CYTOKINES

Cell communication - The basis of life
For the smooth operation of all physiological processes in the organism, all of the approximately 60 trillion cells of the body need to be able to communicate well. This complex communication occurs via two chemical substances:-

- Cytokines
- Cell surface molecules.

Principally, one can make a differentiation between a direct communication via an adhesion molecule (cell-to-cell contact) and an indirect communication via cytokines (messenger substances) bound to receptors. Both of these types of communication take place concomitantly and may also have an influence on one another.

THE IMMUNE REACTION

The immune reaction is made up of a series of defined, controlled and exactly progressing, inter-reactive steps. Depending on the cell type and the individual stimulus, particular cell activation will result in the production of one or more cytokines and/or the expression of a specific receptor pattern on the cell membrane.

CONTROL OF THE IMMUNE REACTION

The specific control of and the sequence of the activating and suppressive steps in the immune response is mediated by the immune cells themselves through the release of both pro-inflammatory and anti-inflammatory cytokines. The Th1 or pro-inflammatory or activating cytokines are TNF, IL-1β, IL-2, IL-6, IFN-α, IFN-γ, and TGF. The Th2 or anti-inflammatory or suppressing cytokines are IL-4 and IL-10.
Figure 1 Anti-inflammatory and pro-inflammatory cytokines

Cytokines

Pro-inflammatory cytokines
- up-regulation of the inflammatory response
  - IL-1
  - IL-2
  - IL-6
  - TNF-α
  - IFN-α
  - IFN-β

Anti-inflammatory cytokines
- inhibition of pro-inflammatory cytokines
  - IL-4
  - IL-10
  - IL-13

- up-regulation of acute phase reactants
  - IL-1
  - IL-6
  - IL-11
  - TNF-α
  - IFN-γ
  - TNF-β

- chemokine IL-8

- stimulation of pro-inflammatory cytokines
  - IL-12
There is also an up or down regulation of adhesion molecules in the immune reaction (i.e. a change in the expression density and pattern of various cell surface molecules). See below.
The balance of these regulatory systems is controlled both locally and systemically by proteolytic serum activity, which should counteract any unphysiological increases in their activity. The proteolytic activity is through such enzymes as: antiproteinases and various hydrolase enzymes, as well as cytokine receptors and cytokine antibodies. These hydrolase enzymes, which are released locally from the cells, do several functions:

- Counteract the excess production of cytokines
- Split or inactivate these cytokines,
- Promote the shedding of cytokine receptors.

Our immune system takes care of most of the damaging toxins without us even being aware of them. The primary defensive reactions generally occur locally and, in practice, without any systemic effects. Any temporary shift in the immune homeostasis is observed only locally.

**DISTURBANCES IN THE IMMUNE REACTION**

Any permanent stress on the immune system may bring about a shift in the immune homeostasis that can no longer be compensated by the organism. This is especially the case with chronic disease and in particular autoimmune and malignant diseases, There is a shift in the balance of cytokines to an excess of the Th1 pro-inflammatory cytokines and a decrease in the Th2 anti-inflammatory cytokines. These disturbances subsequently result, for example, in unphysiological concentrations of immune complexes, antiproteinases, cytokines and adhesion molecules.

Once these cytokines have stopped just appearing locally but now causing systemic effects i.e. in the rest of the body, adverse effects from the subsequent inflammatory response can appear distant to their site of origin. These resultant disturbances in cell communication can lead to the inhibition of a more efficient defensive immune system and set up a vicious spiral of inflammation and disease. Therefore, there is a danger of finding an insufficient
defence system against infection, or against the development or perpetuation of chronic, autoimmune or malignant diseases.

**OVERVIEW OF IMMUNE DEFICIENCY**

The defensive performance can be insufficient or lacking entirely in the event of:

- Gene defects
- Damage to the immune system, during the development of tolerance to the initiator of the defense i.e. the key toxin
- Ageing.

This causes or favours

- The deregulation of certain immune components,
- An increased potential to develop infection,
- The persistence of the antigens associated with the development of chronic inflammations and faulty regeneration
- The development of malignant diseases.

The defensive posture can be excessive in part (extreme antigen exposition, over-stimulation, lacking counter regulation) or inhibited in part (over-expression of immune complexes, cytokines and/or adhesion molecules). The over and under expression will impedes the immune functions or causes such systemic side effects as, for example, the wasting syndrome seen in HIV infections. The immune system then recognises endogenous structures as being foreign (cross-reactions, insufficient tolerance). This is considered to be the cause of autoimmune diseases. The immune system can react excessively to harmless substances (insufficient tolerance) with the subsequent development of allergies.
TYPES OF CYTOKINES

The immune cells secrete numerous soluble and, in part, highly specialised mediators which, in a series of complex interactions, guarantee for the viability, development, differentiation, proliferation and activity in the organism. These cytokines are soluble glycoproteins that function as intercellular signalling molecules.

Cytokines have an effect on practically all cells and not only on the immune cells. Cytokines maintain a rigidly controlled communication network between the individual cell types, even including those of the nervous system.

Cytokines are set free in the course of normal cell functions, especially in response to particular stimulus such as the presence of antigens, immune complexes, complement, enzymes, cytokines, and certain types of cell-to-cell contact.

In order to initiate, regulate and again terminate the individual defensive steps, numerous cytokines are usually secreted concomitantly by the immune cells.

By way of their cytokine receptors, these immune cells are capable of reacting to a wide range of cytokines. Depending on the type of target cell, this reaction may not only consist of the development of acute phase proteins, antibodies and cytokines, but may also involve the suppression of these functions.

The change in the function of the target cells is always accompanied by a change in their receptor density and receptor pattern, especially that of the adhesion molecule on the surface membrane.

In contrast to the reactions seen with many hormones, those attributed to the cytokines such as TNF, interferon (IFN), colony-stimulating factor (CSF) and interleukin (IL)) usually only involve local, short-distance signals. Their release is usually only necessary for a definite purpose, at a defined time and at a locally-limited site.
In chronic inflammation this can be radically different.

**FAMILIES OF CYTOKINES**

There are two families of cytokines:

1) TH-1 family, the immune-stimulatory or pro-inflammatory cytokines (IL-1β, IL-2, IL-6, TNF)
2) TH-2 family, the anti-inflammatory or immune-suppressive cytokines (IL-4, IL-10).

In this way, a deficiency in stimulatory or cytotoxic cytokines can limit the body’s defensive system against both cancer cells and infections.

**EFFECT OF KEY TOXINS**

Certain triggers (key toxins) can trigger the switching of cytokine production from TH-2 anti-inflammatory cytokines to the pro-inflammatory cytokines. These TH-1 cytokines in turn initiate the production of interleukin 2 (IL-2) and interferon γ. These cytokines prompt phagocytes to produce oxygen rich species (ROS) and nitrogen rich species (NOS). These reactive oxygen species initiate the production of a further cytokine, tumour necrosis factor α (TNF-α) which further changes the cell function by interfering with mitochondrial oxidative phosphorylation by upsetting the enzyme NADPH oxidase, the rate-controlling enzyme of the hexose- monophosphate shunt, and stimulates nitric oxide synthase, producing further nitric oxide.

When suppressive cytokines are lacking, however, the local counter-regulatory reactions are also missing and the immune reaction can no longer be stopped.

The cytokine network is a fine regulatory system. Both too much and too little of the individual cytokines has a negative effect and consequently leads to disturbances in the immune system. The unhindered formation of cytokines results in high concentrations of these substances (either alone or bonded to antiproteinases) which are not only seen locally, but are
instead found throughout the entire body in the form of vagabond structures (where they serve as the cause of numerous adverse effects).

An excessive formation of cytokines is evident as an immunopathogenetic mechanism of chronic inflammations especially IL-1 and TNF, in the wasting syndrome, with diseases generating tumours, as well as in chronic infections (TNF), septic shock (TNF) and a breakdown of the immune system (IL-10).
GENERAL ACTIVITY OF CYTOKINES:

- Cytokines involved in acute inflammation
  - TNF-α, IL-1, IL-6, IL-8, IL-11
  - And other chemokines, G-CSF, and GM-CSF
- Cytokines involved in chronic inflammation
  - They can be subdivided into:
    - Cytokines mediating humoral responses IL-4, IL-5, IL-6, IL-7, and IL-13
    - Cytokines mediating cellular responses IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferon (IFN), transforming growth factor-ß (TGF), and tumour necrosis factor-α and ß (TNF).
- Cytokines involved in fever induction:
  - Cytokine IL-1 (Interleukin), TNF-α (Tumour Necrosis Factor) and IL-6 initiate PGE₂ series (Prostaglandin E₂) altering the hypothalamic set point for body temperature causing a fever.
- Cytokines involved in the production of ACTH
  - Cytokine IL-1 and IL-6 inducing the pituitary adrenal axis to produce ACTH (Adrenocorticotropic Hormone) and hence cortisol which will inhibit the production of cytokines – an inhibitory feed back (see following diagram).
ACUTE PHASE REACTANTS

Tissue injury

Release of cytokines

- Cytokines IL-1, TNF-alpha, IL-6
  - Initiate PGE$_2$ series
  - Alteration of the hypothalamic set point for temperature
  - Generation of the febrile response

- Cytokines IL-1 and IL-6
  - act on pituitary-adrenal axis inducing the production of ACTH
  - Initiate production of cortisol
  - Liver synthesises Acute Phase Reactants (APR)

Alteration in metabolism and regulation in the liver

- Liver synthesises Acute Phase Proteins (APP)

Corticosteroids inhibit production of cytokines

Figure 3 Pro-inflammatory cytokine function-1
Cell

IL-1

Target Tissues

Many cell types

Monocytes

Lymphocytes

Phagocytosis, microorganisms, lectins, inflammatory agents, some chemicals, cytokins

Bacterial lipopolysaccharide endotoxin

Antigens

Tissues

Effects

Helper T cells

Bone marrow

Liver

Brain

Fat

IL-1

TNF

T and B cells

Bone marrow

Skeletal muscle

Liver

Brain

Endocrine organs

Uptake of Fe, Zn and amino acids. Synthesis of glucose and acute phase reactant proteins

Inhibition of lipoprotein lipase

Fever, anorexia

Release of ACTH, steroids, insulin, glucagon, growth hormone, stomatostatin

Immune system activation and enhancement

Differentiation and proliferation of myeloid and haemopoetic cells

Figure 4 Cytokine functions - 2
<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>CELLS OF ORIGIN</th>
<th>STIMULUS OF INDUCTION</th>
<th>PRIMARY ACTION</th>
<th>OTHER EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1 (IL-1)</td>
<td>macrophages, monocytes, epithelium, astrocytes</td>
<td>micro-organisms, antigens, inflammatory agents, plant lectins, certain chemicals</td>
<td>Initiates acute inflammatory response. Stimulated production of PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Contributes to cachexia in severe disease</td>
</tr>
<tr>
<td>Interleukin-2 (IL-2)</td>
<td>Activated T helper cells</td>
<td>T cell activation by IL-1 or T cell mutagens</td>
<td>Stimulates T cell formation, Stimulates INF, TNF, LAK and tumour-infiltrating lymphocytes</td>
<td>Synergises with LAK cells in antitumour cytotoxicity</td>
</tr>
<tr>
<td>Interleukin-3 (IL-3)</td>
<td>activated T helper cells</td>
<td>T cell activation by IL-1 or T cell mutagens</td>
<td>Stimulates production of myeloid stem cells and haemopoietic cells</td>
<td>Stimulates histamine-producing cells and histamine release</td>
</tr>
<tr>
<td>Interleukin-4 (IL-4)</td>
<td>activated T helper cells</td>
<td>T cell activation by IL-1, antigen or endotoxin</td>
<td>Stimulates IgG and IgE synthesis by B cells</td>
<td></td>
</tr>
<tr>
<td>Interleukin-5 (IL-5)</td>
<td>activated T helper cells</td>
<td>T cell activation by IL-1, antigen or endotoxin</td>
<td>Enhances synthesis of IgA and IgM and the IL-4 production of IgE</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>activated T helper cells; macrophages, fibroblasts, endothelial cells</td>
<td>IL-1 stimulated B cells; antigens, mutagens, endotoxins</td>
<td>T cells, macrophages and hepatocytes; act on CNS to produce fever</td>
<td></td>
</tr>
<tr>
<td>Interleukin-7 (IL-7)</td>
<td>Marrow stromal cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Interleukin-8 (IL-8)</td>
<td>T lymphocyte</td>
<td>Blood monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour Necrosing Factor (TNF)</td>
<td>Natural killer cells activated macrophages and monocytes</td>
<td>Bacterial endotoxin. Inflammatory agents IL-1 and IFN</td>
<td>Kills tumour cells. Inhibits lipoprotein lipase. Contributes to the inflammatory response. Accelerates lipolysis</td>
<td>May contribute to cachexia by initiating fat depot depletion</td>
</tr>
<tr>
<td>Interferon-α (IFN-α)</td>
<td>Neutrophils</td>
<td>Viral stimulation of neutrophils</td>
<td>Endotoxin</td>
<td>Confers resistance to viral infection on cells</td>
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<tr>
<td>Interferon-β (IFN-β)</td>
<td>Fibroblasts</td>
<td>Cytokins</td>
<td></td>
<td>Confers resistance to viral infection on cells</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Activated T helper cell</td>
<td>T cell activation by IL-1, antigen or endotoxin</td>
<td></td>
<td>Confers resistance to viral infection on cells</td>
</tr>
<tr>
<td>Granulocyte macrophage colony stimulating factor</td>
<td>Macrophages Fibroblasts Epithelial cells</td>
<td>T cell activation by IL-1, antigen or endotoxin; cytokin stimulation</td>
<td></td>
<td>Stimulates the production of TNF, IL-1 and H₂O₂</td>
</tr>
</tbody>
</table>
SPECIFIC CYTOKINES

**IL-1**

IL-1 comes mainly from macrophages and monocytes.
IL-1 is induced by micro-organisms, microbial products, antigens, inflammatory agents, plant lectins, lymphokines and certain chemicals.
IL-1 activates the process known as the acute phase response. This response is characterised by production of a variety of hepatic proteins (e.g., C-reactive protein, serum amyloid A, fibrinogen, complement, alpha 1-antitrypsin).
These include inducing a fever and the synthesis and release of glucocorticoid hormones, prostaglandins and collagenase enzyme.
IL-1 activates adhesion molecules on vascular endothelium. (VCAM)
IL-1 stimulates the production of IL-8 that activates neutrophils.
IL-1elicits the release of histamine from mast cells at the site of inflammation causing increased capillary permeability and vasodilatation.
IL-1 is a potent mitogen for astroglial cells and induces astrocytes to synthesise NGF.
IL-1 has been shown to interfere powerfully with the hypothalamic-hypophyseal-gonadal axis (HHGA). At the CNS level, IL-1 has been shown to decrease plasma LH levels (Luteinising hormone), a phenomenon attributed to the inhibition of hypothalamic secretion of LHRH and LHRH gene expression.

**MAJOR EFFECTS:**
Fever; sleep; anorexia, inflammation; endothelial cell expression of CD54; release of tissue factor; lymphocyte activation; production of IL-6, IL-8 and CSF
**IL-2**

IL-2 generated by T lymphocytes after stimulation by antigen or mitogen. IL-2 is a pro-inflammatory cytokine that plays a critical role in regulating both cellular and humoral chronic inflammatory responses.

The ultimate purpose of the host defence system is to eliminate invading microorganisms. Once an invading organism is recognised as foreign, elimination is accomplished through phagocytosis and antibody formation (in the case of bacteria) and cytotoxic attack (for viral, fungal, or other intracellular pathogens).

Immunologic memory and IL-2

IL-2 is responsible for largely for immunologic memory. The immune system forms a long-term "memory" from antigen exposure so that any future contact will stimulate an immediate defence against that particular antigen.

There are two major classes of lymphocytes: **B lymphocytes** (B cells) derived from the bone marrow and **T-lymphocytes** (T cells) derived from bone marrow but matured by the thymus. B cells express antibody molecules on their surface, and when stimulated by antigen, each B cell becomes a **plasma cell** that secretes antibodies specific for that antigen. T cells also express what appear to be antibody molecules on their surfaces, but unlike B cells, these molecules cannot be secreted. Instead, T cells react to antigen stimulation by secreting other types of molecules; **cytotoxic ("killer") T cells** secrete molecules that kill infected or abnormal cells on contact, while **"helper" T cells** secrete a variety of cytokines involved in the immune response. Thus, a foreign antigen stimulates both B cells and T cells, and the resulting immune response is specific for that antigen alone.

After an antigen binds receptors on an individual T cell, the antigen stimulates the T cell to secrete IL-2 and to make IL-2 receptors. The IL-2 receptor then acts as an on-off switch.
The T lymphocyte has two receptor sites; the first site readily binds IL-2, while the second site attaches more slowly to the IL-2 molecule. It is the interaction at the second site, however, that activates the T cell, causing it to undergo complex changes in morphology, metabolism, expression of surface receptors, and the production of cytokines. The activated T cell starts to synthesise DNA and divides, producing two T cells that can now be activated by antigen. This proliferation continues until there is a clone of identical T cells, each capable of binding antigen. Thus, T-cell proliferation is antigen-specific; that is, only those cells that react with a given antigen survive and multiply. Proliferation is also dependent upon the concentration of IL-2, the density of IL-2 receptors on the cell surface, and the number of IL-2 receptor interactions that occur during a specific interval of time. As antigen declines in concentration, IL-2 dissociates from the second site on the IL-2 receptor (a slow process because of the strength of the bond); signalling stops, and the T cell clone stops proliferating. The lingering cells compose the memory population of the immune system.

In addition to its role as an inducer of T cell proliferation, IL-2 stimulates the proliferation of B cells, helping them to secrete antibodies. Killer T cells, which appear to be in a constant state of activation, also respond to IL-2, indeed, these cells represent the first line of defence against intracellular pathogens because of their immediate responsiveness to IL-2. IL-2 may also influence the maturation and differentiation of lymphocytes in the thymus and bone marrow.

IL-2 has been found to be low in persistent microbial infections and cancer and there is a considerable body of research into the immunostimulatory effect of IL-2.

In summary: -

IL-2 is a major growth factor for both helper and cytotoxic T cells and for lymphokine-activated killer (LAK) cells resulting in:

- B cell development
- Increased lymphokine secretion (hence IFN-γ, lymphotoxin, IL-4, IL-3, IL-5, GM-CSF {macrophage colony stimulating factor})
- Enhanced expression of MHC (Major Histocompatability Complex) class II proteins
- IL-2 and its receptor occur in the brain and this protein promotes the division and maturation of oligodendrocytes and the survival of peripheral nervous system neurones in culture.

Figure 5 IL-2 and cancer flow chart
From The Cancer Journal 1997 Vol 3 Supplement 1 pS117; unbroken lines are stimulatory effects, broken lines are inhibitory effects

**IL-3**

IL-3 is generated from activated T-cells and mast cells.  
IL-3 stimulates eosinophils and B cell differentiation.  
IL-3 inhibits lymphokine-activated killer (LAK) cell activity.  
IL-3 shares several biological activities with GM-CSF.  
IL-3 supports the survival of cholinergic neurones.
**IL-4**

IL-4 is mainly derived from Th2 cells (CD4 cells), mast cells and basophils.
It is a structural homologue of IL-13.
IL-4 suppresses the production of pro-inflammatory cytokines.
IL-4 suppresses the production of IL-8.
IL-4 is involved in the development of Th2 subset.
IL-4 is involved in B-lymphocyte production.
It cause the switching of antibody production to IgE and IgG.
It is the growth factor for mast cells.
Responsible for the induction of CD8.
It stimulates production of VCAM-1.
It enhances expression of MHC class 11 proteins and thus antigen production.
It has some anti-inflammatory action in synovial membranes by inhibiting the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α.
Its actions are antagonised by IFN-γ and vice versa.
It is a synergist with IL-3.

**IL-5**

IL-5 is derived from Th2 cells (CD4 T helper cells) and activated mast cells like IL-4.
IL-5 stimulates growth of eosinophils, which are responsible for killing the helminth family of parasites.
IL-5 stimulates the growth and differentiation of B-cells.
It induces the synthesis of IgA and IgM in mature B-lymphocytes.
Enhancement of T cell cytotoxicity.
**IL-6**

IL-6 is derived from macrophages, fibroblasts, bone marrow, vascular endothelium and some T cells.

IL-6 is also derived from IL-1 stimulated B cells, antigens, mitogens and endotoxins.

IL-6 inhibits the macrophage production of IL-1 (a feedback loop) and Interferon gamma (IFN-\(\gamma\)).

IL-6 stimulates the production of immunoglobulin producing B cells.

IL-6 stimulates proliferation of thymic and peripheral T cells.

With IL-1, IL-6 induces T cell differentiation to “Killer” T lymphocytes.

IL-6 stimulates the liver to produce Acute Phase Proteins such as fibrinogen, serum amyloid protein A and \(\alpha_2\)-macroglobulin.

IL-6 activates Natural Killer cells (NK cells).

IL-6 appears to play an important role in bone metabolism through induction of osteoclastogenesis and osteoclast activity.

The upregulation of IL-6 has been found in type 1 diabetes, inflammatory thyroid disease, RA, systemic sclerosis, psoriasis and various cancers.

IL-6 has major functions in neoplastic processes.

IL-6 may affect cancer progression by its actions on:-

- Cell adhesion and motility
- Thrombopoiesis
- Tumour specific antigen expression
- Cancer cell proliferation.

Depending on the cell type, IL-6 can either inhibit or stimulate cancer cell proliferation. Tumours stimulated by IL-6 include melanoma, renal cell carcinoma, prostate carcinoma, Kaposi's sarcoma, ovarian carcinoma, lymphoma and leukaemia, and multiple myeloma.

**IL-6 and its implications in various disease processes**
1. Ageing

Although a normal physiologic process, ageing is accompanied by a variety of disorders including,

- Alzheimer's disease,
- Arteriosclerosis
- Thyroiditis.

Because IL-6 levels are directly correlated with ageing in a variety of species, it may play an important role in the ageing process. Intriguingly, dietary restriction, the only experimental intervention that reproducibly prolongs maximum lifespan in mammals can restore a variety of physiologic parameters, including IL-6 secretion and serum levels to the level in youth. Similarly, DHEA, currently thought to influence various ageing processes also has been shown to diminish the age-associated rise in serum IL-6.

IL-6 may be an important mediator of several infectious and autoimmune diseases. These include:-

- Human immunodeficiency virus (HIV infection)
- Rheumatoid arthritis
- Castleman's disease
- Para-neoplastic symptoms associated with cardiac myxoma
- Sepsis
- Inflammatory joint disease, particularly rheumatoid arthritis, which is associated with, increased synovial fluid levels of IL-6.

IL-6 and hormonal function

- Glucocorticoids inhibit IL-6 expression.
- During times of stress or inflammation, IL-6 levels are increased. IL-6, in turn, can induce release of corticotrophin-releasing factor, which results in elevated systemic levels of corticosteroids. These findings along
with the observations that natural and synthetic corticosteroids inhibit IL-6 production, from a variety of tissues, provide a mechanism for a negative-feedback loop.

- Oestrogen inhibits IL-6 expression.
- It has been observed that menopause or ovariectomy resulted in increased IL-6 serum levels and increased IL-6 secretion by mononuclear cells. Studies have
demonstrated that oestradiol inhibits bone marrow stromal cell and osteoblastic cell production.

**IL-7**

IL-7 is derived from stromal cells in bone marrow and thymus.  
Stimulates proliferation of B-cell progenitors  
Stimulates mature T-cells  
Enhances cytotoxicity

**IL-8**

IL-8 is derived from many cell types, including mononuclear phagocytes, antigen-activated T cells, endothelial and epithelial cells, and even neutrophils.  
IL-8 activates the chemotactic migration and activation of neutrophils and other cell types (such as monocytes, lymphocytes, basophils, and eosinophils) at sites of inflammation.  
IL-8 stimulate granulocyte activity  
IL-8 up-regulates cell-surface adhesion molecule expression (such as endothelial leukocyte adhesion molecule, ELAM-1, and intracellular adhesion molecule, ICAM-1), thereby enhancing neutrophil adherence to endothelial cells and facilitating their diapedesis through vessel walls.

**IL-9**

IL-9 is generated from CD4 T helper cells and some B lymphomas.  
IL-9 is dependent on IL-4, IL-10 and IL-2.  
Promotes proliferation of T-cells – CH8 T cells.  
It inhibits lymphokine production by IFN-γ-producing CD4+ T cells.  
Increase production of immunoglobulins and proliferation of mast cells.

**IL-10**

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IL-10 is generated from CD4 T cells, activated CD8+ T lymphocytes, B-lymphocytes, macrophages, activated mast cells and epidermal cells. It is an anti-inflammatory cytokine along with IL-4 and IL-13. IL-10 down-regulates the macrophage production of IL-1, IFN-γ and TNF-α. IL-10 inhibits the production of IL-2 induced IFN-γ by NK cells. IL-10 promotes the inhibition of IL-4 and IFN-γ induced MHC class II expression on monocytes. IL-10 inhibits nitric oxide production. IL-10 stimulates B-cell proliferation. IL-10 inhibits macrophage/monocyte activation. IL-10 suppresses activity of peripheral blood lymphocytes (important in graft rejection).

**IL-11**

IL-11 is generated from bone stromal cells and some fibroblasts. IL-11 is a functional homologue of IL-6 and can replace IL-6 in the induction of acute phase proteins in the liver. IL-11 promotes lymphopoiesis. IL-11 promotes the growth of haemopoietic cells. IL-11 produces acute phase proteins in the liver. IL-11 stimulates T cell-dependent B cell immunoglobulin secretion, IL-11 stimulates platelet production. IL-11 induces IL-6 expression by CD4⁺ T cells.

**IL-12**

IL-12 is generated from T and B-lymphocytes, NK cells and macrophages. The production is of IL-12 is inhibited by IL-4 and IL-10.
The stimulatory effect of IL-12 on TH1 development is antagonised by IL-4, a cytokine that promotes TH2 cell development.

IL-12 is a proinflammatory cytokine.

IL-12 enhances cytotoxic T cells

IL-12 stimulates lymphokine-activated killer (LAK) cell generation and activation.

IL-12 induces IFN-γ production by NK cells and T cells, therefore enhancing formation of TH1 cells.

IL-12 inhibits the synthesis of IgE by IL-4-stimulated lymphocytes via IFN-gamma-dependent and other independent mechanisms.

IL-12 stimulates NK cells (natural killer cells).

IL-12 is a growth factor for activated T-cells.

IL-12 is a maturation factor for cytotoxic T-cells

**IL-13**

The gene has been mapped to human chromosome 5, and is closely linked to the gene encoding IL-4, both being anti-inflammatory cytokines.

IL-13 is generated from activated “helper” and cytotoxic “killer” T-cells

IL-13 suppresses the production of pro-inflammatory cytokines (IL-1β, TNF-α, IL-8 and IL-6), chemokines and growth factor by macrophages.

Inhibition of inflammatory cytokine production is also a characteristic of two other cytokines produced by TH2 lymphocytes, namely IL-4 and IL-10.

IL-13 down-regulates the production of nitric oxide.

IL-13 enhances the expression of MHC class 11 proteins (Major Histocompatibility Complex) and thus antigen production.

IL-13 increases CD23 expression.

IL-13 cause the switching of antibody production to IgE and IgG4.

It has been found that, whereas normal cells share receptors between IL-4 and IL-13, most tested established brain tumour lines over-express a receptor for IL-13 and these receptors block IL-4.
IL-14

IL-14 is generated from T-lymphocyte and malignant B-lymphocytes.
Induces proliferation of activated B-cells
Inhibits immunoglobulin production
It has been suggested that IL-14 plays an important role in an aggressive form of B-cell type non-Hodgkin’s lymphoma

IL-15

The human IL-15 gene has been mapped to chromosome 4, similarly to IL-2. IL-15 is generated from epithelial cells, activated monocytes and fibroblasts. IL-15 shares many biologic properties with IL-2 and mediates its activity via a high affinity receptor comprised of a unique alpha chain (to IL-15) and the beta and gamma chains of the IL-2.
IL-15 stimulates T-cell production by binding to IL-2 receptor sites
IL-15 stimulates NK cell proliferation, as well as CTL and LAK activity.
IL-15 enhances B cell expansion and immunoglobulin production.
IL-15 functions also a T lymphocyte chemo-attractant.

IL-16:

IL-16 is the only member of the "C" family of chemokines.
IL-16 is an unusual cytokine in that pre-formed IL-16 is stored in CD8^+ lymphocytes and is secreted upon stimulation with histamine or serotonin.
It induces chemotaxis of CD4^+ T lymphocytes.
Initiates T cell mediated inflammation in asthma.

IL-17:

IL-17 is generated from activated T lymphocytes.
IL-17 stimulates IL-6 and IL-8 production.
IL-17 enhances ICAM-1 expression.

**IL-18 (IFN-gamma-inducing factor: (IGIF) )**

It has been proposed that IGIF be designated as Interleukin-18 (IL-18)

Functions
- It induces IFN-gamma production more potently than does IL-12
- The development of Th1 cells.
- The enhancement of NK cell cytotoxicity
- Augments GM-CSF production
- Decreases IL-10 production.

**INTERFERONS**

The cytokine, interferon, is produced by T “helper” lymphocytes.

There are two types of T cells:
1. Cytotoxic (or "killer") T cells, which aggressively screen other cells for signs of infection and malignancy and secrete toxic molecules to kill any aberrant cells;
2. "Helper" T cells, which co-operate with B cells (lymphocytes that mature in the bone marrow) in the antibody response to antigens such as bacterial toxins.

The helper T cells produce interferon and other cytokines in response to an antigen challenge. Before a B cell can produce and secrete antibodies, it must recognise a specific antigen and receive signals from certain cytokines.

**Overview**
- Interferon fights infectious disease.
- They increase the phagocytic activity of macrophages.
- Interferon increases the cytotoxic activity of lymphocytes.
Interferon inhibits the replication of intracellular pathogens.

**IFN-α and IFN-β**

IFN-α and IFN-β are generated by leukocytes and fibroblasts. They have:

- Antiviral properties.
- Interferon activates phagocytes.
- Anti-proliferative properties.
- Interferon up-regulates MHC class I expression.

**IFN-γ**

IFN-γ is generated by T-lymphocytes and NK cells. Its functions are:

- Antiviral properties.
- The development of T_H1 from T_H0 cells and the inhibition of T_H2 cells.
- It is the body’s most powerful macrophage-activating factor.
- IFN-γ increases the expression of both MHC type 1 and 11 proteins, thus enhancing antigen production.
- Stimulates the expression of VCAM.
- Stimulates the differentiation of cytotoxic T-cells.
- IFN-γ antagonises several of the actions of IL-4.
- IFN-γ promotes the synthesis of IgG2 by activated T cells.

**IFN-gamma-inducing factor: (IGIF)**

- See IL-18
CYTOTOXINS

**TNF-α**

TNF-α is generated by macrophages, mast cells and T-lymphocytes. Macrophages are stimulated to produce TNF by IFN-γ (i.e. TNF-α is produced when IFN produces migration inhibition factor (MIF) when T-lymphocytes are stimulated by an endotoxin.

TNF-α in high concentrations as when it is induced by an endotoxin:
- Pyrogenic properties; either directly via stimulation of PGE\(_2\) synthesis by the vascular endothelium of the hypothalamus, or indirectly by inducing the release of IL-1.
- The production of acute phase proteins.
- TNF-α stimulates production of nitric oxide.

If TNF-α is produced for long periods, then it produces cachexia.

TNF-α when produces in low concentrations:
- Up-regulates the inflammatory response.
- Induces expression of ICAM-1, VCAM-1 and E-selectin.
- Enhances killing of intracellular organisms such as Leishmania and Mycobacterium tuberculosis.
- Activates leukocytes to produce IL-6 and TNF-α itself.

**TNF-β**

TNF-β is produced by activated T-cells.

Its properties are similar to those of TNF-α and include:
- The induction of apoptosis (programmed cell death) in many types of transformed, virally infected, and tumour cells.
- The stimulation of several PMN (polymorphonuclear leukocytes) effector functions.
- TNF-β lyses tumour cells.
- TNF-β activates neutrophils.
- TNF-β increases the adhesion of leukocytes to the vascular endothelium.

**Colony stimulating factors: (G-CSF and GM-CSF)**

There are two forms of Colony Stimulating Factors:

They are derived from monocytes, T lymphocytes, fibroblasts and endothelial cells that are activated by macrophage products such as the pro-inflammatory cytokines IL-1 and TNF.
- They both participate in acute inflammation.
- They both can stimulate neutrophils.
- GM-CSF can also activate effector functions of eosinophils and mononuclear phagocytes.
- In the airway inflammation accompanying asthma, GM-CSF, IL-3 and IL-5 perpetuate the eosinophil activation and survival.

The source of GM-CSF may be the alveolar macrophages, which are reported to produce two to threefold higher levels of GM-CSF than control macrophages. Another possible source for all three cytokines is the T-lymphocytes present in the airways.

Other factors in the inflammation of asthma might be IL-4 and IL-13 (both stimulatory) and IFN-γ (inhibitory) that are probably involved in the control of IgE synthesis, while IL-1 and TNF-α up-regulation the expression of the endothelial adhesion molecule.

**Transforming growth factor: (TGF)**

TGF is a family of cytokines that includes five isoforms; TGF-β1, β2, and β3 that are from separate genes yet, bind to the same high affinity receptor. TGFs are
trophic polypeptides that stimulate the proliferation and differentiation of different cell types.

**TGF-β**

TGF-β is derived from T cells, platelets, and monocytes:

- TGF-β inhibits T cell and NK cell proliferation and activation.
- At a site of injury, TGF-β that is stored in platelets will be released upon the degranulation of mast cells. TGF-β then attracts monocytes and other leukocytes to the site, thus participating in the initial step of chronic inflammation. TGF-β then positively regulates its own production and the production and deposition of extracellular matrix components as well as the expression of integrins resulting in enhanced cell adhesion.
- TGF-β up-regulates IL-1, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and TNF-α.
- TGF-β inhibits collagenase production, and if the TGF-β expression is prolonged, this may result in progressive fibrosis analogous to unregulated tissue repair.

Conditions in which a role for TGF-β has been suggested include:

- Mesangial proliferative glomerulonephritis
- diabetic nephropathy
- pulmonary fibrosis
- systemic sclerosis

**Epidermal growth factor**

Epidermal growth factor and transforming growth factors (TGFs) are related acidic proteins of approximately 50 amino acids, and are members of a larger protein family that includes certain viral proteins. Recently discovered growth factors belonging to this family are:
• Amphiregulin,
• Heparin binding growth factor, and
• Schwanoma-derived growth factor (SDGF)

Epidermal growth factor functions:
EGF is a trophic polypeptides that stimulate the proliferation and differentiation of different cell types.
• EGF is also shown to be a potent stimulator of astrocyte proliferation.
• EGF is not synthesized by the developing neuronal cells, (but its homologue, TGF-alpha, is expressed in the brain.)
• During gliogenesis, EGF is detected in tissues and in the blood.
• EGF is known to strongly affect the morphology of astrocytes and induce upregulation of the glutamine. viii

Fibroblast growth factors (FGFs)
The family of fibroblast growth factors (FGFs) include several forms:
FGFs occur in many peripheral tissues, and they are potent mitogens for a large number of cell types. The brain and pituitary are rich sources of bFGF.

CELL SURFACE MOLECULES
To date, about 300 different cell surface structures have been discovered on the membranes of various body cells. The term cell surface molecule is a collective term including adhesion molecules, MHC complexes, cytokine receptors, etc.
The ligands are bound according to the lock-and-key principle, whereby this process generally involves mediators, adhesion molecules of other cells or matrix proteins (structural proteins such as collagen, fibrin, etc.).
Aside from a few exceptions, the binding of a ligand is associated with a signal transfer in the cells. The manner of this signal transduction in the cell is still more or less unknown.
These cell surface molecules, when they react to a specific stimulus (cell-to-cell contact, cytokines, toxins, hypoxia, thermal and mechanical irritations, etc.), are
able to induce the cells to be able to increase the expression density of some of the receptors or even create other types. According to the particular receptor type, its expression may already occur after only a matter of minutes. After 2 - 6 hours, their density reaches its maximum and then returns to normal values within 24 hours. When the receptors have performed their work, they are either shed or reabsorbed.

According to their functional condition (and activity), and also dependent on their objectives, the cells constantly alter the type and the density of their surface molecules. This adaptability is especially intense in the immune cells.

**ADHESION MOLECULES**

When there is an altered inflammation state, the inflammatory markers or inflammation-producing cells of the immune system secrete a class of molecules called intracellular adhesion molecules (ICAMs)\textsuperscript{ix}. Each cell type has a characteristic, basic structure with specific cell surface molecules, which allow them to adhere to other cells or to tissues. Many immune cells are even identified based upon these adhesion molecules (e.g. CD4, CD8). Decisive defensive steps in the inflammatory pathways require a direct cell-to-cell contact via adhesion receptors. These steps are:-

- Antigen recognition and presentation,
- Induction of a humoral immune response,
- Activation of cytotoxic T cells or macrophages,
- Immune cell migration,

The up and down regulation of adhesion receptors primarily occurs via their interaction with stimulatory and inhibitory cytokines which are secreted locally. In the living organism, the function of the different adhesion receptors, as well as that of the cytokines, cannot be considered individually. They interact with one another and mediate complex "messages".
The importance of adhesion molecules for a functional defensive system is recognised in the role they play in the pathogenesis of chronic inflammatory diseases, autoimmune diseases and metastases.

Chronic and autoimmune inflammatory responses are associated with these stimuli from key toxins that are constantly present. These induce an excessive supply of adhesion receptors and increase the migration of further immunocytes into the affected area or areas.

In the localised arterial wall, these ICAMs create an opportunity for macrophages and monocytes to bind to and infiltrate the arterial wall and be converted into foam cells.

Foam cells subsequently release oxidants that convert normal LDL into atherogenic, oxidised LDL. These oxidised LDL create the opportunity for altered gene expression in the arterial wall. It begins replicating as a benign tumour called a monoclonal hyperplasia, and it becomes an atheroma.

**TYPES OF ADHESION MOLECULES**

1. Selectins – a series of proteins that resemble lectins that bind to sugars with a high affinity. They are present when the endothelium is stimulated by :-
   - Bacterial endotoxins
   - IL-1
   - Thrombin
   - TNF-α (Tumour Necrosis Factor)

2. Members of the gene immunoglobulin super-family
   - VCAM (Vascular Adhesion Molecule)
   - ICAM-1 (Inter-cellular Adhesion Molecule)
   - ICAM-2
   - IL-1 and TNF-α stimulate the adhesion molecules.
   - Integrons
   - Cartilage link proteins.
The inflammatory activity correlates with the expression density of the adhesion molecule. An over-expression of selectin leads to a substantial increase in the migration of neutrophilic cells and is responsible, in part, for the preservation of chronic inflammation.

The over-expression of ICAM-1 on the endothelium in areas altered by inflammation helps to perpetuate this inflammation and even enables the passage of lymphocytes through the blood-brain barrier in patients with multiple sclerosis.

Figure 7 ICAM flow chart

- ICAM hyper
- Associated with increased risk of heart disease
- Challenge against: giardia, helicobacter, chlamydia
- Upregulates inflammatory response in multiple sclerosis
- Challenge inflammatory biomarkers and supplement accordingly
<table>
<thead>
<tr>
<th>Cytokine</th>
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<tr>
<td>Bone Morphogenetic Proteins</td>
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<td>HIV/AIDS</td>
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The other three intercellular signalling molecules are neurotransmitters, endocrine hormones and autacoids.

The Cancer Journal from Scientific American 1997; 3: Supplement 1

MHC type 1 is always recognised by lymphocytes bearing cell surface protein CD8; MHC type 11 is always recognised by lymphocytes bearing CD4


IL-15 may be responsible for the recruitment and activation of T lymphocytes in the synovium of patients with rheumatoid arthritis where its levels have been found to be elevated

Interferon α-2 was the first pure human protein found to be effective in the treatment of cancer, and it has served as a prototype for the clinical development of other immune modulators such as IL-2 and various growth-regulating cytokines. Interferon α-2 has been used to treat chronic myelogenous leukaemia and other myeloprolific disorders, perhaps by inhibiting oncogene expression. Used in combination with retinoids, interferon α-2 has induced regression in advanced squamous carcinomas of the skin and cervix, suggesting that the cytokine may influence cell differentiation. It also inhibits vascular and endothelial cell proliferation and thus has a place in treatment of melanomas, hypernephromas, and haemangiomias. Because it can increase the intensity of antigen expression on certain tumours (ovarian and colorectal carcinomas), interferon α-2 has potential for diagnostics (imaging) and therapeutics (monoclonal antibodies).

Multiple sclerosis (MS) is an autoimmune disease that might be mediated by T-cells that attack the myelin components of the CNS, specifically myelin basic protein (MBP) and proteolipid protein (PLP).

When animals are injected with MBP or PLP plus adjuvant, a disease is induced that is similar to MS. There is strong evidence that MS develops in patients who have a myelin configuration resembling certain components of common viruses, in particular the measles virus.

MS is more common and more difficult to treat in females than males, and it tends to run in families. It is chronic and progressive; patients are ultimately confined to wheelchairs or, in severe cases, bedridden. An estimated 300,000 Americans are afflicted with MS.
Most are treated with diet and exercise. Steroids and other immuno-suppressants are reserved for acute exacerbation of the disease. Studies in the use of interferon-γ for MS were disappointing; the compound stimulated the immune system but exacerbated the MS. Interferon-β, on the other hand, appears to have an inhibitory effect on a hyperactive immune system, perhaps by moderating the activity of γ-interferon and might prove to be more successful.

In the peripheral nervous system (PNS) studies have indicated that individual growth factors act as critical determinants of transmitter type. In the brain, however, the initiation of neurotransmitter specific genes appears to involve more complex mechanisms, requiring the obligatory interaction of multiple signal molecules.

(Journal of Immunology. 1994;153:2681)