Functional Medicine University's Functional Diagnostic Medicine Training Program

Module 5 * FMDT 541C

The Biology and Physiology of Inflammation

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The Biology and Physiology of Inflammation

As you learned in the "Immune System" module, there is an intimate relationship between the mechanism of inflammation and the immune system response. Inflammation is the body's normal physiological response to injury. The cause of tissue injury is attributed to trauma, autoimmune, microbial, heat and toxins (chemicals).

When tissue injury occurs, numerous substances are released by the injured tissues, which cause changes to the surrounding uninjured tissues. Some of the tissue products that cause the inflammatory reaction include: histamine (which increases permeability, causes contraction of smooth muscle, and constriction of the bronchioles), serotonin, lipid mediators (prostaglandins, leukotrienes, and lipoxins and platelet-activator factor), bradykinin, products of the complement system, products of the blood clotting system, and substances released by the sensitized lymphocytes (lymphokines). These substances are the messengers of the inflammation process, and have been viewed as areas of therapeutic intervention. Collectively they are called autocoids. Autocoids are substances released from the cells in response to various stimuli to elicit normal physiological responses locally. An imbalance in the synthesis and release of the autocoids contributes significantly to pathological conditions such as inflammation, allergy, hypersensitivity and ischemia-reperfusion[4].

General Classifications of Autocoids

1. Biogenic amines – histamine, serotonin(5-HT)
2. Biogenic peptides – kinins (bradykinin, kallidin), angiotensin
3. Small peptides – cytokines (e.g. interleukins, chemokines, lymphokines, interferon, tumor necrosis factor) Cytokines are small soluble proteins with low molecular weight. Their function is to act as chemical messengers for regulation of innate and acquired immunity and stimulate hematopoiesis. They are produced in just about all cells involved with immunity, but in particular the T-helper cells.
4. Membrane derived – leukotrienes, prostaglandins, thromboxane A_2, platelet-activating factor, prostacyclin, lipoxins and hepoxylins
5. Endothelial derived – nitric oxide

Note: The effects of histamine are mediated through H1 and H2 receptors. H1 receptor activation causes vasodilation, dermal pain, itching, smooth muscle contraction (uterine, bronchial and intestinal), and increased mucous production. H2 receptor activation causes increased gastric acid production, increased mucous production and activation of suppressor T-cells. Platelet activating factor (PAF) is a phospholipid that has messenger functions. It is synthesized by platelets, endothelial cells, basophils, mast cells, neutrophils, monocytes and macrophages from mast cells and platelets. PAF is a mediator of platelet aggregation, inflammation and anaphylaxis.

Note: Inhibition of ACE (angiotensin converting enzyme) with ACE inhibitors will cause a decrease in degradation of bradykinin. One of the adverse side-effect of ACE inhibitors is the development of a dry cough, which can be cause by increased bradykinin levels due to decreased degradation.

Inflammation is characterized by the following events:

1. Vasodilatation of the local blood vessels
2. Increased capillary permeability (which causes an increase in interstitial fluid)
3. Clotting of the interstitial fluid (caused by fibrinogen)
4. Migration of monocytes and granulocytes
5. Swelling of the tissues

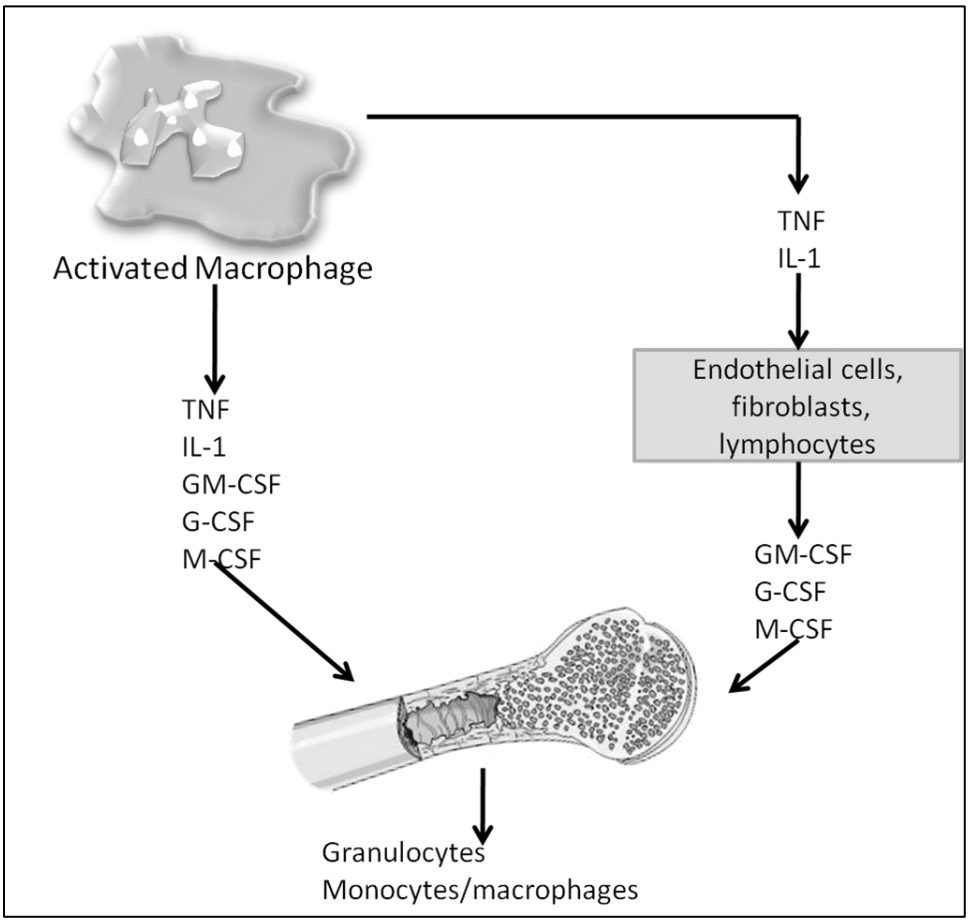
As you can read from the preceding description the *inflammatory process is connected to the vasculature.* You may recall the cardinal features of inflammation: tumor, calor, rubor and dolor.

The Four Lines of Defense

Tissue macrophages are the first line of defense, followed by neutrophil invasion, the second line of defense, and subsequent neutrophilia. A third line of defense occurs with a secondary macrophage invasion into the injured area. The fourth line of defense is the increased production of granulocytes and monocytes by the bone marrow. Activated macrophages produce and release numerous growth factors in the inflamed tissue. The dominant factors (cytokines) released by the macrophages include the following:

1. TNF (tumor necrosis factor)
2. IL-1 (interleukin-1)
3. GM-CSF (granulocyte-monocyte colony-stimulating factor)
4. G-CSF (granulocyte colony-stimulating factor)
5. M-CSF (monocytes colony-stimulating factor)

These factors cause an increase in production of granulocytes and monocytes by the bone marrow. These factors also provide a powerful feedback mechanism to help remove the cause of the inflammation.



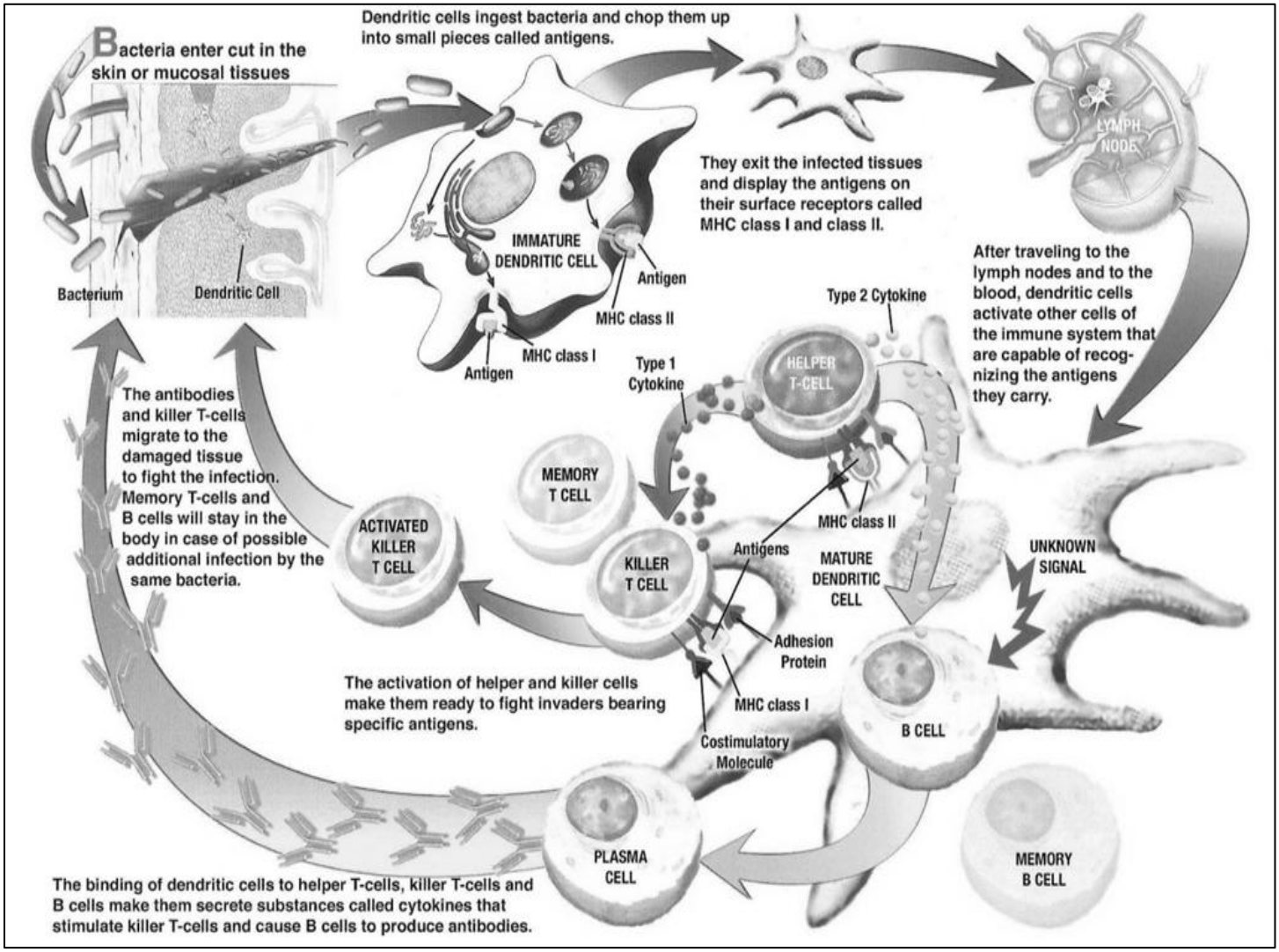
Review of Innate and Acquired Immunity

You should recall, from the immune system module, the distinction between innate immunity and acquired immunity. Innate immunity consists of the following:

1. Phagocytosis by the tissue macrophage system
2. Stomach acid and digestive enzymes
3. Resistance of the skin
4. Presence of certain substances in the blood (lysozymes, polypeptides, complement, and natural killer lymphocytes)

Acquired (adaptive) immunity forms antibodies and activated lymphocytes that attack and destroy specific organisms and toxins.

Interactions of Cellular and Humoral Immunity as Defense Against Invaders



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An Overview of Inflammatory Disease

Tissue Injury
↓
Host Defense Mechanism
[Immune Response (Innate and Acquired)]
↓
Acquired/Congenital Deficiencies or Defects of the Immune System
↓
Aberration(s) of the Immune Response
(Immune system Imbalance)
↓
Chronic Inflammation/Disease
(Autoimmune, Cardiovascular, Gastrointestinal, Musculoskeletal, and *Cancer*)

Triggers of the Immune Response and Inflammation

There are a host of stimuli that can activate the immune response, and therefore inflammation. The following is a list of some of the triggers:

- Oxidative stress (reactive oxygen species, especially oxidized lipids)
- Radiation
- Psychological stress
- Injury
- Food and environmental allergens
- Viral infections
- Nutrient deficient/poor diet (e.g. diet high in refined sugar, SAD diet)
- Intestinal hyperpermeability (leaky gut)
- Pathogen-associated molecular patterns (PAMPs) – PAMPs are molecules that trigger an immune response by activating toll-like receptors. Toll-like receptors are transmembrane proteins expressed by cells of the innate immune system and the antigen presenting cell. Once activated, the toll-like receptors signal the pathways of the inflammatory response, namely nuclear factor kappa B (NF-kB).[Note: MAMPs (microbial-associated molecular patterns) is the term sometimes used in conjunction with non-pathogenic microbes]

The following is a list of some of the PAMPs:

- **lipopolysaccharide** (LPS) from the outer membrane of the gram-negative cell wall
- **bacterial lipoproteins and lipopeptides**
- **porins** in the outer membrane of the gram-negative cell wall
- **peptidoglycan** found abundantly in the gram-positive cell wall and to a lesser degree in the gram-negative cell wall
- **lipoteichoic acids** found in the gram-positive cell wall
- **lipoarabinomannan** found in acid-fast cell walls
- **mannose-rich glycans** (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar). These are common in microbial glycoproteins and glycolipids but rare in those of humans
- **flagellin** found in bacterial flagella
- **bacterial and viral nucleic acids**.
- *N*-**formylmethionine**, an amino acid common to bacterial proteins
- **double-stranded viral RNA**
- **single-stranded viral RNA**
- **lipoteichoic acids, glycolipids, and zymosan** from yeast cell walls
- **phosphorylcholine and other lipids** common to microbial membranes

- Arachidonic acid metabolites (PG-E2)
- Environmental toxins

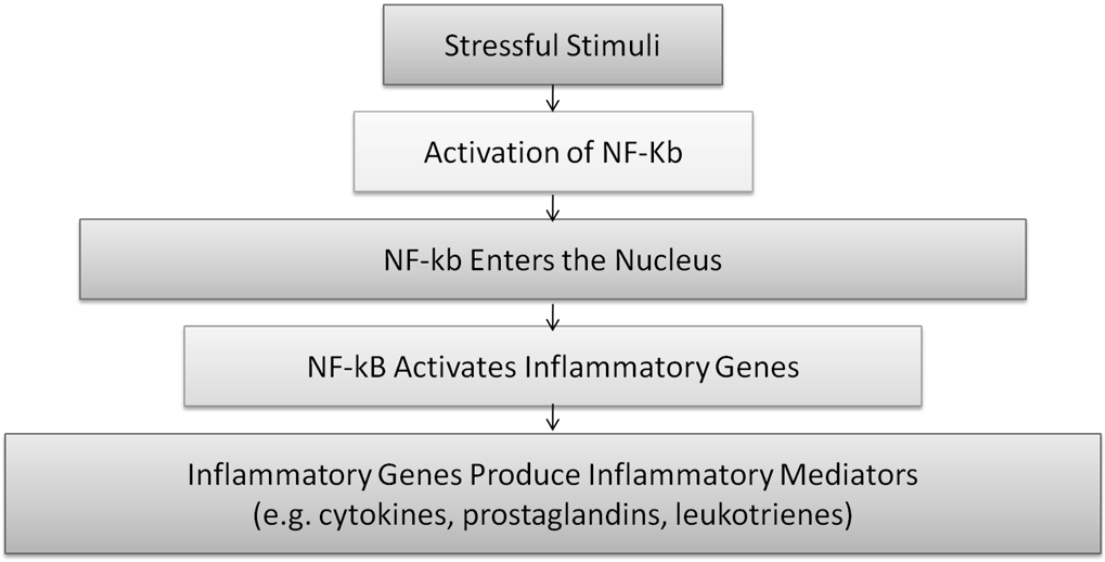
The above mentioned triggers will then activate a pro-inflammatory transcription factor called nuclear factor kappa B (NF-kB). NF-kB is a protein made up of two protein subunits; p50 and p65. It exists in the cytoplasm in an inactive form by a regulatory protein called inhibitor kappa-B (IKB). Exposure to the **stressful stimuli**, as previously mentioned, activates a protein called inhibitory kappa-B kinase (IKK), which phosphorylates inhibitor kappa-B, thereby destroying it. The destruction of IKB allows NF-kB to enter the nucleus and bind with DNA to activate pro-inflammatory gene expression.

NK-kB is highly activated at sites of inflammation in diverse diseases and can induce transcription of pro-inflammatory cytokines, chemokines, adhesion molecules, MMPs (matrix metalloproteinases), Cox-2, and inducible nitric oxide (iNOS). In Rheumatoid arthritis, for example, NF-kB is over-expressed in the inflamed synovium, where its activity may enhance recruitment of inflammatory cells and production of pro-inflammatory mediators like IL-1, IL-6, IL-8, and TNF-α.[1]

Inflammatory airway disease in humans has also been associated with cytokine and adhesion molecule expression. This correlates with activation of NF-kB in bronchial biopsies from asthma patients. Increased NF-kB activity with nuclear localization was observed especially in the airway epithelial cells, where there is abundant expression of pro-inflammatory cytokines, chemokines, iNOS, and Cox-2.[1]

Helicobacter pylori-associated gastritis is also marked by increased NF-kB activity in gastric epithelial cells. The number of NF-kB positive cells correlates with the degree of gastritis. Similarly, there is evidence of NF-kB activation in inflammatory bowel disease, where lamina propria macrophages display activated p50 and especially p65.[1]

Note: An article in the Journal of Inflammation, November 2007, stated that there are other pathways independent of inhibitor kappa B (IKK) that can release NF-kB.



Some of the Pro-Inflammatory Molecules Regulated By NF-kB and Their Physiological Effects

A. *Tumor necrosis factor*
   1. TNF-α (TNF-α is a pleiotrophic inflammatory cytokine that serves as a mediator in various pathologies. Some examples are: cancer, multiple sclerosis, rheumatoid arthritis, diabetes and AIDS.
   2. TNF-β (TNF-β acts on a plethora of different cells. It induces the synthesis of GM-CSF, G-CSF, IL-1, and prostaglandin-E2 in fibroblasts. It also promotes the proliferation of fibroblasts and is involved in wound healing.)

B. Colony stimulating factors

1. G-CSF (granulocyte colony stimulating factor) G-CSF stimulates the bone marrow to produce granulocytes)
2. GM-CSF (granulocyte-monocyte colony stimulating factor) Stimulates stem cells to produce granulocytes and monocytes)

C. Interleukins

1. IL-1 (IL-1 is produced by macrophages, monocytes, dendritic cells and fibroblasts, as well as other cells. IL-1 is released into the local environment as a part of the inflammatory reaction. IL-1 cause the endothelial cells to secrete chemokines such as MCP-1 and upregulate the expression of vascular adhesive molecules such as E-selectin, ICAM-1 and VCAM-1)
2. IL-2 (IL-2 was initially identified as a T cell growth factor. IL-2 can stimulate the growth and differentiation of B cells, natural killer cells, monocytes, macrophages and oligodendrocytes.
3. IL-6 (IL-6 play a role in acute phase reactions, hematopoiesis, bone metabolism, and cancer progression.)
4. Il-12 (IL-12 is produced by macrophages and B cells and has been shown to have multiple effects on T cells and natural killer cells.

D. Interferon

1. IFN-β (IFN-β is known to upregulate and downregulate a wide variety of genes, most of which are involved in the antiviral immune response. (Research on IFN-β has been focused on its used to treat MS.)

E. Chemokines

1. IL-8 (IL-8 is a chemoattractant for neutrophils, basophils, eosinophils and T cells.)
2. GROα, GROβ, GROγ (IL-8 related chemotactic cytokines- activates neutrophils and basophils)
3. RANTES (A cytokines that is a member of the IL-8 family. It attracts T memory cells and monocytes)
4. MCP-1/JE (monocytes chemotactic protein)

F. Adhesion molecules

1. ICAM-1 (Intercellular adhesion molecule-1)
2. E-selectin (E-selectin is expressed on inflamed endothelial cells by cytokine activity and is refer to as endothelial leukocyte adhesion molecule-1.
3. V-CAM (vascular cell adhesion molecule) V-CAM mediates adhesion of lymphocytes, monocytes, eosinophils and basophils to the vascular endothelium. V-Cam is upregulated by a variety of cytokines. V-CAM-1 plays a role in the formation of atherosclerosis[7].

G. Enzymes

1. COX-2 (Cyclooxygenase-2 is an enzyme that catalyzes the production of pro-inflammatory prostaglandins.)
2. iNOS (inducible Nitric Oxide Synthase) INOS plays a part in the oxidative burst from the macrophages. Recall that the burst participate in anti-microbial and anti-tumor activity under normal circumstances.

Overview of Environmental Stimuli Into Biochemical Inflammation

Environmental Stimuli (triggers)
↓
Activation of NF-kB
↓
Increased expression of pro-inflammatory genes
↓
Production of cytokines
Pro-inflammatory enzymes (iNOS-inducible nitric oxide synthase, Cox-2 –cylcooxygenase-2, Lipox- lipoxygenase)
Adhesion molecules
↓

- (IL-6 stimulates production of CRP)
- (Cyclooxygenase-2 transforms arachidonic acid into thromboxanes and prostaglandins-E2)
- (IL-1 induces the production of collagenase/matrix metalloproteinases both of which destroy connective tissue)
- (Lipooxygenase acts on arachidonic acid to produce leukotrienes)
- (inducible nitric oxide synthase→ nitric oxide)
- (Tumor necrosis factor-α)
- (Adhesion molecules)

↓

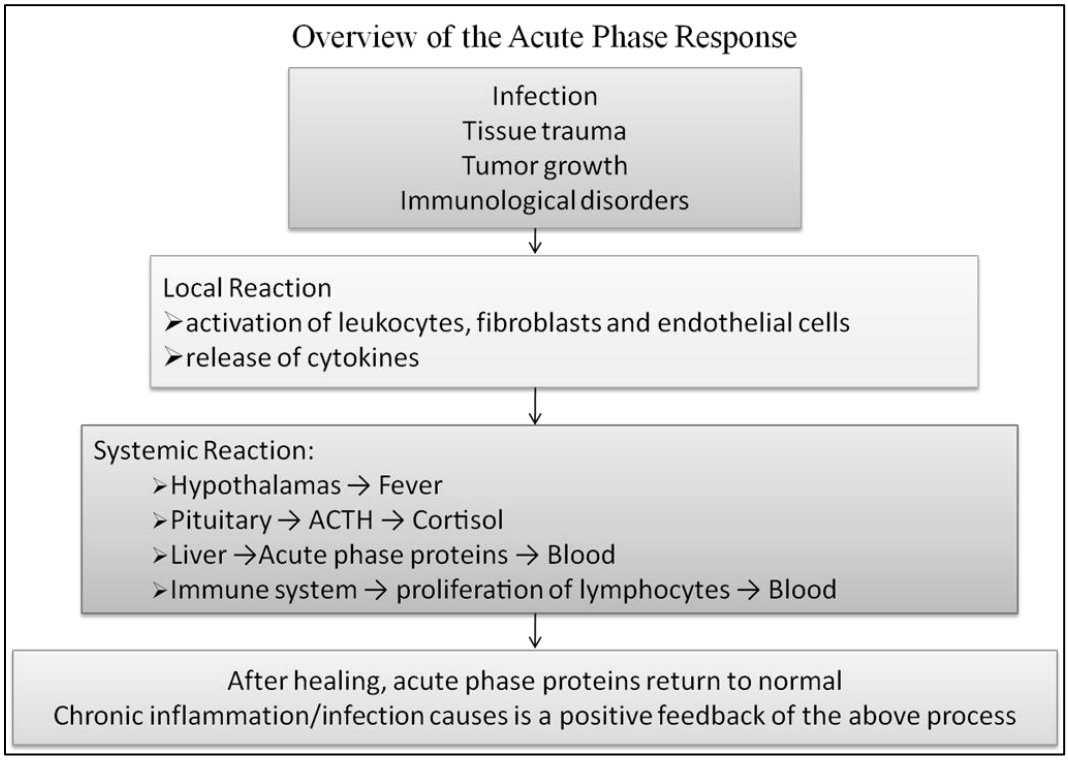
Autoimmune disease, cardiovascular disease, insulin resistance, neurodegenerative disease, cancer, chronic inflammation

Acute Phase Response and Acute Phase Proteins

The acute phase response is the answer of the organism to disturbances of its homeostasis due to infection, tissue injury, neoplastic growth, or immunological disorders. The acute phase response is thought to be beneficial to the injured organism with the aim of restoring the disturbed physiological homeostasis[5]. Acute phase proteins are mostly comprised of glycoproteins that are found in the serum. They are mainly synthesized by the liver cells and released into the bloodstream in response tissue injury from a variety of sources including: trauma, acute infections, chronic infections, thermal injury, and malignancy. IL-1, IL-6, and TNF are the cytokines thought to stimulate their production. The acute phase response is general and nonspecific. A research study investigating the role of protein malnutrition and acute phase reactants indicated that a protein deficient diet by itself induces IL-6 production[10]. There are numerous acute phase proteins. Below is a list of some of the acute phase proteins.

Acute Phase Proteins:

- *C-reactive protein* - CRP is increased in connective tissue disorders, bacterial infections and neoplastic disease. CRP is an independent risk factor of the development of type 2 diabetes[6]. CRP is synthesized in the liver in response to IL-1. CRP binds to membrane phospholipids in microbial membranes. CRP functions as an opsonin (a molecule that promotes phagocytosis by binding to a microbe). A research study on sleep loss and inflammatory markers revealed both acute total and short-term partial sleep deprivation resulted in elevated high-sensitivity CRP concentrations[8]. The conclusion was that failure to obtain adequate amounts of healthy sleep promotes low-level systemic inflammation.
- *Alpha-1 acid glycoprotein* – promotes of fibroblasts, interaction with collagen
- *Alpha-1 antitrypsin* – protease inhibitor
- *Haptoglobins* – hemoglobin scavenger
- *Ceruloplasmin* – copper transport protein
- *Serum amyloid A* – cholesterol scavenger
- *Fibrinogen* – clotting formation of fibrin matrix repair
- *Ferritin* – iron transport protein (increased in inflammation, malignancy and liver disease)
- *Complement components*



Natural Interventions that Inhibit NF-kB[9]

- Vitamin D
- Turmeric-curcumin
- Alpha Lipoic Acid
- Green Tea Extract
- Rosemary
- Propolis
- Resveratrol
- Essential Fatty Acid (The anti-inflammatory effects of EFA may result from the inhibitory effects of oxidized omega-3 fatty acids on NF-kB activity via a PPARα-dependent pathway. PPARs means stands for peroxisome proliferator-activated receptors. PPARs are nuclear receptors that control many cellular and metabolic processes. They respond to changes in lipid and glucose homeostasis.)

In Summary

In summary, the physiological response to tissue injury (trauma, infection, surgery, radiation, oxidative stress) involves both local and systemic reactions. The normal inflammatory response maintains homeostasis and allows for tissue healing. Chronic inflammation leads to multiple organ dysfunctions. From a functional medicine perspective, you need to keep in mind the body systems involved in acute and chronic inflammation, which include; the sympathetic nervous system, the immune system, the endocrine system, the gastrointestinal system, and the vascular endothelium.

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