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Cytokines

This module will help you

- learn the general properties of cytokines and naming conventions.
- understand how cytokines regulate immunity.
- test your knowledge and immunology problem solving skills.

Properties of Cytokines

Cytokines are small secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis. They must be 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 membrane receptors, which then signal the cell via **second messengers**, often tyrosine kinases, to alter its behavior (**gene expression**). Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules.

Cytokine is a general name; other names include **lymphokine** (cytokines made by lymphocytes), **monokine** (cytokines made by monocytes), **chemokine** (cytokines with chemotactic activities), and **interleukin** (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (**autocrine action**), on nearby cells (**paracrine action**), or in some instances on distant cells (**endocrine action**).

It is common for different cell types to secrete the same cytokine or for a single cytokine to act on several different cell types (**pleiotropy**; see the table below.) Cytokines are **redundant** in their activity, meaning similar functions can be stimulated by different cytokines. Cytokines are often produced in a **cascade**, as one cytokine stimulates its target cells to make additional cytokines. Cytokines can also act **synergistically** (two or more cytokines acting together) or **antagonistically** (cytokines causing opposing activities).

Their short half life, low plasma concentrations, pleiotropy, and redundancy all complicated the isolation and characterization of cytokines. Searches for new cytokines is now often conducted at the DNA level, identifying genes similar to known cytokine genes.

Cytokine Activities

Cytokine activities are characterized using recombinant cytokines and purified cell populations *in vitro*, or with **knock-out mice** for individual cytokine genes to characterize cytokine functions *in vivo*. Cytokines are made by many cell populations, but the predominant producers are helper T cells (Th) and macrophages.

The largest group of cytokines stimulates immune cell proliferation and differentiation. This group includes Interleukin 1 (IL-1), which activates T cells; IL-2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5, and IL-

6, which stimulate proliferation and differentiation of B cells; Interferon gamma (IFNγ), which activates macrophages; and IL-3, IL-7 and Granulocyte Monocyte Colony-Stimulating Factor (GM-CSF), which stimulate hematopoiesis.

Selected Immune Cytokines and Their Activities*

Cytokine	Producing Cell	Target Cell	Function**
GM-CSF	Th cells	progenitor cells	growth and differentiation of monocytes and DC
IL-1α IL-1β	monocytes macrophages B cells DC	Th cells	co-stimulation
		B cells	maturation and proliferation
		NK cells	activation
		various	inflammation, acute phase response, fever
IL-2	Th1 cells	activated T and B cells, NK cells	growth, proliferation, activation
IL-3	Th cells NK cells	stem cells	growth and differentiation
		mast cells	growth and histamine release
IL-4	Th2 cells	activated B cells	proliferation and differentiation IgG_1 and IgE synthesis
		macrophages	MHC Class II
		T cells	proliferation
IL-5	Th2 cells	activated B cells	proliferation and differentiation IgA synthesis
IL-6	monocytes macrophages Th2 cells stromal cells	activated B cells	differentiation into plasma cells
		plasma cells	antibody secretion
		stem cells	differentiation
		various	acute phase response
IL-7	marrow stroma thymus stroma	stem cells	differentiation into progenitor B and T cells
IL-8	macrophages endothelial cells	neutrophils	chemotaxis
IL-10	Th2 cells	macrophages	*cytokine production*
		B cells	activation
IL-12	macrophages B cells	activated Tc cells	differentiation into CTL (with IL-2)
		NK cells	activation
IFN-α	leukocytes	various	*viral replication* MHC I expression
IFN-β	fibroblasts	various	*viral replication* MHC I expression
IFN-γ	Th1 cells, Tc cells, NK cells	various	*Viral replication*
		macrophages	MHC expression
		activated B cells	Ig class switch to IgG_{2a}
		Th2 cells	*proliferation*
		macrophages	pathogen elimination
MIP-1α	macrophages	monocytes, T cells	chemotaxis

MIP-1β	lymphocytes	monocytes, T cells	chemotaxis
TGF-β	T cells, monocytes	monocytes, macrophages	chemotaxis
		activated macrophages	IL-1 synthesis
		activated B cells	IgA synthesis
		various	*proliferation*
TNFα	macrophages, mast cells, NK cells	macrophages	CAM and cytokine expression
		tumor cells	cell death
TNF-β	Th1 and Tc cells	phagocytes	phagocytosis, NO production
		tumor cells	cell death

* CTL: cytotoxic T lymphocytes; DC: dendritic cells; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; IL: interleukin; IFN: Interferon; TGF: Tumor Growth Factor; TNF: Tumor Necrosis Factor.
** Italicized activities are inhibited.

Additional information is available at
http://www.copewithcytokines.de/
http://cmbi.bjmu.edu.cn/cmbidata/cgf/CGF_Database/cytweb/
http://www.beckmancoulter.com/products/instrument/flowcytometry/ecatalog/cdchart.asp

Other groups of cytokines include interferons and chemokines. **Interferons** IFNα and IFNβ inhibit virus replication in infected cells, while IFNγ also stimulates antigen-presenting cell MHC expression. **Chemokines** attract leukocytes to infection sites. Chemokines have conserved cysteine residues that allow them to be assigned to four groups. The groups, with representative chemokines, are C-C chemokines (RANTES, MCP-1, MIP-1α, and MIP-1β), C-X-C chemokines (IL-8), C chemokines (Lymphotactin), and CXXXC chemokines (Fractalkine). Some cytokines are predominantly inhibitory. For example, IL-10 and IL-13 inhibit inflammatory cytokine production by macrophages.

Helper T cells have two important functions: to stimulate cellular immunity and inflammation, and to stimulate B cells to produce antibody. Two functionally distinct subsets of T cells secrete cytokines which promote these different activities. Th1 cells produce IL-2, IFNγ, and TNFβ, which activate Tc and macrophages to stimulate cellular immunity and inflammation. Th1 cells also secrete IL-3 and GM-CSF to stimulate the bone marrow to produce more leukocytes. Th2 cells secrete IL-4, IL-5, IL-6, and IL-10, which stimulate antibody production by B cells.

T cells are initially activated as Th0 cells, which produce IL-2, IL-4 and IFNγ. The nearby cytokine environment then influences differentiation into Th1 or Th2 cells. IL-4 stimulates Th2 activity and suppresses Th1 activity, while IL-12 promotes Th1 activities. Th1 and Th2 cytokines are antagonistic in activity. Th1 cytokine IFNγ inhibits proliferation of Th2 cells, while IFNγ and IL-2 stimulate B cells to secrete IgG_{2a} and inhibit secretion of IgG_1 and IgE. Th2 cytokine IL-10 inhibits Th1 secretion of IFNγ and IL-2; it also suppresses Class II MHC expression and production of bacterial killing molecules and inflammatory cytokines by macrophages. IL-4 stimulates B cells to secrete IgE and IgG_1. The balance between Th1 and Th2 activity may steer the immune response in the direction of cell-mediated or humoral immunity. (See The Big Picture: Immunity to Infection.)

Cytokine Receptors

Cytokines act on their target cells by binding specific membrane receptors. The receptors and their corresponding cytokines have been divided into several families based on their structure and activities. **Hematopoietin family** receptors are dimers or trimers with conserved cysteines in their extracellular domains and a conserved Trp-Ser-X-Trp-Ser sequence. Examples are receptors for IL-2 through IL-7 and GM-CSF. **Interferon family** receptors have the conserved cysteine residues but not the Trp-Ser-X-Trp-Ser sequence, and include the receptors for IFNα, IFNβ, and IFNγ. **Tumor Necrosis Factor family** receptors have four extracellular domains; they include receptors for soluble TNFα and TNFβ as well as membrane-bound CD40 (important for B cell and macrophage activation) and Fas (which signals the cell to undergo apoptosis). **Chemokine family** receptors have seven transmembrane helices and interact with G protein. This family includes receptors for IL-8, MIP-1 and RANTES. Chemokine receptors CCR5 and CXCR4

are used by HIV to preferentially enter either macrophages or T cells.

Hematopoietin cytokine receptors are the best characterized. They generally have two subunits, one cytokine-specific and one signal transducing. An example is the GM-CSF subfamily, where a unique α subunit specifically binds either GM-CSF, IL-3, or IL-5 with low affinity and a shared β subunit signal transducer also increases cytokine-binding affinity. Cytokine binding promotes dimerization of the α and β subunits, which then associate with cytoplasmic tyrosine kinases to phosphorylate proteins which activate mRNA transcription. GM-CSF and IL-3 act on hematopoietic stem cells and progenitor cells and activate monocytes. With IL-5, they also stimulate eosinophil proliferation and basophil degranulation. All three receptors phosphorylate the same cytoplasmic protein. Antagonistic GM-CSF and IL-3 activities can be explained by their competition for limited amounts of β subunit.

The IL-2R subfamily of receptors for IL-2, IL-4, IL-7 , IL-9, and IL-15 have a common signal-transducing γ chain. Each has a unique cytokine-specific α chain. IL-2 and IL-15 are trimers, and share an IL-2R β chain. Monomeric IL-2R α has low affinity for IL-2, dimeric IL-2R $\beta\gamma$ has intermediate affinity, and trimeric IL-2R $\alpha\beta\gamma$ binds IL-2 with high affinity. IL-2R α chain (**Tac**) is expressed by activated but not resting T cells. Resting T cells and NK cells constitutively express low numbers of IL-2R $\beta\gamma$. Antigen activation stimulates T cell expression of high affinity IL-2R trimers as well as secretion of IL-2, allowing autocrine stimulation of T cell proliferation in an antigen-specific manner. Antigen specificity of the immune response is also maintained by the close proximity of antigen-presenting B cells and macrophages with their helper T cells, so that cytokines are secreted in the direction of and close to the membrane of the target cell. **X-linked severe combined immunodeficiency** (**X-*scid***) is caused by a defect in IL-2R family γ chain, which results in loss of activity from this family of cytokines.

Cytokine activity can be blocked by **antagonists**, molecules which bind cytokines or their receptors. IL-1 has a specific antagonist that blocks binding of IL-1α and IL-1β to their receptor. During immune responses, fragments of membrane receptors may be shed and then compete for cytokine binding. Microbes also influence cytokine activities. For example, Vaccinia virus (Smallpox and Cowpox) encodes soluble molecules which bind IFNγ, while Epstein-Barr virus (Infectious Mononucleosis) encodes a molecule homologous to IL-10 that suppresses immune function in the host.

The TNF receptor family molecules CD40 and Fas bind cell surface ligands on effector T cells: CD40L and FasL. CD40 is expressed on B cell and macrophage plasma membranes. T cell CD40L binding to B cell CD40 stimulates B cell proliferation and isotype switching. T cell CD40L binding to macrophage CD40 stimulates macrophages to secrete TNFα and become much more sensitive to IFNγ. T cell FasL binding to Fas leads to the activation of caspase proteases that initiate apoptosis of the cell expressing membrane Fas. Activated lymphocytes express Fas, so that FasL-positive Tc cells can regulate the immune response by eliminating activated cells. An immune deficiency disease linked to expression of a mutant Fas is characterized by over-proliferation of lymphocytes.