CYTOKINES

Dr Dinesh Kumar Sharma
Department of Microbiology
Ch. Charan Singh University, Meerut

INTRODUCTION

Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own.

The term "cytokine" is derived from a combination of two Greek words - "cyto" meaning cell and "kinos" meaning movement. Cytokines are cell signalling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma.

Cytokines exist in peptide, protein and glycoprotein (proteins with a sugar attached) forms. The cytokines are a large family of molecules that are classified in various different ways due to an absence of a unified classification system.

In general, cytokines are soluble molecules, although some also exist in membrane-bound forms. The interaction of a cytokine with its receptor on a target cell can cause changes in the expression of adhesion molecules and chemokine receptors on the target membrane, thus allowing it to move from one location to another.

Cytokines can also signal an immune cell to increase or decrease the activity of particular enzymes or to change its transcriptional program, thereby altering and enhancing its effector functions. Finally, they can instruct a cell when to survive and when to die.

Note: Low molecular weight proteins, <30kD
High affinity for receptors
Active in picomole amounts

Note: CKs are primarily produced by immune system but many other organs (liver, brain, endocrine glands) make CKs to influence immune response.

NOMENCLATURE

Nomenclature has been a problem because these molecules were originally named after the activity that they described, or cell types they derived from. This results in a large number of 3 or 4 letter acronyms. Names such as interleukines, chemokines, monokines, interferons, colony-growth factors are the general terms to describe cytokines fallen into different categories.

Cytokines are small secreted proteins that mediate and regulate immunity, inflammation, and hematopoiesis. However, it has been shown that membrane bound forms of cytokines, such as membrane bound TNF alpha (mTNF), also exhibit biological activities.

Cytokines are produced de novo in response to an immune stimulus. They generally (although not always) act over short distances and short time spans and at very low concentration. They act by binding to specific cell surface receptors, which then signal the cell via kinase cascades, often tyrosine kinases, to modulate gene expression. The gene products (proteins) participate in the cell proliferation, differentiation, migration, and apoptosis activities.

ACTIONS OF CYTOKINES

Cytokines bind to specific receptors on the membranes of target cells, triggering signal transduction pathways that ultimately alter enzyme activity and gene expression.

The susceptibility of a target cell to a particular cytokine is determined by the presence of specific membrane receptors.

Cytokines regulate the *intensity and duration of the* immune response by stimulating or inhibiting the activation, proliferation, and/or differentiation of various cells, by regulating the secretion of other cytokines or of antibodies, or in some cases by actually inducing programmed cell death in the target cell.

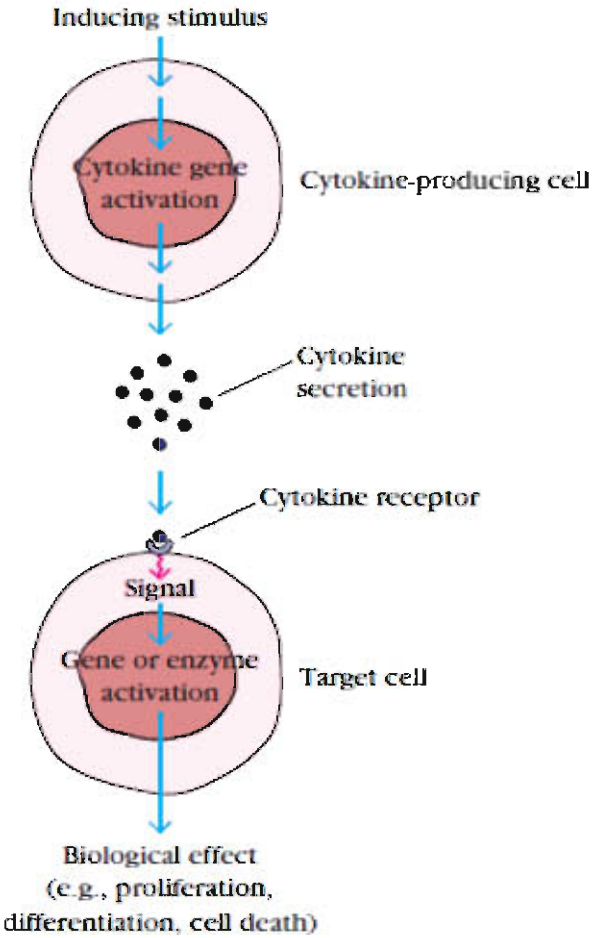


FIGURE 4-2 Overview of the induction and function of cytokines. An inducing stimulus, which may be an antigen or another cytokine, interacts with a receptor on one cell, inducing it to secrete cytokines that in turn act on receptors of a second cell, bringing about a biological consequence. In the case of IL-2, both cells may be antigen-activated T cells that secrete IL-2, which acts both on the secreting cell and on neighboring, activated T cells.

ACTIONS OF CYTOKINES

- Autocrine acts on same cell that produced it.
  IL-2 for T cell activation

- Paracrine acts on nearby cells.
  T cell help for B cells

- Endocrine acts on cell at a distance (through bloodstream).
  Inflammatory cytokines.

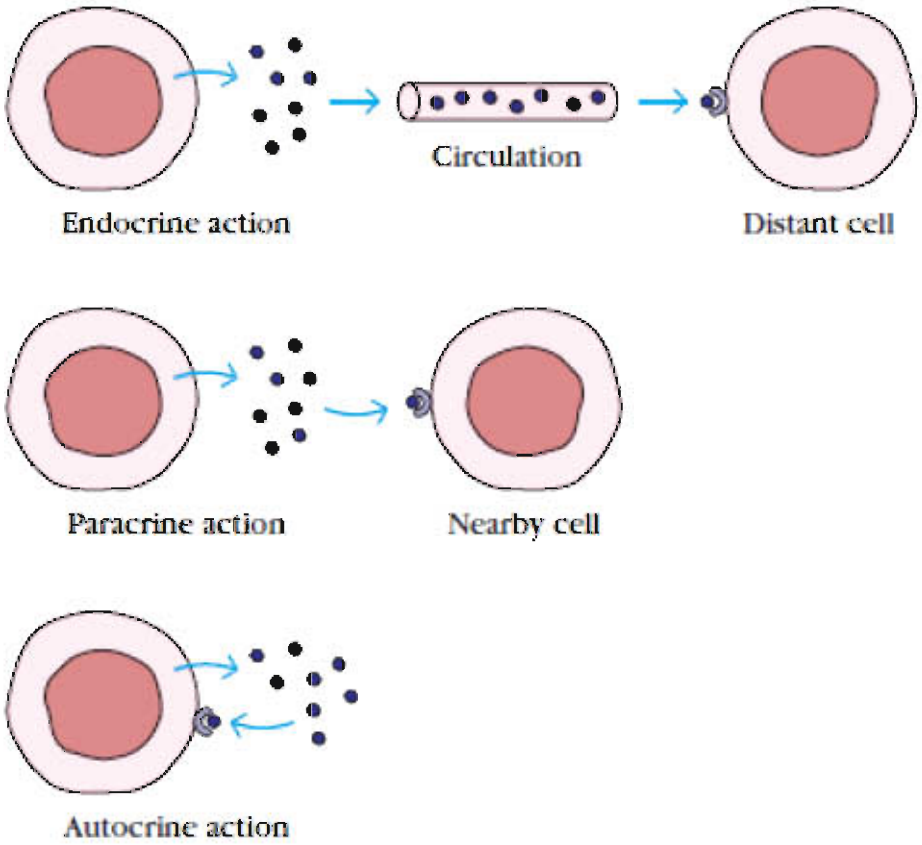


FIGURE 4-1 **Most immune system cytokines exhibit autocrine and/or paracrine action; fewer exhibit endocrine action.**

PROPERTIES OF CYTOKINES

Pleiotropy

Affects multiple cell types

Redundancy

Multiple cytokines affects cells of the same type

Synergy

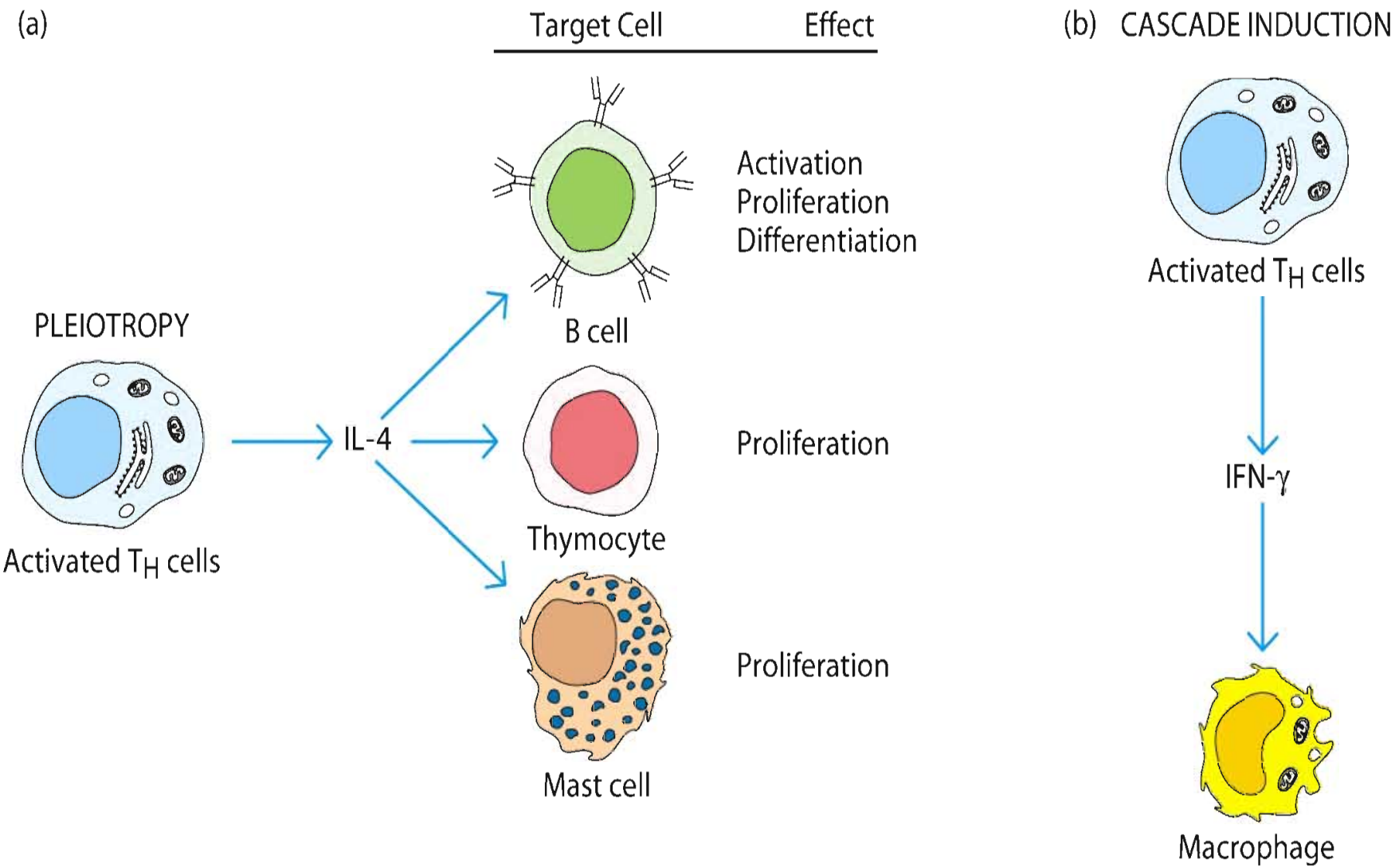
Cytokines acting in concert on the same cell

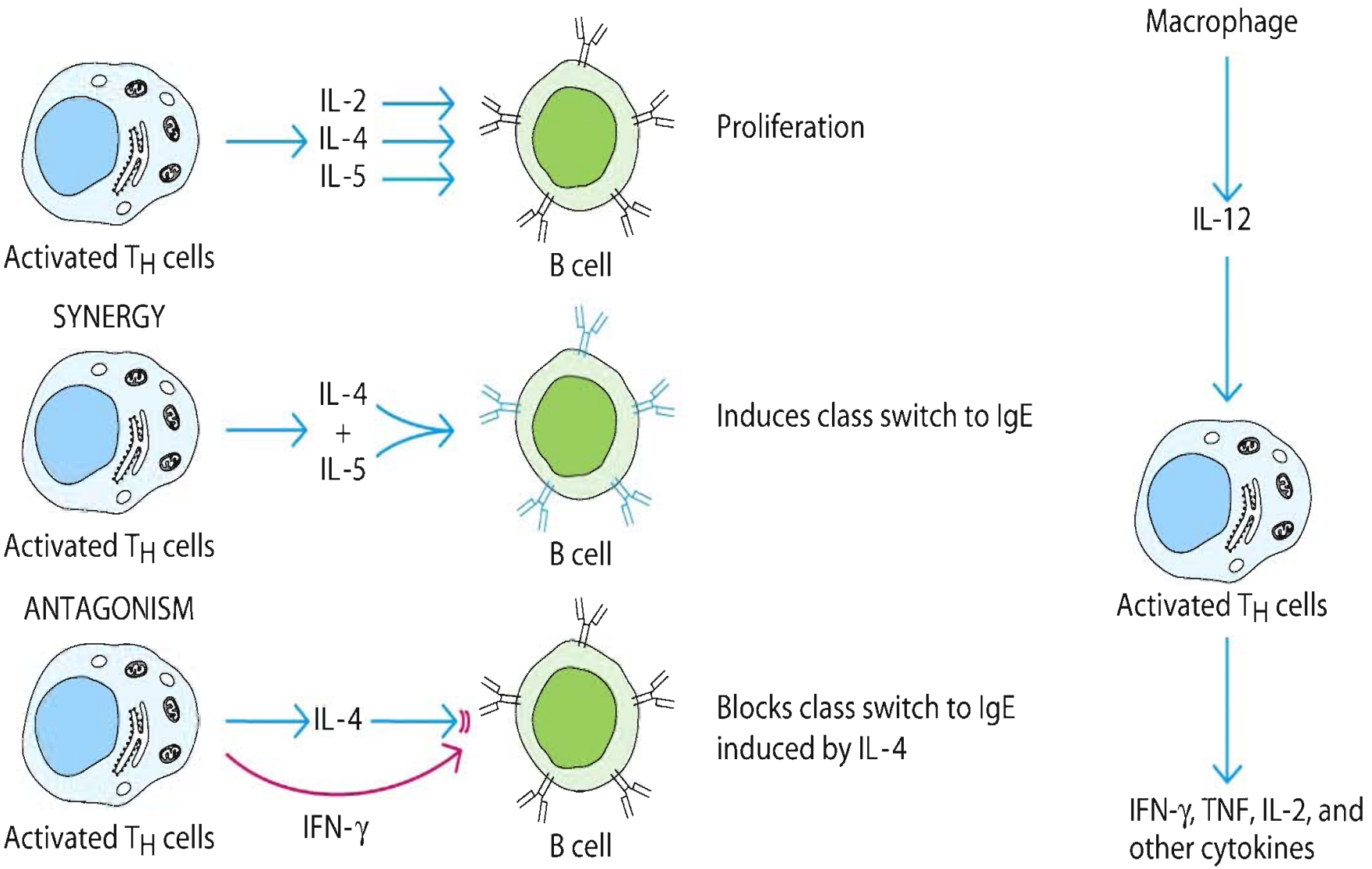
Antagonism

Competing actions

Cascading

Cytokines acting sequentially


(a)
Target Cell
Effect
PLEIOTROPY
Activated T_H cells
IL-4
B cell
Activation
Proliferation
Differentiation
Thymocyte
Proliferation
Mast cell
Proliferation
(b) CASCADE INDUCTION
Activated T_H cells
IFN-γ
Macrophage


Macrophage
IL-12
Activated T_H cells
IFN-γ, TNF, IL-2, and other cytokines
IL-2
IL-4
IL-5
Activated T_H cells
B cell
Proliferation
SYNERGY
IL-4
+
IL-5
Activated T_H cells
B cell
Induces class switch to IgE
ANTAGONISM
IL-4
IFN-γ
Activated T_H cells
B cell
Blocks class switch to IgE induced by IL-4

Cytokine-generating Cells

- Innate immunity
  - Macrophages
  - Endothelial cells
  - Fibroblasts
- Adaptive immunity
  - T lymphocytes
  - Macrophages
  - NK cells

CYTOKINE NETWORK

- CKs are part of complex system that regulates the immune system.

- CKs are primarily produced by immune system but many other organs (liver, brain, endocrine glands) make CKs to influence immune response.

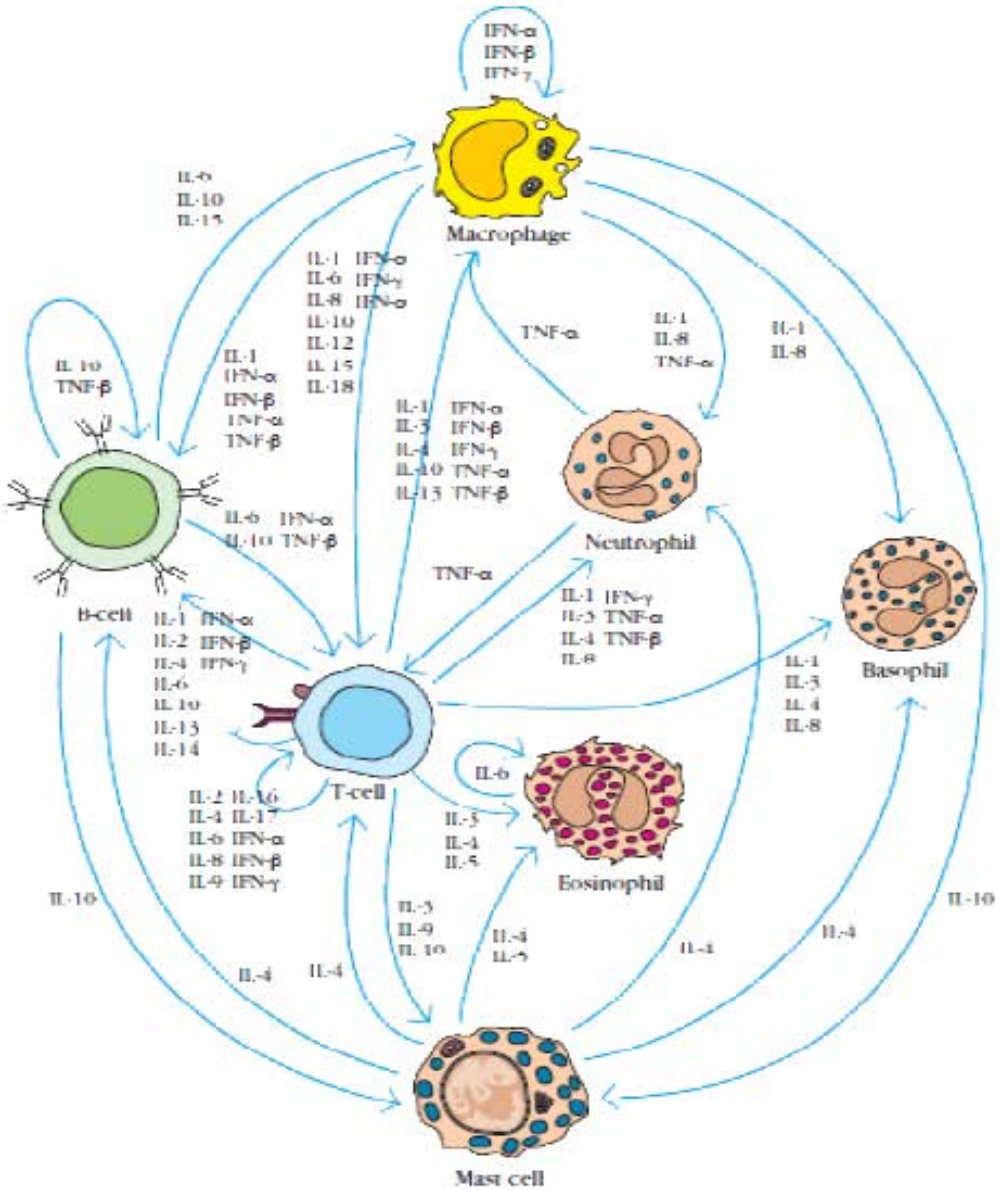


FIGURE 4-4 The cells of the immune system are subject to control by a network of cytokine actions.

VISUALIZING CONCEPTS

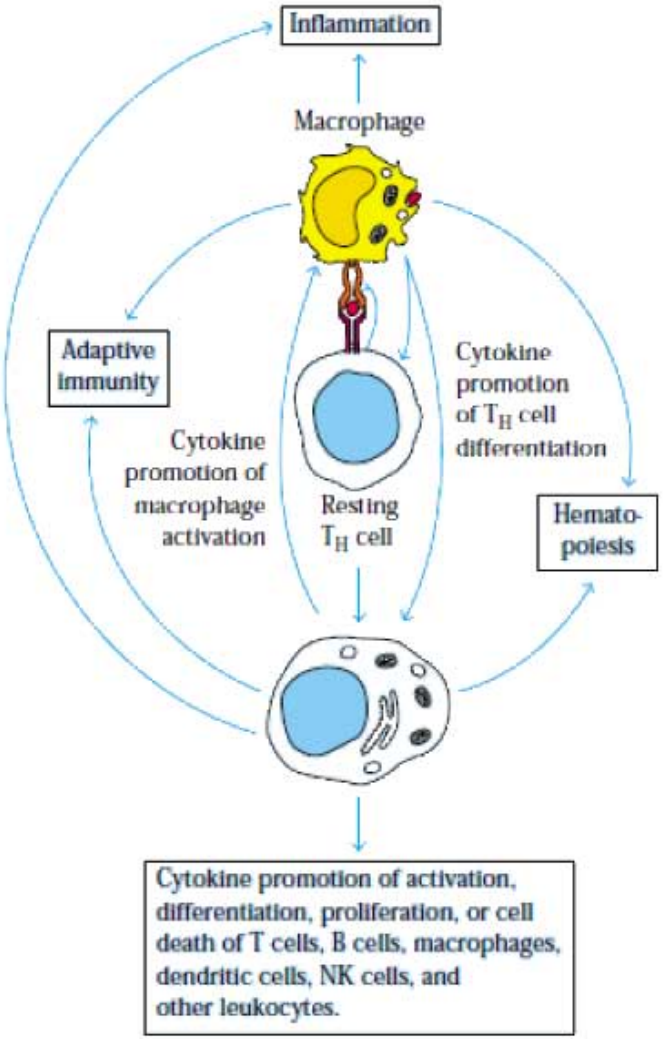


FIGURE 12-5 Interaction of antigen with macrophages and the subsequent activation of resting T_H cells leads to release of numerous cytokines (blue arrows), generating a complex network of interacting cells in the immune response.

CYTOKINE RECEPTORS

To exert their biological effects, cytokines must first bind to specific receptors expressed on the membrane of responsive target cells. Because these receptors are expressed by many types of cells, the cytokines can affect a diverse array of cells.

Cytokine Receptors Fall Within Five Families

Receptors for the various cytokines are quite diverse structurally, but almost all belong to one of five families of receptor proteins:

- Immunoglobulin superfamily receptors
- Class I cytokine receptor family (also known as the hematopoietin receptor family)
- Class II cytokine receptor family (also known as the interferon receptor family)
- TNF receptor family
- Chemokine receptor family

Many of the cytokine-binding receptors that function in the immune and hematopoietic systems belong to the class I cytokine receptor family.

The members of this receptor family have conserved amino acid sequence motifs in the extracellular domain consisting of four positionally conserved cysteine residues (CCCC) and a conserved sequence of tryptophan serine-(any amino acid)-tryptophan-serine (WSXWS, where X is the nonconserved amino acid).

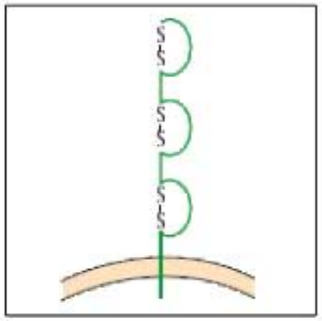
The receptors for all the cytokines classified as hematopoietins belong to the class I cytokine receptor family, which also is called the hematopoietin receptor family.

The class II cytokine receptors possess the conserved CCCC motifs, but lack the WSXWS motif present in class I cytokine receptors. Initially only the three interferons, α, β and γ, were thought to be ligands for these receptors. However, recent work has shown that the IL-10 receptor is also a member of this group.

RECEPTOR FAMILY

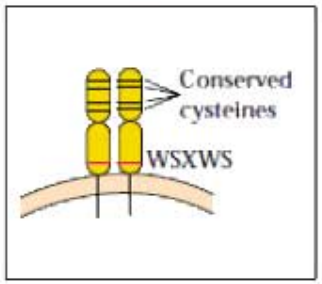
LIGANDS

(a) Immunoglobulin superfamily receptors



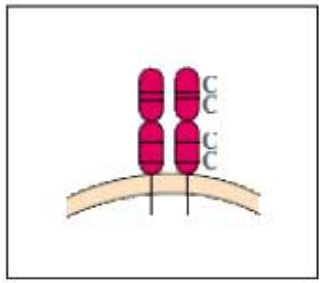
IL-1
M-CSF
C-Kit

(b) Class I cytokine receptors (hematopoietin)



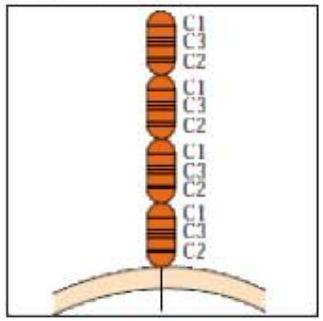
IL-2 IL-13
IL-3 IL-15
IL-4 GM-CSF
IL-5 G-CSF
IL-6 OSM
IL-7 LIF
IL-9 CNTF
IL-11 Growth hormone
IL-12 Prolactin

(c) Class II cytokine receptors (interferon)



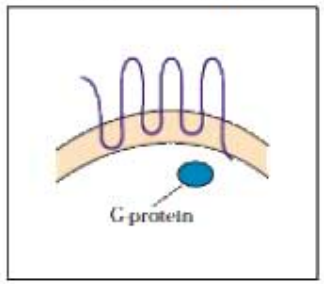
IFN-α
IFN-β
IFN-γ
IL-10

(d) TNF receptors



TNF-α
TNF β
CD40
Nerve growth factor (NGF)
FAS

(e) Chemokine receptors



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

FIGURE 12-6 Schematic diagrams showing the structural features that define the five types of receptor proteins to which most cytokines bind. The receptors for most of the interleukins belong to the class I cytokine receptor family. C refers to conserved cysteine.

TABLE 12-1 Functional groups of selected cytokines[1]

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_H1 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_H2 cells; mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Interleukin 25 (IL-25)	Unknown	Induces secretion of T_H2 cytokine profile
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 IgE; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells; $CD8^+$ cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

[1]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.

INTERLEUKINS

The name "interleukin" was chosen in 1979, to replace the various different names used by different research groups to designate interleukin 1 (lymphocyte activating factor, mitogenic protein, T-cell replacing factor III, B-cell activating factor) and interleukin 2.

The term *interleukin* derives from (*inter*-) "as a means of communication", and (-*leukin*) "deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes".

Interleukins (ILs) are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes). ILs can be divided into four major groups based on distinguishing structural features. However, their amino acids sequence similarity is rather weak (typically 15–25% identity). The human genome encodes more than 50 interleukins and related proteins.

INTERLEUKIN-1

IL-1 is produced predominantly by macrophages and macrophage-like cells but also by endothelial and epithelial cells.

IL-1 has two forms, IL-α and IL-β, encoded by two separate genes, which bind to the same IL-1 receptors. Two IL-1 receptors bind to the two forms of IL-1 with different affinities. They are distributed on a variety of cells in different concentrations throughout the body.

During development, IL-1 production by fetal macrophages in response to LPS up-regulates G-CSF production by monocytes from the bone marrow and liver of human fetuses.

During inflammation, IL-1 up-regulates the expression of endothelial adhesive glycoproteins, such as ICAM-1, promoting neutrophil attachment. IL-1 also promotes IL-6, IL-8, and other chemokine production by macrophages. IL-1, along with TNF-α, induces prostaglandin E_2 production in the hypothalamus, raising the body's temperature.

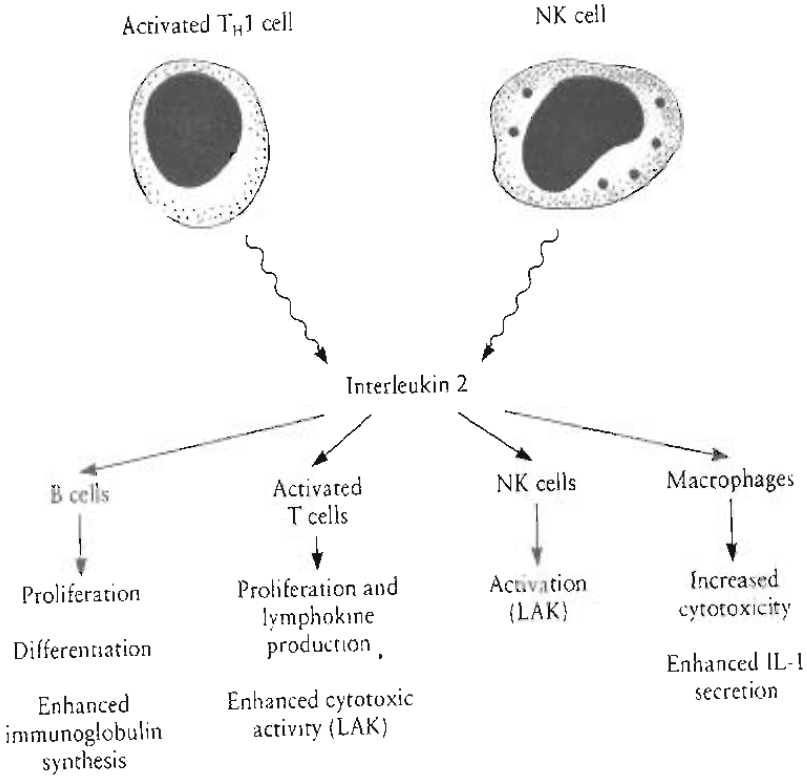
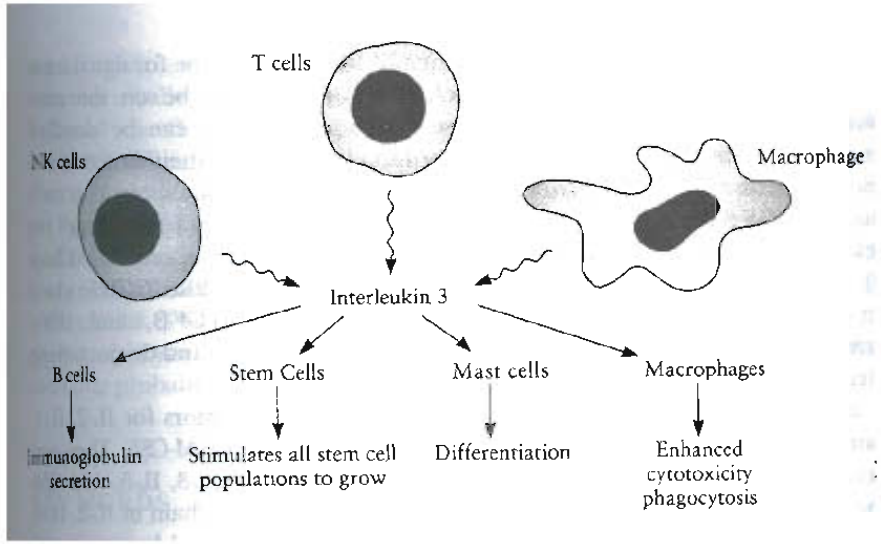
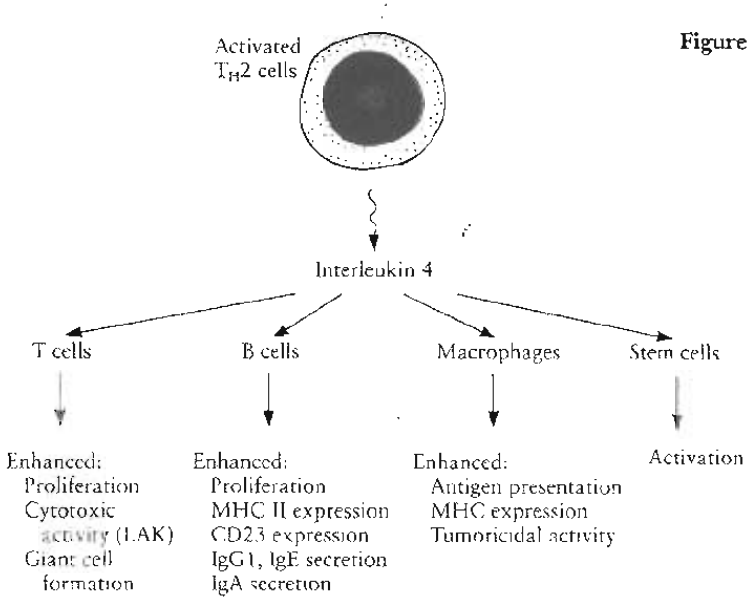


Figure 12–2 The origins and targets of interleukin-2.





Figure

DISCOVERY OF INTERFERONS

- 1957
- Isaacs and Lindenmann
- Did an experiment using chicken cell cultures
- Found a substance that interfered with viral replication and was therefore named interferon
- Nagano and Kojima also independently discovered this soluble antiviral protein

INTERFERONS

Interferons are a group of signaling proteins made and released by host cells in response to the presence of several viruses. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.

IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens.

Interferons are named for their ability to "interfere" with viral replication by protecting cells from virus infections. IFNs also have various other functions: they activate immune cells, such as NK cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigen. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

- There are three types of interferons (IFN), alpha, beta and gamma. IFN-alpha is produced in the leukocytes infected with virus, while IFN-beta is from fibroblasts infected with virus. IFN-gamma is induced by the stimulation of sensitized lymphocytes with antigen or non-sensitized lymphocytes with mitogens. It is believed that IFN-alpha and beta originated from the same ancestral gene, whereas IFN-gamma did not.
- IFN has not only an antiviral activity, but also various kinds of biological activities including cell growth inhibition, immunosuppressive effects, enhancement of macrophage, natural killer (NK) cell, and neutrophil functions, and cell differentiation-inducing activity.
- IFN also shows the antitumor activity resulting from the integration of the above-mentioned biological activities. IFN is also deeply involved in the pathogenesis of various diseases, e.g., collagen diseases such as SLE and rheumatoid arthritis, insulin-dependent diabetes mellitus, severe pancreatitis, nephritis, multiple sclerosis, allergic diseases, and atherosclerosis.
- At present, IFN is clinically used in therapy against virus infections such as hepatitis B and C, and for malignancies such as renal cell carcinoma, multiple myeloma, malignant melanoma, glioblastoma, skin cancers, malignant lymphoma and chronic myelogenous leukemia.

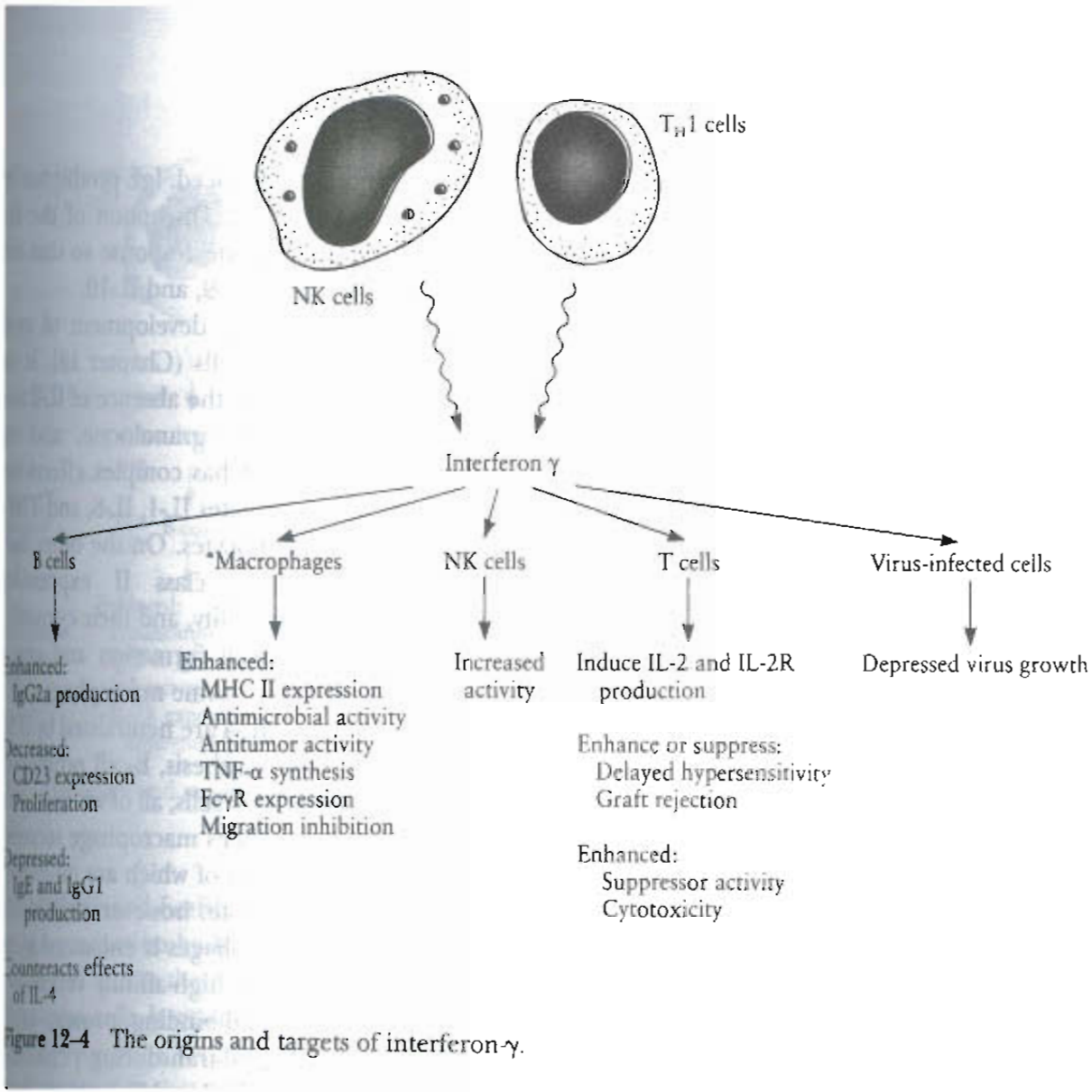


Figure 12–4 The origins and targets of interferon-γ.

TYPE I INTERFERON (IFN-α, IFN-β)

- Produced by macrophages and virus-infected cells
- Inhibits viral replication in cells via PKR and RNaseL
- Increases expression of MHC I and Tc mobilization
- Stimulates production of IFN-γ by activated T cells
- Activate NK cells

TYPE II INTERFERON (IFN-γ)

- Produced primarily by Th1
- Induce ICAM production in endothelial cells
- Activate NK cells
- Increase MHC I and MHC II expression to help Th cell and APC interaction
- Promotes B cell differentiation to plasma cell
- Promotes cytotoxic T cell differentiation

TUMOR NECROSIS FACTOR

Tumor necrosis factor is a cytokine, i.e. a small protein used by the immune system for cell signalling. If macrophges (certain white blood cells) detect an infection, they release TNF in order to alert other cells of the immune system as well as cells of other tissues, leading to inflammation.

TNF is involved in systemic inflammation and is one of the cytokines that initiate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as T helper cells, NK cells, neutrophils, mast cells, eosinophils, and neurons.
The Tumor Necrosis Family (TNF) family of cytokines regulates the development, effector function, and homeostasis of cells participating in the skeletal, neuronal, and immune systems, among others.

The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, inflammation and to inhibit tumorigenesis, viral replication, and respond to sepsis via IL-1 and IL-6-producing cells.

There are two eponymous (having the same name as) members of the TNF family: TNF**-α and TNF-*β, though*** TNF- ***β*** is more commonly known as Lymphotoxin**-α, or *LT-* α.** Both of these are secreted as soluble proteins.

TNF- **α** (frequently referred to simply as TNF) is a proinflammatory cytokine, produced primarily by activated macrophages, but also by other cell types including lymphocytes, fibroblasts, and keratinocytes (skin cells), in response to infection, inflammation, and environmental stressors.

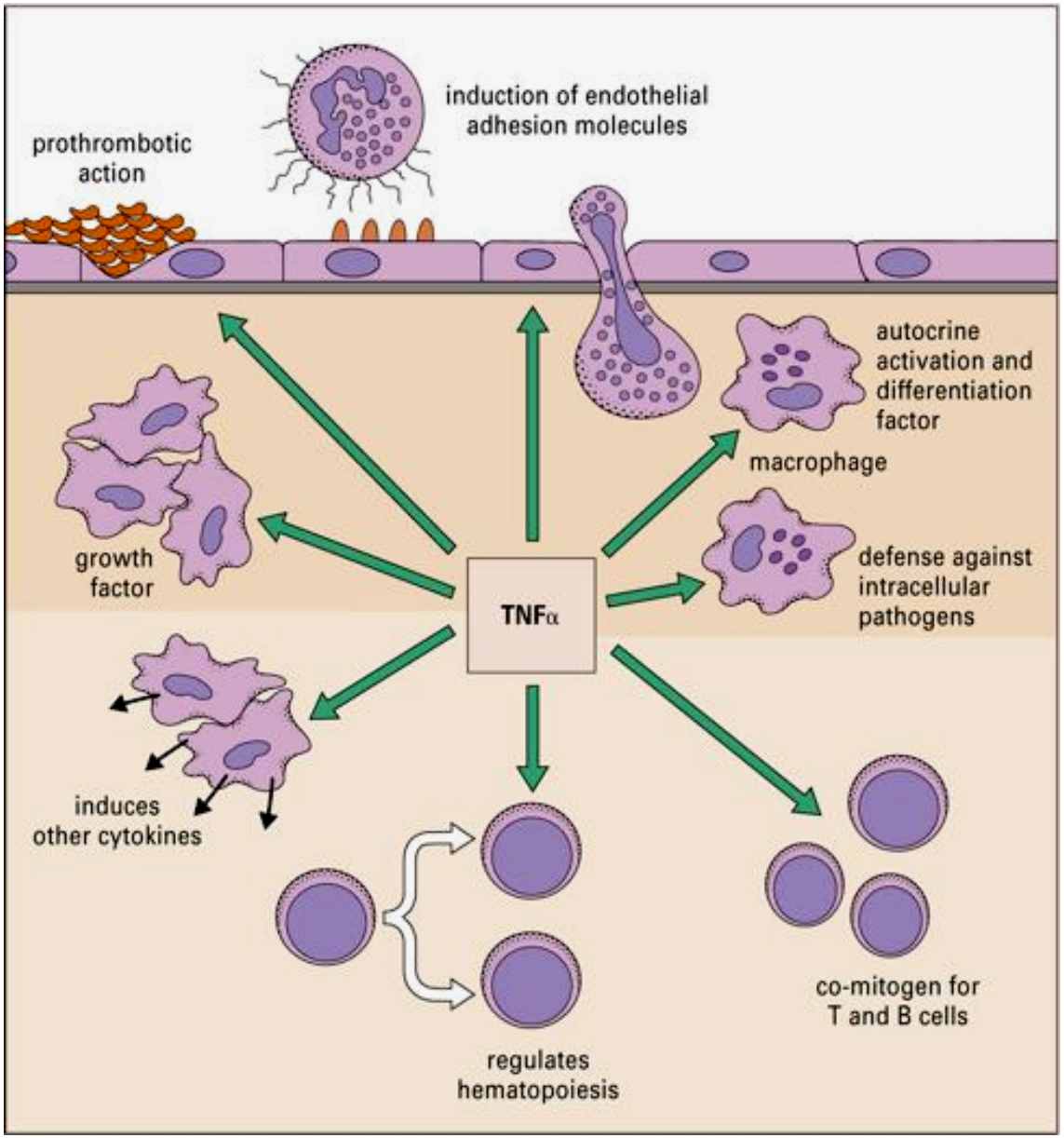
TNF elicits its biological effects by binding to its receptors, TNF-R1 or TNF-R2, which are described below. Lymphotoxin- is produced by activated lymphocytes and can deliver a variety of signals. On binding to neutrophils, endothelial cells, and osteoclasts (bone cells), Lymphotoxin- delivers activation signals; in other cells, binding of Lymphotoxin- can lead to increased expression of MHC glycoproteins and of adhesion molecules.

Tumor Necrosis Factor-α (TNF-α)

- Produced by activated macrophages and T cells.
- Most important mediator of acute inflammation in response to microbes, such as LPS.
- Induces production of myeloid CSFs, IFN-g, IL-6, IL-8 and other chemokines.
- Mediate recruitment of neutrophils and microphages to site of inflammation by stimulating cells to produce adhesion molecules (*e.g.* ICAM-1).
- Stimulates endothelial cells and macrophages to produce chemokines.
- A potent pyrogen causing fever by direct action or via IL-1.
- Promotes production of acute phase proteins, such as CRP.
- Roles in rheumatoid arthritis, psoriasis, tuberculosis, …

prothrombotic

hematopoiesis



macrophage activation

lymphocyte development

CHEMOKINES

- Produced by many leukocytes and other types of cells
- Large family of molecules (over 50)
- Have significant structural homology and overlapping functions
- Chemotactic for leukocytes, such as PMN, T and B cells
- Recruit leukocytes to sites of infection and inflammation
- Involved in lymphocytes trafficking, wound healing, metastasis, angiogenesis, lymphoid organ development….

TABLE 12-5 Haematopoietic cytokines

Haematopoietic 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/c-*kit* 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_H1 and T_H2), macrophages, mast cells	Macrophage, granulocyte production; dendritic cell maturation and activation
IL-3	T cells (T_H1 and T_H2), macrophages	Stem cells and myeloid progenitor cell growth; mast cells
IL-5	Activated helper T cells –T_H2 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-11	As above	As LIF
IL-7		T-cell survival

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.

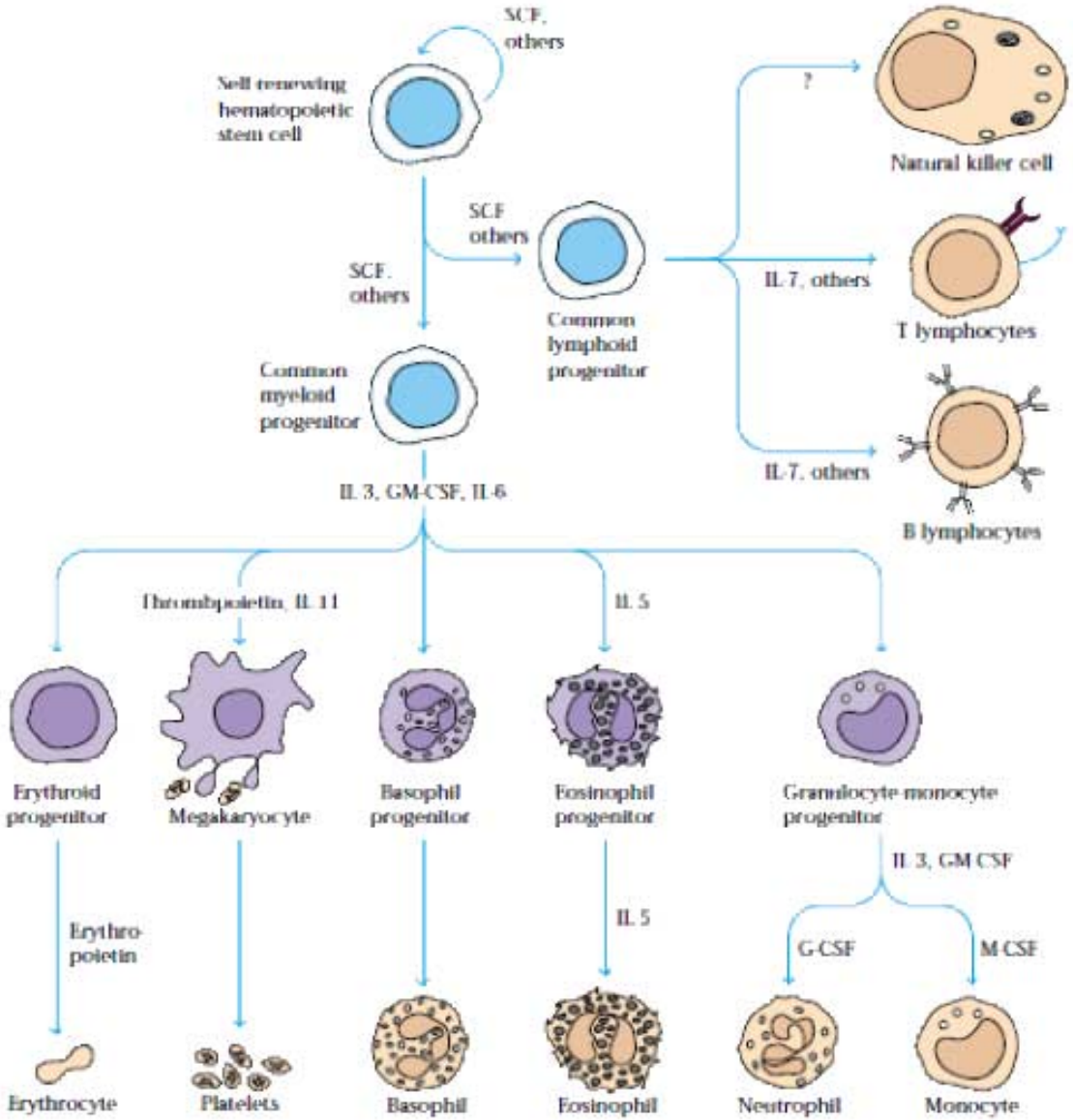


FIGURE 12-16 Hematopoietic cytokines and hematopoiesis. A variety of cytokines are involved in supporting the growth and directing the differentiation of hematopoietic cells. Note that additional factors may be required for some of the developmental pathways shown in the diagram. CFU, colony-forming unit, a cell capable of generating a colony of cells from which the fully differentiated cell type emerges.

SUMMARY

1. Cytokines are low-molecular-weight proteins that are produced and secreted by a variety of cell types. They play major roles in the induction and regulation of the cellular interactions involving cells of the immune, inflammatory and hematopoietic systems.

2. The biological activities of cytokines exhibit pleiotropy, redundancy, synergy, antagonism, and, in some instances, cascade induction.
3. There are over 200 different cytokines, most of which fall into one of the following families: hematopoietins, interferons, chemokines, and tumor necrosis factors.
4. Cytokines act by binding to cytokine receptors, most of which can be classified as immunoglobulin superfamily receptors, class I cytokine receptors, class II cytokine receptors, members of the TNF receptor family, and chemokine receptors.
5. A cytokine can only act on a cell that expresses a receptor for it. The activity of particular cytokines is directed to specific cells by regulation of the cell's profile of cytokine receptors.

THANKS