Cytokines: Physiology and Clinical Relevance

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A shift in medicine is taking place. It is characterized by the emergence of neuroendoimmunology (NEI), a convergence of scientific disciplines aimed at understanding the interconnectedness of the nervous, endocrine, and immune systems. Optimal health is dependent upon homeostatic balance within the complex NEI supersystem. When homeostatic balance is compromised, it has far reaching effects on the entire system. For example, inflammation - the body's primary response to cellular injury - is marked by blood capillary dilation, immune cell infiltration to injury site, redness, heat, and pain. The inflammation process is characterized by the activation of pro-inflammatory cytokines within the immune system. It has been implicated in a broad range of complex disease states such as; metabolic syndrome, mood disorders, cardiovascular health, and many others. Understanding how inflammation impacts the function of the nervous, endocrine, and other systems, is one aspect of the rapidly emerging field of neuroendoimmunology.

NEI has significant implications for improving clinical practice. The discipline sheds light on how chemical messengers, including neurotransmitters, hormones, and cytokines, string systems together, and sets the stage for more intuitive ways to measure and manage health and disease. Below you will find a brief description of the individual cytokines, and their role as novel biomarkers of inflammation and chronic disease.

Cytokine Introduction

Cytokines are multifunctional molecules that mediate a wide range of physiological responses, primarily host defense (immunity and inflammation). In addition to their role in immunity and inflammation, cytokines are involved in a variety of pathophysiological conditions in the central nervous system (CNS) and the peripheral nervous system (PNS), and thus serve as neuro-immunomodulators. Additionally, the immune system utilizes cytokine-like messengers, called chemokines, to recruit and activate specific white blood cell subtypes.

Cytokines are generally classified by their ability promote or inhibit inflammatory responses (see reference chart).

Pro-inflammatory cytokines	Anti-inflammatory cytokines	Chemokines
IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, IFN-γ, TNF-α	IL-4, IL-5, IL-10, IL-13, TGF-β	IL-8, MCP-1, MIP-1ß

Cytokines can also be grouped based on the type of T-lymphocytes with which they are associated (see reference chart). Immune challenges signal T-lymphocytes and other immune cells to produce unique profiles of cytokines that recruit and activate highly specific defense responses. These profiles are termed Th1, Th2, and Th17.

Th1 cytokine profile	Th2 cytokine profile	Th17 cytokine profile
IL-2, IL-12, IFN- γ, TNF-α	IL-4, IL-5, IL-10, IL-13	IL-6, IL-17, TNF-α, TGF-β

Pathophysiological Significance

Cytokine levels differ dramatically from baseline in acute and chronic pathological conditions. In many disease states, marked local inflammatory responses cause cytokines to spill into general circulation, resulting in detectable levels in biological fluids, such as serum and plasma. Changes in the circulating levels of these proteins have been linked to many disease states, making them valuable functional biomarkers. Excessive or diminished cytokine levels are associated with many clinical conditions and diseases, including:

- Central Nervous System Disorders
- Autoimmunity
- Fibromyalgia
- Bacterial infections
- Tumors
- Cardiac Diseases
- Toxicity
- Viral infections
- Allergies
- Asthma
- Diabetes

Cytokine Analysis

Cytokine analysis can provide information regarding an individual's inflammatory status. This information may be useful for objectively establishing the presence of an activated immune response as well as guide targeted therapeutic regimens designed to reduce inflammation and its secondary effects. Cytokines can be accurately measured in biological fluids via two methodologies, serum cytokine analysis and whole blood stimulated cellular analysis. Aspects of each methodology are listed below.

Serum Cytokine Analysis-

- Sample type: Serum
- Assay principle: Direct cytokine measurement
- Indication: Cost-effective method for suspected acute inflammation. Differentiates among bacterial, viral, allergic, and asthmatic immune responses

Whole Blood Stimulated Cellular Analysis (functional cytokine test)-

- Sample Type: Whole blood
- Assay principle: Cytokine measurement from stimulated immune cells
- Indication: ***Preferred method for chronic disease and inflammation management***

Why Perform a Stimulated Cellular Analysis?

Frequently, patients with chronic illness have only mildly elevated serum cytokine levels, yet may be suffering from the effects of inflammation. For chronic conditions, stimulated cellular cytokine analysis is recommended. Stimulated cellular cytokine analysis is performed by comparing baseline, non-stimulated cytokine levels with cytokine responses to immune cell stimulants phytohemagglutinin (PHA) (non-specific) and lipopolysaccharide (LPS) (specific). Stimulation induces the proliferation of immune cells that may be involved in previous and/or active immune responses. ***Abnormal cytokine responses may indicate impaired host defenses, infections, or underlying inflammatory disorders.*** Stimulated cytokine analysis is useful in the evaluation of patients with:

- Complex or chronic conditions
- Hormone imbalances
- Persistent neurotransmitter imbalances
- Challenged immune systems

A cytokine analysis has two main advantages over a complete blood count (CBC) with differential in evaluating inflammation. A stimulated cytokine analysis will evaluate an adaptive as well as an innate immune response, whereas a CBC with differential will only evaluate an innate immune response. Only the adaptive immune response has memory of previous immune challenges. Secondly, a cytokine analysis will describe the ACTIVITY of all leukocytes, whereas a CBC with differential will only evaluate the number of leukocytes present.

Methods of Stimulation:

PHA: Phytohemagglutinin (PHA) is a plant mitogen used for non-specific mitotic stimulation of human lymphocytes. The yield of soluble factors (such as cytokines) in response to PHA stimulation of peripheral blood mononuclear cells (PBMCs) is considered to be an indication of T-cell activation and of activated monocytes response to T-cell-derived stimuli. Stimulation of PBMCs with PHA results in a marked up-regulation of cytokine expression, which can be associated with the development of a Th1 or Th2 response.

LPS: Lipopolysaccharide (LPS) is the main constituent of Gram-negative bacteria cell walls. LPS has been demonstrated to induce inflammatory reactions *in vitro* as well as *in vivo*, where it has been associated with lethal shock. PBMCs are known to be reactive to LPS. Since LPS is one of the major components of the bacterial cell wall, relative cytokine levels may be used to indicate whether a patient's immune status has been challenged with an ***acute bacterial infection***, or with a ***previous bacterial encounter***.

Cytokine Descriptions and Clinical utility:

Interleukin-1β (IL-1β)

IL-1β is a potent pro-inflammatory cytokine produced by white blood cells (monocytes, macrophages and dendritic cells). The principal effects of IL-1β are:

- Co-stimulation of antigen presenting cells (APCs) and T cells
- B-cell growth
- Immunoglobulin (Ig) production
- Acute-phase response
- Phagocyte activation
- Inflammation
- Fever
- Hematopoiesis

Under immune stimulation, IL-1β also helps leukocytes pass through blood vessel walls to the site of infection and leads to fever by affecting areas of the brain that control body temperature. Increased IL-1β production is associated with:

- Sepsis
- Atherosclerosis
- Sleep disorders
- Rheumatoid arthritis
- Inflammatory bowel disease
- Type 2 Diabetes
- Schizophrenia
- Colitis
- Myasthenia gravis
- Other inflammatory diseases
- Leukemia
- Depression
- Periodontitis
- Other autoimmune diseases

Interleukin-2 (IL-2)

The principal source of IL-2 is antigen or mitogen activated T lymphocytes. Its action is restricted to antigen-activated T cells and natural killer (NK) cells. IL-2 promotes the proliferation, differentiation and survival of these target cells and proliferation and expansion of antigen-specific CD4+ and CD8+ T cells. IL-2 and its receptors are elevated in:

- Hodgkin's disease
- Multiple sclerosis
- Type 1 diabetes
- Severe burn trauma
- Allograft rejection
- Rheumatoid arthritis

Interleukin-4 (IL-4)

IL-4 cytokines are produced by activated T cells, eosinophils, mast cells, and basophils. IL-4 induces differentiation of naive helper T cells to Th2 cells, and is a key regulator in humoral and adaptive immunity. Other biological roles include the stimulation of activated B-cells and T-cell proliferation. IL-4 stimulates production of antibody-producing B cells, leading to the production of immunoglobulin (Ig), and class-switching to IgE. IL-4 also inhibits the production of pro-inflammatory cytokines (IL-1β, TNFα). Increased IL-4 levels indicate elevated activity of eosinophils and mast cells. Increased expression of IL-4 has been associated with:

- Allergy
- Malaria
- Leishmaniasis
- Asthma
- Hepatitis C infection
- Cutaneous T cell lymphoma
- Atopic syndrome
- Intracellular infections

Interleukin-5 (IL-5)

IL-5 is produced by Th2 cells and mast cells. IL-5 plays a specific role in the control of eosinophil production and differentiation and is primarily involved in:

- Asthma
- Allergy
- Acute and chronic atopic diseases
- Hashimoto's thyroiditis
- Hodgkin's disease
- Cutaneous T cell lymphoma
- Grave's disease
- Other Autoimmune diseases

Interleukin-6 (IL-6)

IL-6 is produced by a variety of cell types. The principal cell sources for IL-6 are macrophages, fibroblast cells, and endothelial cells. Other cell sources are T cells, B cells, eosinophils, mast cells, glial cells, and astrocytes. IL-6 is pleiotropic (multi-functional) and acts as both a pro-inflammatory and an anti-inflammatory cytokine. It is one of the key mediators of fever and of the acute phase response. Furthermore, elevated IL-6 levels have been observed in inflammatory diseases and myelomas. Over-production of IL-6 has been associated with:

- Rheumatoid arthritis
- Multiple myeloma
- Lennert syndrome
- Kawasaki disease
- Castleman's disease
- Cardiac tumors
- Schizophrenia
- Liver cirrhosis
- Systemic lupus erythematosus
- Inflammatory disease
- Alzheimer's disease
- Multiple sclerosis
- Viral meningitis
- Bacterial meningitis
- CNS trauma (stroke, head injury)
- Other bacterial diseases

Interleukin-7 (IL-7)

The main sources for IL-7 production are bone marrow stromal cells, thymic stromal cells, keratinocytes, and epithelial cells (thymic and intestinal). IL-7 promotes the survival and proliferation of T cell precursors. IL-7 has been associated with:

- Osteolytic cancer
- Lymphopenia
- Dermatitis
- Severe combined immunodeficiency syndrome (SCID)
- HIV infection

Interleukin-8 (IL-8)

IL-8 is a pro-inflammatory chemokine produced by macrophages, epithelial cells, and other somatic cells. IL-8 functions as a chemoattractant cytokine and plays a pivotal role in acute inflammation by recruiting and activating neutrophils. IL-8 is also a potent angiogenic factor. Elevated IL-8 levels have been associated with:

-- Rheumatoid arthritis
- Colitis
- Uveitis
- Crohn's disease
- Other Autoimmune diseases
- Acute inflammatory disease
- Dermatitis
- Acute respiratory distress syndrome
- Idiopathic pulmonary fibrosis
- Urinary tract infection
- Central nervous system trauma
- Central nervous system inflammation
- Inflammatory diseases

Interleukin-10 (IL-10)

IL-10 is an anti-inflammatory cytokine produced by activated monocytes, T cells, and B cells. IL-10 is involved in the function of a number of cells, and influences many physiological processes, including angiogenesis, tumorigenesis, and infection. IL-10 down-regulates the expression of Th1 cytokines and is an important B cell and mast cell growth factor. Elevated IL-10 production has been associated with:

- Systemic inflammation
- Streptococcal infection
- Leprosy

- Septicemia
- Multiple sclerosis
- Lung injury
- Leishmaniasis
- Filariasis
- Other autoimmune diseases
- Other infectious diseases
- Septic shock
- Keratitis
- Allergic airway inflammation
- Trypanosomiasis
- HIV infection
- Hepatitis B infection
- Pancreatitis
- Peritonitis
- Rheumatoid arthritis
- Malaria
- Uveitis
- Hepatitis C infection

Interleukin-12 (IL-12)

The main producers of IL-12 are antigen presenting cells (APCs), dendritic cells and macrophages. IL-12 is involved in the differentiation of näive T cells into Th0 cells. Th0 cells further develop into Th1 cells, which induce the host's cell-mediated immunity. IL-12 plays an important role in the cytotoxic activities of NK cells and CD8+ cytotoxic T lymphocytes. IL-12 has been associated with:

- Autoimmune diseases
- Multiple sclerosis
- Viral and bacterial infections
- Tumors/cancers
- HIV infection
- Rheumatoid arthritis
- Graft vs. host disease
- Glomerulonephritis
- Type 2 diabetes

Interleukin-13 (IL-13)

IL-13 is predominantly produced by Th2 cells, mast cells, and NK cells. IL-13 has an anti-inflammatory effect on monocytes and macrophages, and induces B cells to proliferate. IL-13 is an important mediator of:

- Allergic inflammation
- Atopic syndrome
- Allergic asthma
- Chronic obstructive pulmonary disease (COPD)

Interleukin-17 (IL-17)

IL-17 is produced exclusively by T cells upon activation, most often acting as a pro-inflammatory cytokine that stimulates the release of secondary cytokines and chemokines. IL-17 induces the recruitment of neutrophils for antimicrobial effect. Elevated IL-17 levels have been associated with:

- Inflammatory diseases
- Rheumatoid arthritis
- Cancers
- Crohn's disease
- Multiple sclerosis
- Inflammatory skin disorders
- Gastric Candidiasis
- Inflammatory airway diseases
- Other autoimmune diseases

Interferon-γ (IFN-γ)

IFN-γ is produced mainly by activated lymphocytes (T cells and NK cells). IFN-γ regulates cellular activities responsible for inflammation. It further modulates the antigen specific immune response by affecting both APCs and antigen-recognizing lymphocytes. IFN-γ also promotes innate and adaptive immune responses in the host against a variety of infectious agents, tumors, trauma, and autoimmunity. IFN-γ is associated with a variety of diseases, including:

- Multiple sclerosis
- Type 2 diabetes
- Bacterial infections
- Tumors
- Hepatitis B
- Systemic lupus erythematosus
- Rheumatoid arthritis
- Dermatitis
- Graft vs. host disease
- Hepatitis C

- Other inflammatory diseases
- Other viral infections
- Other autoimmune diseases

Tumor Necrosis Factor-α (TNF-α)

TNF-α is a pleiotropic (multi-functional), pro-inflammatory cytokine produced by:

- Monocytes
- Macrophages
- NK cells
- Astrocytes
- Kupffer cells
- T cells
- B cells
- Glial cells
- Basophils
- Eosinophils

The primary role of TNF-α is the regulation of immune cells. TNF-α is also able to induce programmed cell death (apoptosis), elicit inflammation, and inhibit tumorigenesis and viral replication. TNF-α plays a pivotal role in:

- Bacterial infection
- Rheumatoid arthritis
- Type 1 and Type 2 Diabetes
- Depression
- Other autoimmune diseases
- Alzheimer's disease
- Hepatitis C infection
- Chronic lymphatic leukemia
- Viral replication
- Multiple sclerosis
- Crohn's disease
- Schizophrenia
- Sleep disorders
- CNS disorders
- Psoriasis
- Other autoimmune diseases
- Septic shock
- Celiac disease
- Systemic lupus erythematosus
- Other inflammatory diseases
- Parkinson's disease
- Hepatitis B infection
- Tumors/cancers
- Other viral diseases

Granulocyte colony-stimulating factor (G-CSF)

G-CSF is produced by endothelial cells, monocytes, macrophages, and a number of other immune cells. G-CSF and its receptor have also been reported to be expressed on neurons and neural progenitor cells. The normal, physiological role of G-CSF is the survival, proliferation, differentiation, and production of neutrophils for nonspecific host defense against microbial infection. G-CSF reduces inflammatory activity by inhibiting the production or activity of the primary inflammatory mediators IL-1β, TNF-α, and IFN-γ. Elevated levels of G-CSF have been found in patients with:

- Acute bacterial infections.
- Neutropenia

Granulocyte macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a protein secreted by macrophages, T cells, mast cells, endothelial cells, and fibroblasts. GM-CSF acts as a hematopoietic growth factor to stimulate bone marrow to increase the number of two types of white blood cells that fight infection -granulocytes (neutrophils) and monocytes- to make those cells more effective. It inhibits neutrophil migration and enhances the functional activity of mature endothelial cells. GM-CSF is produced by the body in response to infection or inflammation. Elevated levels of GM-CSF have been observed in:

- Allergic patients with late phase cutaneous reactions
- Inflammatory arthritis and other inflammatory reactions
- Other bacterial infections
- Toxic Shock
- Autoimmunity
- Asthma

Macrophage inflammatory protein-1β (MIP-1β or CCL4)

MIP-1β (also known as CCL4) is a pro-inflammatory chemokine that plays a discrete role in normal physiological activities as a modulator of homeostatic processes, as well as providing host-protective inflammatory responses. MIP-1β participates in the response to invading bacterial, viral, parasitic, and fungal pathogens by regulating the trafficking and activation of selected subgroups of inflammatory cells such as macrophages. It selectively attracts T helper

lymphocytes and NK cells. MIP-1β can also be a potent chemoattractant for B cells, eosinophils and dendritic cells. MIP-1β also induces synthesis and release of other pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) from fibroblasts and macrophages. MIP-1β has been implicated in a wide range of acute and chronic inflammatory conditions such as:

- Bacterial sepsis
- Rheumatoid arthritis
- Allograft rejection
- Plasmodium (malaria)
- Colitis
- Bacterial meningitis
- Multiple sclerosis
- Central nervous system disorders
- HIV Infection
- Viral meningitis
- Asthma
- Sarcoidosis
- Acute infections

Monocyte chemoattractant protein-1 (MCP-1 or CCL2)

MCP-1 (also known as CCL2), is a pro-inflammatory chemokine. MCP-1 activity is necessary for full pathophysiological manifestation of diseases. MCP-1 is major chemoattractant for monocytes in inflammation and immune responses. It also attracts memory T cells and NK cells and induces histamine release from basophils. MCP-1 has been implicated in a wide variety of inflammatory diseases such as:

- Atherosclerosis
- Multiple sclerosis
- Congestive heart failure
- Alzheimer's disease
- Neoplasm
- Glomerulonephritis
- Sepsis
- Myocardial infarction
- HIV infection
- Rheumatoid arthritis
- Idiopathic pulmonary fibrosis
- Chronic inflammatory disorder
- Allergy

Transforming growth factor-β (TGF-β)

TGF-β is the most multifunctional cytokine. It affects a vast range of biological processes including regulation of cellular differentiation and growth, inflammation, wound healing, and bone formation. TGF-β contributes to the pathogenesis of diseases as diverse as autoimmune diseases and carcinogenesis. TGF-β is different from other cytokines in that it has the ability to limit cell growth. It is a strong suppressor of T cell activation and of B cell antibody production. Pathological and healing roles of TGF-β include:

- Wound healing
- Fibrotic disease
- Rheumatoid arthritis
- Scleroderma
- Leukemia
- Parasitic infections
- Celiac disease
- Alzheimer's disease
- Carcinogenesis
- Melanoma
- Liver cirrhosis
- Fibrosarcomas
- Myocarditis
- Lymphoma
- Autoimmune diseases
- Immunologic intolerance
- Parkinson's disease
- Chronic inflammatory disorders
- Radiation-induced fibrosis
- Idiopathic pulmonary fibrosis
- Arteriosclerosis
- Coronary artery disease
- Neurodegenerative diseases
- Immunosuppressive disorders
- Systemic lupus erythematosus