14.4: Stimulating Cells to Secrete Cytokines

Learning Objectives

1. Define cytokine and explain what is meant by “cytokines are pleiotropic, redundant, and multifunctional.”
2. Name 3 cytokines that regulate innate immune responses by triggering an inflammatory response.
3. Name the group of cytokines that regulates innate immunity by preventing translation of viral mRNA and by degrading both viral and host cell RNA.
4. Name 4 cytokines that regulate adaptive immune responses.
5. Name 2 cytokines that stimulate hematopoiesis.

Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems. They are produced by virtually all cells involved in innate and adaptive immunity, but especially by T helper (Th) lymphocytes. The activation of cytokine-producing cells triggers them to synthesize and secrete their cytokines. The cytokines, in turn, are then able to bind to specific cytokine receptors on other cells of the immune system and influence their activity in some manner. Cytokines are pleiotropic, redundant, and multifunctional.

- Pleiotropic means that a particular cytokine can act on a number of different types of cells rather than a single cell type.
- Redundant refers to the ability of a number of different cytokines to carry out the same function.
- Multifunctional means the same cytokine is able to regulate a number of different functions.

Some cytokines are antagonistic in that one cytokine stimulates a particular defense function while another cytokine inhibits that function. Other cytokines are synergistic wherein two different cytokines have a greater effect in combination than either of the two would by themselves. There are three functional categories of cytokines:
1. Cytokines that regulate innate immune responses,
2. Cytokines that regulate adaptive immune responses, and
3. Cytokines that stimulate hematopoiesis.

Cytokines that Regulate Innate Immunity

a. Cytokines that regulate innate immunity are produced primarily by mononuclear phagocytes such as macrophages and dendritic cells, although they can also be produced by T-lymphocytes, NK cells, endothelial cells, and mucosal epithelial cells. They are produced primarily in response to pathogen-associated molecular patterns (PAMPs) such as LPS, peptidoglycan monomers, teichoic acids, unmethylated cytosine-guanine dinucleotide or CpG sequences in bacterial and viral genomes, and double-stranded viral RNA. Cytokines produced in response to PRRs on cell surfaces, such as the inflammatory cytokines IL-1, IL-6, IL-8, and TNF-alpha, mainly act on leukocytes and the endothelial cells that form blood vessels in order to promote and control early inflammatory responses (Figure \(\PageIndex{1}\)).

![Figure \(\PageIndex{1}\): Diapedesis During Inflammation. Integrins on the surface of the leukocyte bind to adhesion molecules on the inner surface of the vascular endothelial cells. The leukocytes flatten out and squeeze between the endothelial cells to leave the blood vessels and enter the tissue. The increased capillary permeability also allows plasma to enter the tissue. Cytokines produced in response to PRRs that recognize viral nucleic acids, such as type I interferons, primarily block viral replication within infected host cells (Figure \(\PageIndex{2}\)).]
Viral replication stimulates the infected host cell to produce type I interferons. Produced by immune-activated cells or virus-infected cells in response to the double-stranded RNA (dsRNA) that many viruses produce as a part of their life cycle, interferons exert their antiviral activity by binding to uninfected neighboring cells and inducing them to produce enzymes that degrade mRNA. This not only prevents translation of viral mRNA into viral protein but also eventually kills the host cell, the factory producing the viruses. Interferons also enhance body defenses against viruses by enhancing the activities of CTLs, macrophages, NK cells, and antibody-producing cells.

Examples include:

1. Tumor necrosis factor-alpha (TNF-α): TNF-α is the principle cytokine that mediates acute inflammation. In excessive amounts, it also is the principal cause of systemic complications such as the shock cascade. Functions include acting on endothelial cells to stimulate inflammation and the coagulation pathway; stimulating endothelial cells to produce selectins and ligands for leukocyte integrins (Figure \(\PageIndex{1}\)) during diapedesis; stimulating endothelial cells and macrophages to produce chemokines that contribute to diapedesis, chemotaxis, and the recruitment of leukocytes; stimulating macrophages to secrete interleukin-1 (IL-1) for redundancy; activating neutrophils and promoting extracellular killing by neutrophils; stimulating the liver to produce acute phase proteins, and acting on muscles and fat to stimulate catabolism for energy conversion. In addition, TNF is cytotoxic for some tumor cells; interacts with the hypothalamus to induce fever and sleep; stimulates the synthesis of collagen and collagenase for scar tissue formation; and activates macrophages. TNF is produced by monocytes, macrophages, dendritic cells, Th1 cells, and other cells.

2. Interleukin-1 (IL-1): IL-1 function similarly to TNF in that it mediates acute inflammatory responses. It also works synergistically with TNF to enhance inflammation. Functions of IL-1 include promoting inflammation; activating the coagulation pathway, stimulating the liver to produce acute phase proteins, catabolism of fat for energy conversion, inducing fever and sleep; stimulating the synthesis of collagen and collagenase for scar tissue formation; stimulating the synthesis of adhesion factors on endothelial cells and leukocytes (Figure \(\PageIndex{1}\)) for diapedesis; and activates macrophages. IL-1 is produced primarily by monocytes, macrophages, dendritic cells, endothelial cells, and some epithelial cell.

3. Chemokines: Chemokines are a group of cytokines that enable the migration of leukocytes from the blood to the tissues at the site of inflammation. They increase the affinity of integrins on leukocytes for ligands on the vascular wall (Figure \(\PageIndex{1}\)) during diapedesis, regulate the polymerization and depolymerization of actin in leukocytes for movement and migration, and function as chemoattractants for leukocytes. In addition, they trigger some WBCs to release their killing agents for extracellular killing and induce some WBCs to ingest the remains of damaged tissue. Chemokines also regulate the movement of B-lymphocytes, T-lymphocytes, and dendritic cells through the lymph nodes and the spleen. When produced in excess amounts, chemokines can lead to damage of healthy tissue as seen in such disorders as rheumatoid arthritis, pneumonia, asthma, adult respiratory distress...
syndrome (ARDS), and septic shock. Examples of chemokines include IL-8, MIP-1a, MIP-1b, MCP-1, MCP-2, MCP-3, GRO-a, GRO-b, GRO-g, RANTES, and eotaxin. Chemokines are produced by many cells including leukocytes, endothelial cells, epithelial cells, and fibroblasts.

4. Interleukin-12 (IL-12): IL-12 is a primary mediator of early innate immune responses to intracellular microbes. It is also an inducer of cell-mediated immunity. It functions to stimulate the synthesis of interferon-gamma by T-lymphocytes and NK cells; increases the killing activity of cytotoxic T-lymphocytes and NK cells; and stimulates the differentiation of naive T4-lymphocytes into interferon-gamma producing Th1 cells. It is produced mainly by macrophages and dendritic cells.

5. Type I Interferons: Interferons modulate the activity of virtually every component of the immune system. Type I interferons include 13 subtypes of interferon-alpha, interferon-beta, interferon omega, interferon-kappa, and interferon tau. (There is only one type II interferon, interferon-gamma, which is involved in the inflammatory response.)

The most powerful stimulus for type I interferons is the binding of viral DNA or RNA to toll-like receptors TLR-3, TLR-7, and TLR-9 in endosomal membranes.

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Signaling pattern recognition receptors located in the cytoplasm of cells such as RIG-1 and MDA-5 also signal synthesis and secretion of type-I interferons.

Type I interferons, produced by virtually any virus-infected cell, provide an early innate immune response against viruses. Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell (Figure \(\PageIndex{2}\)). In addition, type I interferons also cause infected cells to produce enzymes that interfere with transcription of viral RNA or DNA. They also promote body defenses by enhancing the activities of CTLs, macrophages, dendritic cells, NK cells, and antibody-producing cells.
Antiviral Action of Interferon Interferon induces uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

Type I interferons also induce MHC-I antigen expression needed for recognition of antigens by cytotoxic T-lymphocytes; augment macrophage, NK cell, cytotoxic T-lymphocytes, and B-lymphocyte activity; and induce fever. Interferon-alpha is produced by T-lymphocytes, B-lymphocytes, NK cells, monocytes/macrophages; interferon-beta by virus-infected cells, fibroblasts, macrophages, epithelial cells, and endothelial cells.

6. Interleukin-6 (IL-6): IL-6 functions to stimulate the liver to produce acute phase proteins; stimulates the proliferation of B-lymphocytes; and increases neutrophil production. IL-6 is produced by many cells including T-lymphocytes, macrophages, monocytes, endothelial cells, and fibroblasts.

7. Interleukin-10 (IL-10): IL-10 is an inhibitor of activated macrophages and dendritic cells and as such, regulates innate immunity and cell-mediated immunity. IL-10 inhibits their production of IL-12, co-stimulator molecules, and MHC-II molecules, all of which are needed for cell-mediated immunity. IL-10 is produced mainly by macrophages, and TH2 cells.

8. Interleukin 15 (IL-15): IL-15 stimulates NK cell proliferation and proliferation of memory T8-lymphocytes. IL-15 is produced by various cells including macrophages.

9. Interleukin-18 (IL-18): IL-18 stimulates the production of interferon-gamma by NK cells and T-lymphocytes and thus induces cell-mediated immunity. It is produced mainly by macrophages.

Cytokines that Regulate Adaptive Immune Responses (Humoral Immunity and Cell-Mediated Immunity)

Cytokines that regulate adaptive immunity are produced primarily by T-lymphocytes that have recognized an antigen specific for that cell. These cytokines function in the proliferation and differentiation of B-lymphocytes and T-lymphocytes after antigen recognition and in the activation of effector cells.

Examples include:

1. Interleukin-2 (IL-2): IL-2 is a growth factor for NK cells and antigen-stimulated T-lymphocytes and B-lymphocytes. IL-2 also increases the killing ability of NK cells; increases the synthesis of other cytokines; increases Fas-mediated apoptosis; and stimulates antibody synthesis by B-lymphocytes. IL-2 is produced mainly by T4-lymphocytes and to a lesser extent T8-lymphocytes.

2. Interleukin-4 (IL-4): IL-4 is a major stimulus for production of the antibody isotype IgE and the development of TH2 cells for defense against helminths and arthropods. It also antagonizes the effects of interferon-gamma and thus inhibits cell-mediated immunity. IL-4 is produced mainly by TH2 cells and mast cells.

3. Interleukin-5 (IL-5): IL-5 is a growth and activating factor for eosinophils as a defense against helminths and arthropods. It also stimulates the proliferation and differentiation of antigen-activated B-lymphocytes and the production of IgA. IL-5 is produced mainly by TH2 cells.

4. Interferon-gamma (IFN-?): Interferons modulate the activity of virtually every component of the immune system. Type I interferons include more than 20 types of interferon-alpha, interferon-beta, interferon omega, and interferon tau. There is only one type II interferon, interferon-gamma. Type II interferon is produced by activated T-lymphocytes as part of an immune response and functions mainly to promote the activity of the components of the
cell-mediated immune system such as CTLs, macrophages, and NK cells. IFN-? is the principal cytokine for activating macrophages. It also induces the production of MHC-I molecules, MHC-II molecules, and co-stimulatory molecules by APCs in order to promote cell-mediated immunity and activates and increases the antimicrobial and tumoricidal activity of monocytes, macrophages, neutrophils, and NK cells. IFN-? stimulates the differentiation of T4-lymphocytes into T\textsubscript{H1} cells and inhibits the proliferation of T\textsubscript{H2} cells; stimulates the production of IgG subclasses that activate the complement pathway and promote opsonization; and augments or inhibits other cytokine activities. IFN-? is produced primarily by T\textsubscript{H1} cells, CD8\textsuperscript{+} cells, and NK cells.

5. Transforming growth factor-beta (TGF-ß): TGF-ß functions to inhibit the proliferation and effector function of T-lymphocytes; inhibit the proliferation of B-lymphocytes; and inhibits macrophage function. It also promotes tissue repair. TGF-ß is produced by T-lymphocytes, macrophages, and other cells.

6. Lymphotoxin (LT): LT plays a role in the recruitment and activation of neutrophils and in lymphoid organogenesis. Being chemically similar to TNF, LT is also a mediator of acute inflammatory responses. LT is made by T-lymphocytes.

7. Interleukin-13 (IL-13): IL-13 increases the production of IgE by B-lymphocytes, inhibits macrophages, and increases mucus production. IL-13 is made primarily by T\textsubscript{H2} cells.

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**Cytokines that Stimulate Hematopoiesis**

Produced by bone marrow stromal cells, these cytokines stimulate the growth and differentiation of immature leukocytes.

Examples include:

1. **Colony-stimulating factors (CSF):** Promote the production of colonies of the different leukocytes in the bone marrow and enhance their activity. Examples include granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF). In addition to their role in promoting production of leukocyte colonies, the CSFs also appear to promote their function. For example, when GM-CSF binds to receptors on neutrophils, eosinophils, and monocytes, it activates these cells and inhibits their apoptosis. GM-CSF increases adhesion of these cells to capillary walls during diapedesis, enhances their phagocytosis and extracellular killing, and increases both superoxide anion generation and antibody-dependent cytotoxicity. The various CSFs are produced by T-lymphocytes, macrophages, and other cells.

2. **Stem cell factor:** Stem cell factor makes stem cells in the bone marrow more responsive to the various CSFs. It is made mainly by bone marrow stromal cells.

3. **Interleukin-3 (IL-3):** IL-3 supports the growth of multilineage bone marrow stem cells. IL-3 is made primarily by T-lymphocytes.

4. **Interleukin-7 (IL-7):** IL-7 plays a role in the survival and proliferation of immature B-lymphocyte and T-lymphocyte precursors. IL-7 is produced mainly by fibroblasts and bone marrow stromal cells.

Some viruses cause infected host cells to secrete molecules that bind and tie up cytokines, preventing them from binding to normal cytokine receptors on host cells.

- Poxviruses cause infected host cells to secrete molecules that bind interleukin-1 (IL-1) and interferon-gamma (IFN-gamma).
- Cytomegaloviruses (CMV) cause infected host cells to secrete molecules that bind chemokines.
Summary

1. Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems.

2. Cytokines are pleiotropic, meaning that a particular cytokine can act on a number of different types of cells rather than a single cell type.

3. Cytokines are redundant, meaning that a number of different cytokines to carry out the same function.

4. Cytokines are multifunctional, meaning the same cytokine is able to regulate a number of different functions.

5. There are three functional categories of cytokines: Cytokines that regulate innate immune responses; cytokines that regulate adaptive immune responses; and cytokines that stimulate hematopoiesis.

6. Type I interferons provide an early innate immune response against viruses. Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

Contributors and Attributions

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