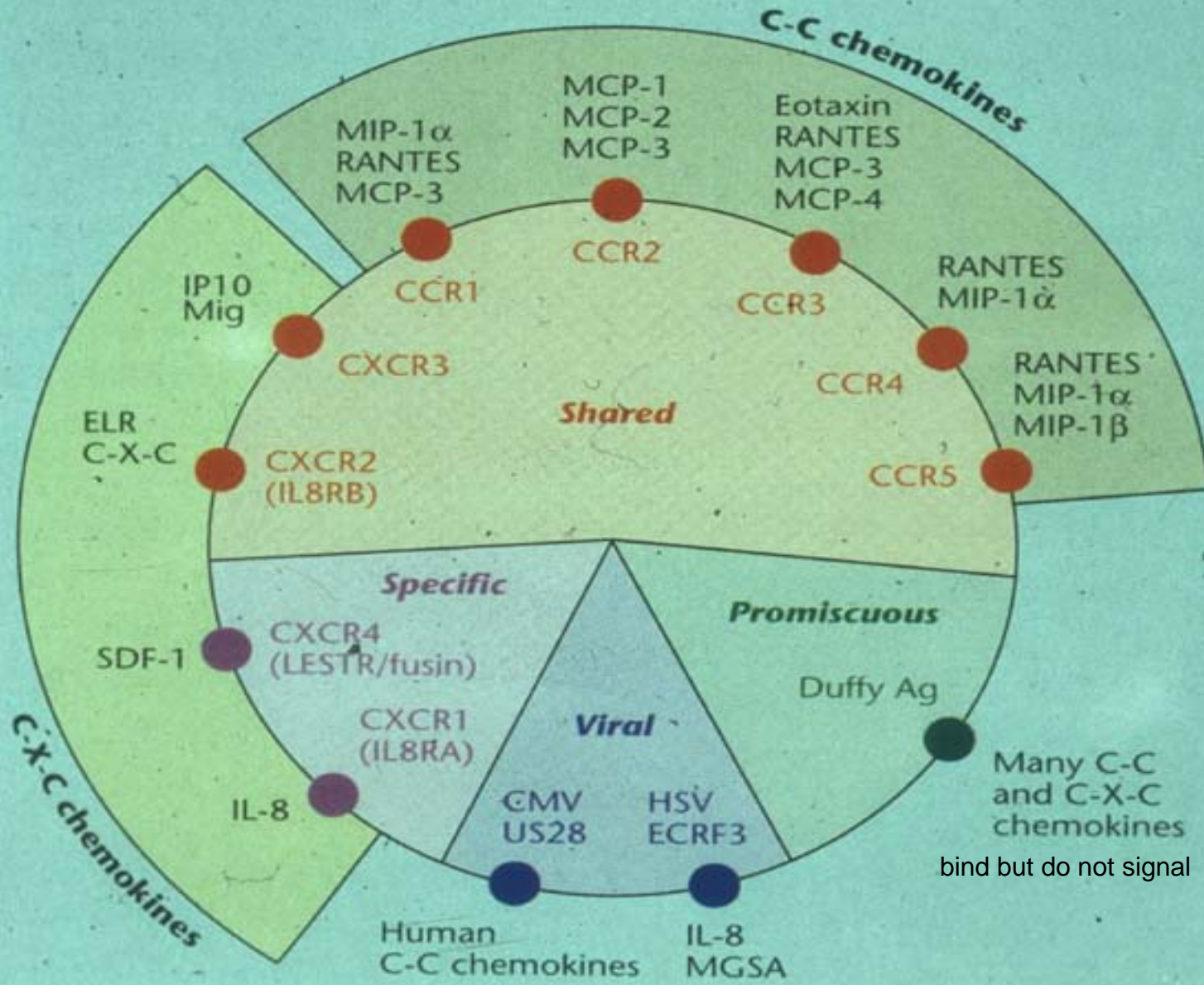
**FIGURE 15-8**



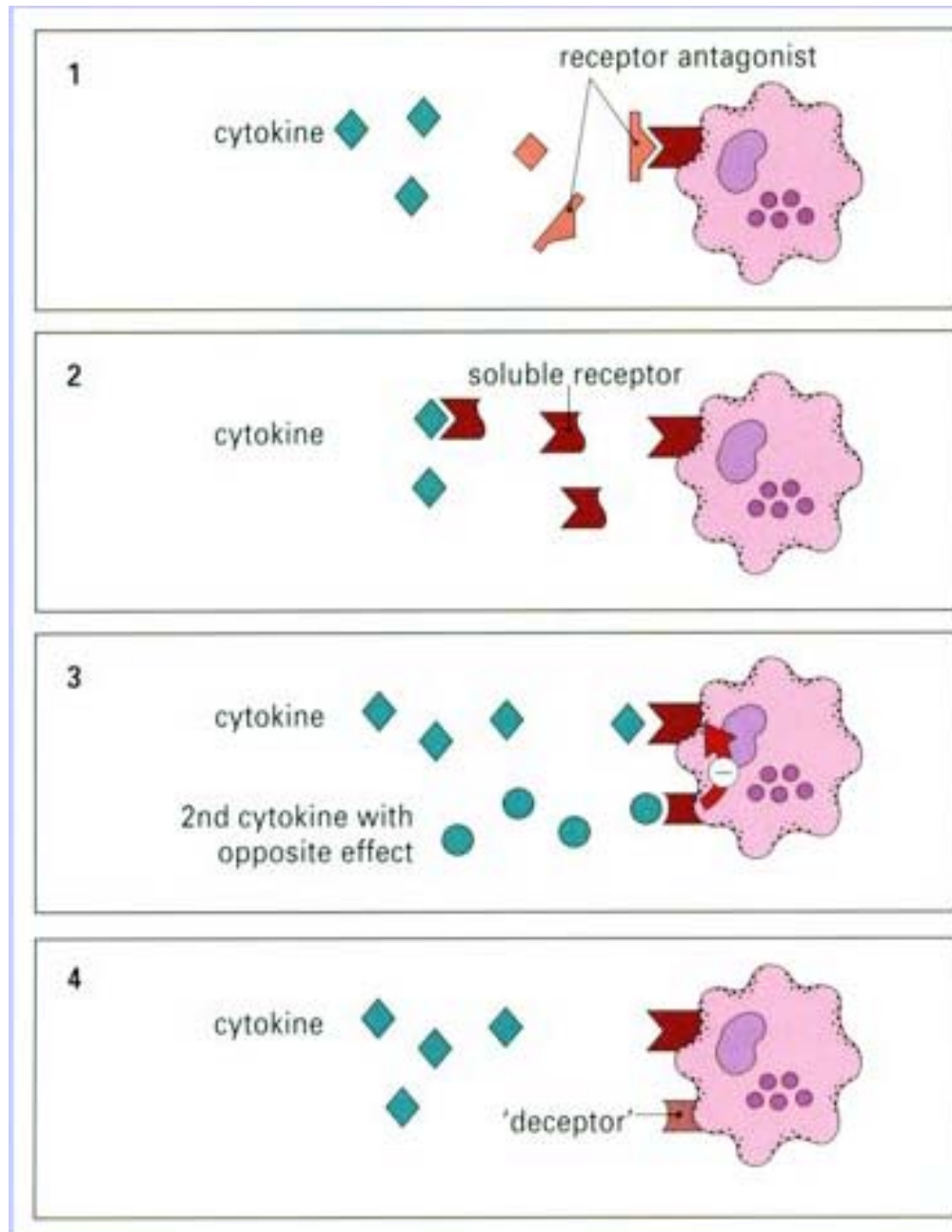
Chemokines signal through receptors coupled with heterotrimeric large G proteins. Binding of a chemokine to its receptor activates many signal-transduction pathways, resulting in a variety of modifications in the physiology of the target cell. If the signal-transduction pathway is not known or incompletely worked out, dashed lines and question marks are used here to represent probable pathways. [Adapted from Premack et al., 1996, Nature Medicine 2: 1174.]

Goldsby et al

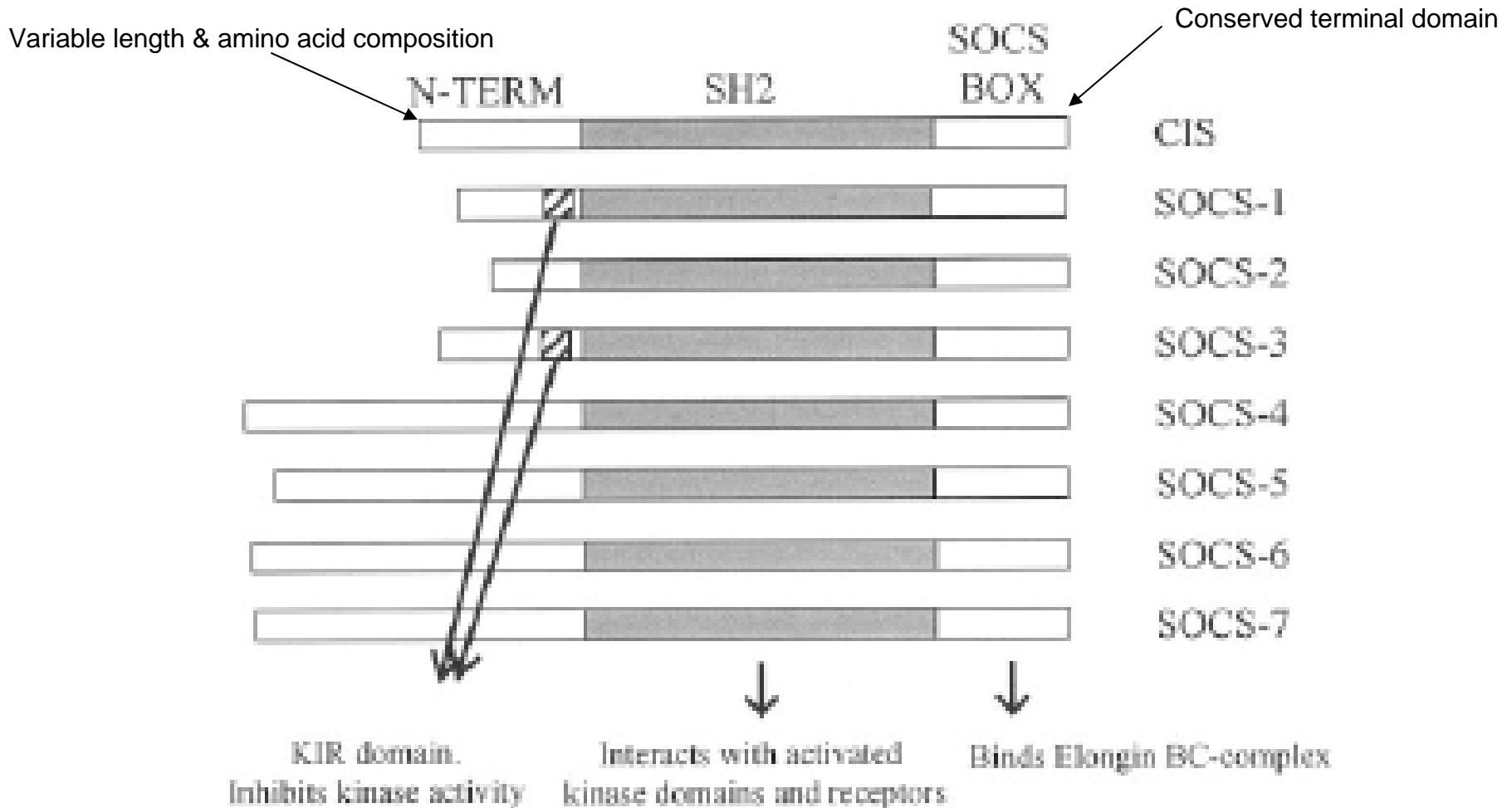
# Chemokine receptor classification and ligand specificity



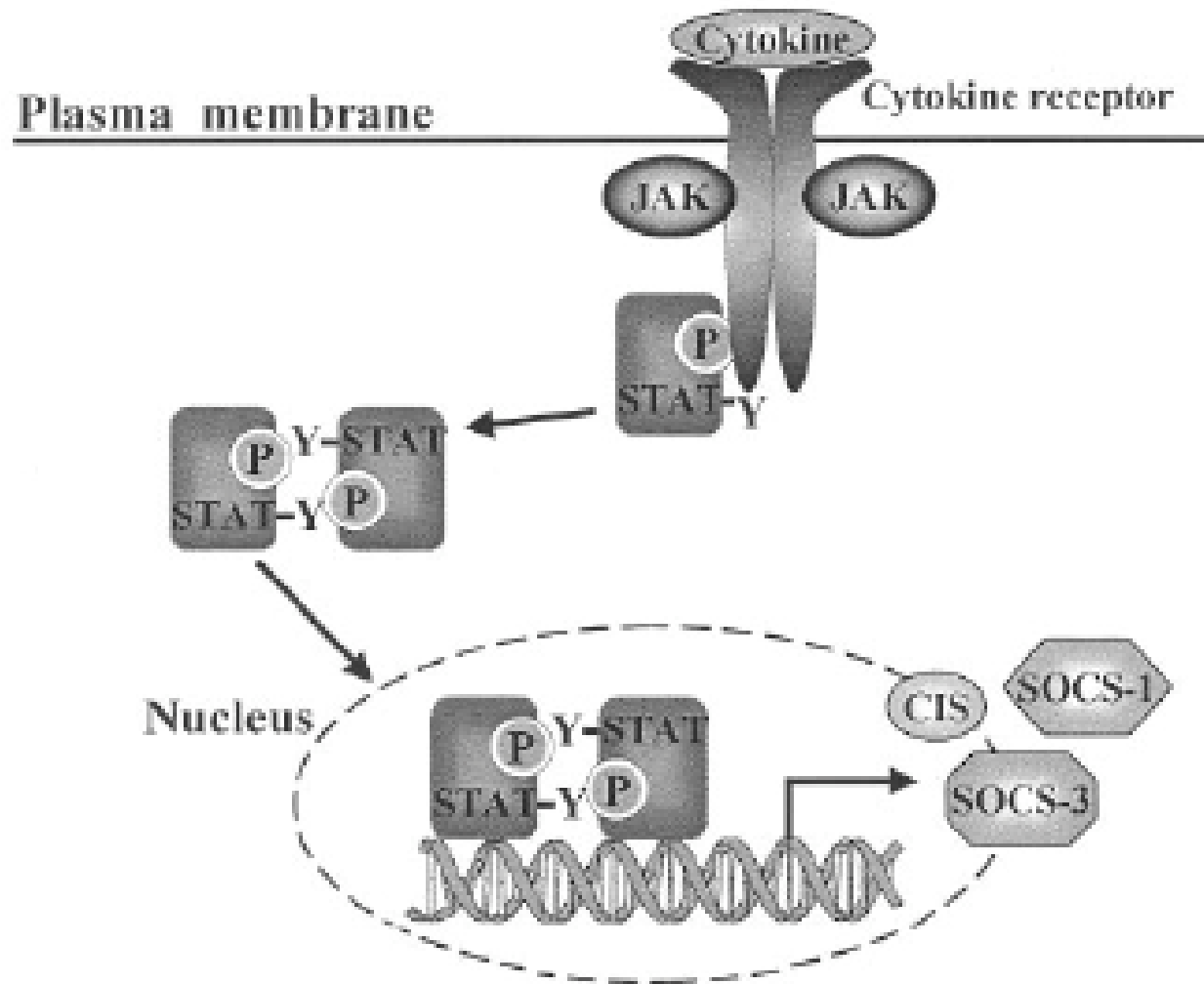
# Regulation of the cytokine network



# Suppressors of Cytokine Signaling (SOCS)

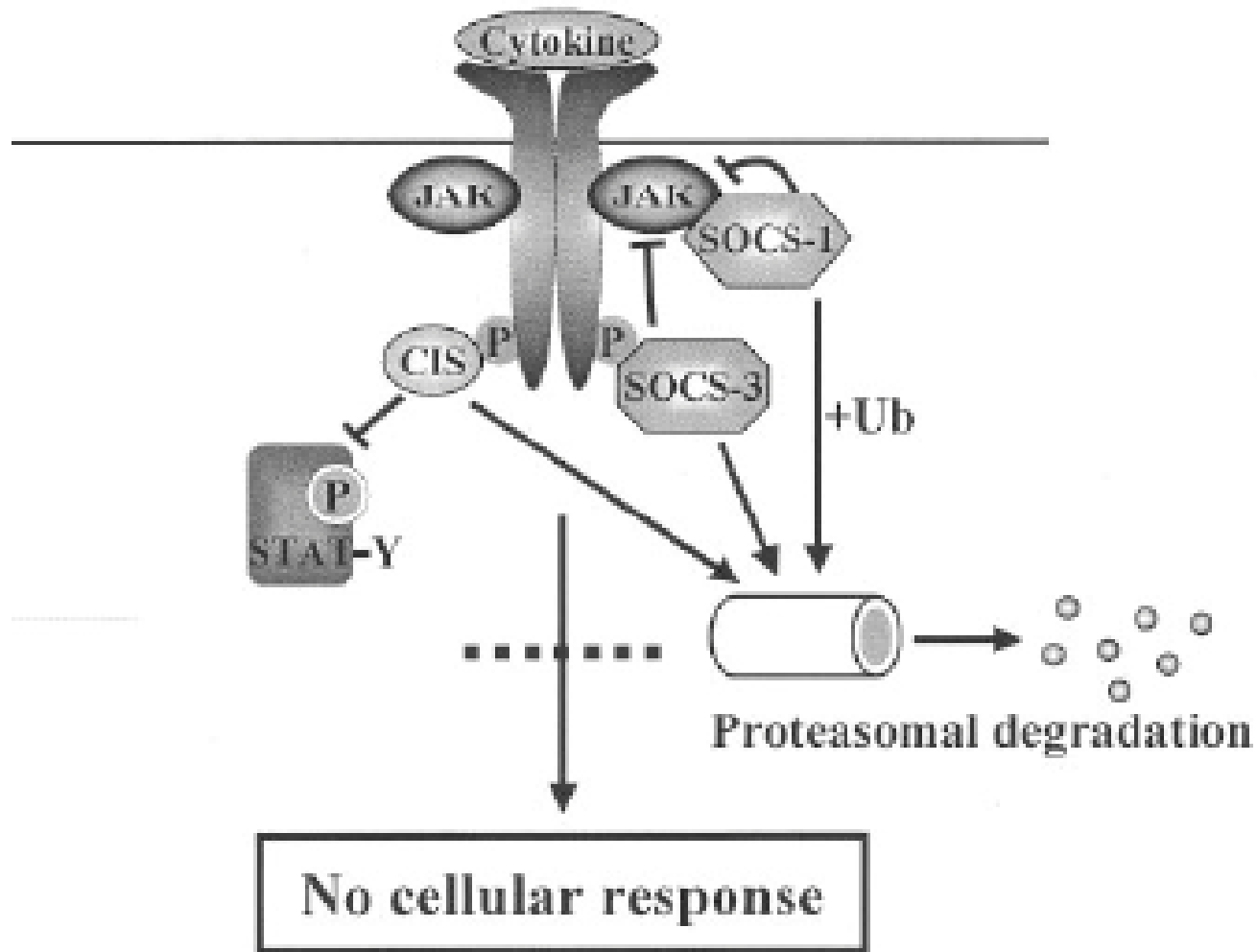


# A Induction of SOCS expression



SOCS protein expression - constitutively low, induced by cytokines, regulated by STAT proteins

## B SOCS inhibition of signalling



SOCS-mediated downregulation: (1) direct binding of SH2 domain to JAK  
(2) all SOCS proteins may target bound signaling molecules for ubiquitination via their SOCS box

# Viral strategies to thwart cytokine activity

- Cytokine homologs
- Soluble cytokine-binding proteins
- Homologs of cytokine receptors
- Interference with intracellular signaling
- Interference with cytokine secretion
- Induction of cytokine inhibitors in the host cell.

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

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

**Table 12-3**  
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See also A. Alcami, *Nature Reviews/Immunology* 3:36-50,2003

# Therapeutic issues

- General rule: cytokines and chemokines with more restricted target cell spectra are easier to develop and apply in a systemic setting.
- But - with appropriate dosages and schedules - pleiotropic cytokines such as the interferons are being used.

<b>Cytokine-based therapies in clinical use</b>		
<b>Agent</b>	<b>Nature of agent</b>	<b>Clinical application</b>
Enbrel	Chimeric TNF-receptor/IgG constant region	Rheumatoid arthritis
Remicade or Humira	Monoclonal antibody against TNF- $\alpha$ receptor	Rheumatoid arthritis Crohn's disease
Roferon	Interferon $\alpha$ -2a*	Hepatitis B Hairy-cell leukemia Kaposi's sarcoma
Intron A	Interferon $\alpha$ -2b	Hepatitis C <sup>†</sup> Melanoma
Betaseron	Interferon $\beta$ -1b	Multiple sclerosis
Avonex	Interferon $\beta$ -1a	Multiple sclerosis
Actimmune	Interferon $\gamma$ -1 $\beta$	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 <math>\alpha</math>-2a is also licensed for veterinary use to combat feline leukemia.</p> <p><sup>†</sup>Normally used in combination with an antiviral drug (ribavirin) for hepatitis C treatment.</p>		

# Possible adverse events in the use of TNF antagonists in Crohn's Disease

- Serious infection
- Demyelinating disorders
- Congestive heart failure
- Autoimmunity
- Malignancy

Cytokines and chemokines serve many functions that are critical in host defense against pathogens.

1. They provide links between innate and adaptive immunity.
2. They regulate the magnitude of the immune response.
3. They provide important amplification mechanisms that enable small numbers of lymphocytes that are specific for an antigen to activate a variety of mechanisms to eliminate the antigen.
4. Excessive production or actions of cytokines can lead to pathologic consequences