

Immune System: Cytokines

Cytokines are a group of proteins and peptides that are signalling compounds produced by animal and plant cells to communicate with one another. They act via cell-surface cytokine receptors. The cytokine family consists mainly of smaller water-soluble proteins and glycoproteins (proteins with an added sugar chain) with a mass of between 8 and 30 kDa. They act like hormones and neurotransmitters but whereas hormones are released from specific organs into the blood and neurotransmitters are produced by neurons, cytokines are released by many types of cells. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory, and infectious diseases. However, not all their functions are limited to the immune system, as they are also involved in several developmental processes during embryogenesis.

When the immune system is fighting pathogens, cytokines signal immune cells such as T-cells and macrophages to travel to the site of infection. In addition, cytokines activate those cells, stimulating them to produce more cytokines.

Cytokines are produced by a wide variety of cell types (both haemopoietic and non-haemopoietic), and can have effects on both nearby cells or throughout the organism. Sometimes these effects are strongly dependent on the presence of other chemicals and cytokines.

Effects

Each cytokine binds to a specific cell-surface receptor. Subsequent cascades of intracellular signaling then alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcription factors, in turn resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition.

The effect of a particular cytokine on a given cell depends on the cytokine, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface, and downstream signals activated by receptor binding; these last two factors can vary by cell type. Cytokines are characterized by considerable "redundancy", in that many cytokines appear to share similar functions.

Generalization of functions is not possible with cytokines; nonetheless, their actions may be grouped as:

- autocrine, if the cytokine acts on the cell that secretes it
- paracrine, if the action is restricted to the immediate vicinity of a cytokine's secretion
- endocrine, if the cytokine diffuses to distant regions of the body (carried by blood or plasma) to affect different tissues.

It seems to be a paradox that cytokines binding to antibodies have a stronger immune effect than the cytokine alone. This may lead to lower therapeutic doses and perhaps fewer side-effects.

Overstimulation of cytokines can trigger a dangerous syndrome known as a cytokine storm; this may be the cause of severe adverse events during the clinical trial of some drugs.

Nomenclature

Cytokines have been variously named as lymphokines, interleukins, and chemokines, based on their presumed function, cell of secretion, or target of action. Because cytokines are characterized by considerable redundancy and pleiotropism, such distinctions, allowing for exceptions, are obsolete.

- The term *interleukin* was initially used by researchers for those cytokines whose presumed targets are principally leukocytes. It is now used largely for designation of newer cytokine molecules discovered every day and bears little relation to their presumed

function. The vast majority of these are produced by T-helper cells.

- The term *chemokine* refers to a specific class of cytokines that mediates chemoattraction (chemotaxis) between cells.

IL-8 (interleukin-8) is the only chemokine originally named an interleukin.

Classification

Structural

Structural homology has been able to partially distinguish between cytokines that do not demonstrate a considerable degree of redundancy so that they can be classified into four types:

- The four α -helix bundle family - Member cytokines have three-dimensional structures with four bundles of α -helices. This family in turn is divided into three sub-families:
 1. the IL-2 subfamily
 2. the interferon (IFN) subfamily
 3. the IL-10 subfamily.

The first of these three subfamilies is the largest. It contains several non-immunological cytokines including erythropoietin (EPO) and thrombopoietin (THPO). Also, four α -helix bundle cytokines can be grouped into *long-chain* and *short-chain* cytokines.

- the IL-1 family, which primarily includes IL-1 and IL-18
- the IL-17 family, which has yet to be completely characterized, though member cytokines have a specific effect in promoting proliferation of T-cells that cause cytotoxic effects
- Chemokines.

Functional

A classification that proves more useful in clinical and experimental practice divides immunological cytokines into those that promote the proliferation and functioning of helper T-cells, type 1 (IFN- γ etc.) and type 2 (IL-4, IL-10, IL-13, TGF- β , etc.), respectively.

A key focus of interest has been that cytokines in one of these two sub-sets tend to inhibit the effects of those in the other. This tendency is under intensive study for its possible role in the pathogenesis of autoimmune disorders.

Cytokine receptors

Cytokine receptor

In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics, and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleiomorphism of cytokines are, in fact, a consequence of their homologous receptors, many authorities are now of the opinion that a classification of cytokine receptors would be more clinically and experimentally useful.

A classification of cytokine receptors based on their three-dimensional structure has, therefore, been attempted. Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.

- Immunoglobulin (Ig) superfamily, which are ubiquitously present throughout several cells and tissues of the vertebrate body, and share structural homology with immunoglobulins (antibodies), cell adhesion molecules, and even some cytokines. Examples: IL-1 receptor types.

- Haemopoietic Growth Factor (type 1) family, whose members have certain conserved motifs in their extracellular amino-acid domain. The IL-2 receptor belongs to this chain, whose γ -chain (common to several other cytokines) deficiency is directly responsible for the x-linked form of Severe Combined Immunodeficiency (X-SCID).
- Interferon (type 2) family, whose members are receptors for IFN β and γ .
- Tumor necrosis factors (TNF) (type 3) family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like CD40, CD27 and CD30, besides the ligands on which the family is named (TNF).
- Seven transmembrane helix family, the ubiquitous receptor type of the animal kingdom. All G-protein coupled receptors (for hormones and neurotransmitters) belong to this family. Chemokine receptors, two of which act as binding proteins for HIV (CXCR4 and CCR5), also belong to this family.

Cysteine-knot cytokines

Members of the transforming growth factor beta superfamily belong to this group, including TGF- β 1, TGF- β 2 and TGF- β 3.

Cell Interactions

Types of cell-cell communication. Essentially these can be divided into:

- a) communication by cell-cell contact
- b) communication by secretion of cytokines

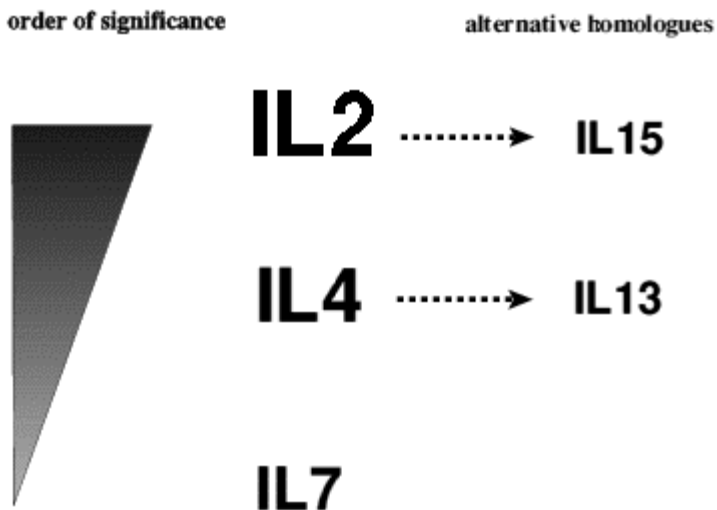
Cytokines are basically polypeptide hormones but the term is often used to denote those molecules which are the products of cells of the immune system or which act upon such cells. In common with other hormones, cytokines exert their effects by binding to specific cell-surface receptors which signal to their target cells. They act at very

low concentrations (typically 10^{-10} - 10^{-15} M). They are short-lived and may act locally, either on other cell types (paracrine) or on the same cell type (autocrine), or systemically (endocrine).

The cytokine network is very complex and many of the details are beyond the breadth of a single lecture. There are 2 general principles. The cytokine system demonstrates great redundancy and great pleiotropism. Redundancy in this context means that most functions of cytokines can be performed by many different cytokines.

For example many different cytokines can act to stimulate cell division in activated T cells.

T cell growth factors



Pleiotropism means that a single cytokine has many different functional effects, literally on many different cell types but in fact sometimes even on the same cell. The practical manifestation of these properties means, for instance, that blocking or genetically ablating (by "knockout" transgenic technology) a particular cytokine rarely has widespread or dramatic effects, and conversely that over expression or exogenous administration of a single cytokine frequently has several diverse effects.

The Immune System as a "Sensory Organ"

A key attribute of an effective Immune system is to be able to detect the presence of trouble - this could be dead or damaged cells, tumour cells or infection with viruses, bacteria or eukaryotic parasites. Some aspects of this have already been dealt with; acute inflammation is the major system for sensing trauma. However, the Immune system needs to be able to detect more subtle changes and also needs to encourage a two-way communication between the innate and adaptive immune responses.

How is this achieved?

Critical sensory detectors

- Complement
- Phagocyte receptors for prokaryotic products
- Sensing of foreign nucleic acid

In terms of protection against infection the ability of certain cells to detect the presence of prokaryotic molecules is of primary importance. Though there is considerable sophistication and subtlety to the mechanisms which do this we can focus here on 3 cells types.

Mast cells primarily detect danger via receptors for activated complement through acute inflammation route; they also use antibodies as sensing tools via Fc receptors for both IgE (high affinity) and IgG (low affinity).

The 2 cell types are the Macrophage and Dendritic cell. These cells share a number of receptors which bind structures which are specific to bacteria or fungi, mostly carbohydrates. In fact blood monocytes may be induced to differentiate into either macrophages or dendritic cells under appropriate distinct conditions. The response of the cells to the ligation of these receptors includes phagocytosis etc but the critical issues relevant in this context are

- cytokine production
- antigen presentation

LPS (bacterial endotoxin) induces the TNF cascade

Macrophages are exquisitely sensitive to the lipopolysaccharides (LPS) produced by certain bacteria. They respond by producing cytokines notably TNF α , but also IL1 and IL6. These mediators induce the Acute Phase Response, which is a rapid systemic response which massively increases the concentration of many key serum proteins to aid the host defence response. C reactive protein (CRP) and Mannose binding protein are natural activators of the Complement system. To know more about the acute phase response, which increases the production of many more proteins, try looking at Horst Ibelgauf's web page on the subject (choose A then Acute Phase Reaction and Acute Phase Proteins)

Although macrophages produce a number of other cytokines in response to LPS, or to stimulation of a variety of other innate receptors, 2 are especially relevant.

IL8 is a chemokine; chemokines are a very rapidly expanding family of small protein cytokines which are primarily involved in chemotaxis. IL8 attracts neutrophils and in addition activates them.

IL12 is produced by both macrophages and dendritic cells. It has a key role to play in regulating the sort of adaptive immune response produced.

Antigen presentation

T cells must see antigen in the form of peptides bound to self MHC molecules and class II MHC is specially adapted to present extra cellular antigens. One question is how the extra cellular antigens gain access to the class II loading compartment. Although it is possible for dendritic cells, activated macrophages or B cells to present any soluble protein in theory, in physiological situations the concentration

of antigen is rarely high enough for this inefficient method of antigen capture. Most real antigen presentation situations require the binding to a receptor which triggers endocytosis. This receptor can be for complement or the Fc of IgG or it can be an innate, carbohydrate receptor on macrophages/dendritic cells.

In normal situations the critical antigen presenting cell (APC) is the dendritic cell. The role of dendritic cells is to act as antigen sensors. Not only do they recognize antigen via their innate (carbohydrate or complement) receptors but these receptors can trigger activation (up regulation of antigen presenting and accessory functions) and migration from the peripheral tissue to the lymph node via the afferent lymphatics. Within the lymph node the antigen presenting DC can interact with the many T cells constantly trafficking through.

In addition to the MHC/peptide - TCR interaction there are other non-antigen specific membrane bound ligand-receptor pairs which are important for the dendritic cell -T cell interaction. The principal one is the association of the CD28 molecule on the T cell with either of 2 ligands B7.1 (CD80) and B7.2(CD86) molecule on the dendritic cell. These molecules are termed accessory molecules and the CD28 molecule delivers an essential second signal to the T cell without which the T cell does not become activated (indeed it becomes unresponsive).

The second essential cell-cell contact is between the activated T cell and an antigen-specific B cell. Most antigens are what we term T-dependent, that is the antibody response to them absolutely requires T cell help. This help is delivered by both cytokines and cell-cell contact. B cells bind specific antigen via their surface Ig, endocytose, process and present it on their class II MHC molecules. This enables them to be recognized by T cells specific for helper epitopes from their specific antigen. This cell-cell interaction also requires CD28 binding to B7 on the B cell. In addition, activation of the T cell has induced expression of a molecule called CD40 ligand or CD40L which binds to CD40 on B cells. Cross linking CD40 promotes B cell

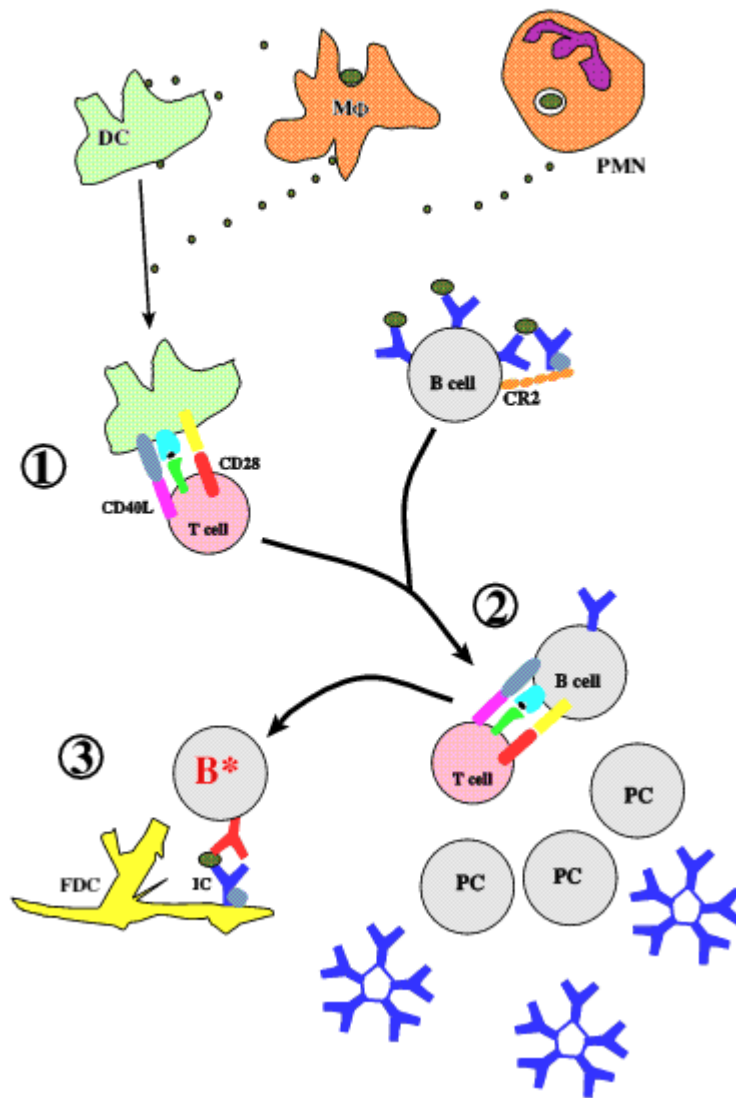
proliferation, prevents apoptosis of germinal-centre B cells and promotes immunoglobulin isotype switching.

Patients with hyper IgM syndrome have IgM producing B cells but do not form germinal centres in response to foreign antigen because the gene for CD40L is defective. Their B cells are normal since they produce IgG and IgE *in vitro* (in the test tube) in response to cross linking with mAbs specific for CD40 and addition of the appropriate lymphokines.

The CD28-B7 and CD40-CD40L receptor ligand interactions are both essential for the dialogue between B and T cells that result in their mutual activation.

The third cell-cell interaction essential in immune responses is the binding of activated B cells to follicular dendritic cells (FDC). FDC are specialised stromal cells which hold intact, i.e. unprocessed, antigen on their surface in the form of long-lived immune complexes. They are only present in B cell follicles and enable the activated B cell to form germinal centres. Among other molecules FDC express CD23 which binds to germinal centre B cells via their CR2 receptor and stimulates differentiation to plasma cells.

Chronology of an Antibody response



The picture above shows the sequence of events in developing an antibody response.

First, antigen is acquired by tissue dendritic cells. While in the peripheral tissues these cells are optimised for taking up samples of their environment, for example they actively endocytose via

carbohydrate receptors like mannose-fucose receptor and they are very active in fluid-phase pinocytosis. They are poor, however, at digesting whole microorganisms and it may be that they rely on macrophages and PMN to help them acquire bacterial or fungal proteins.

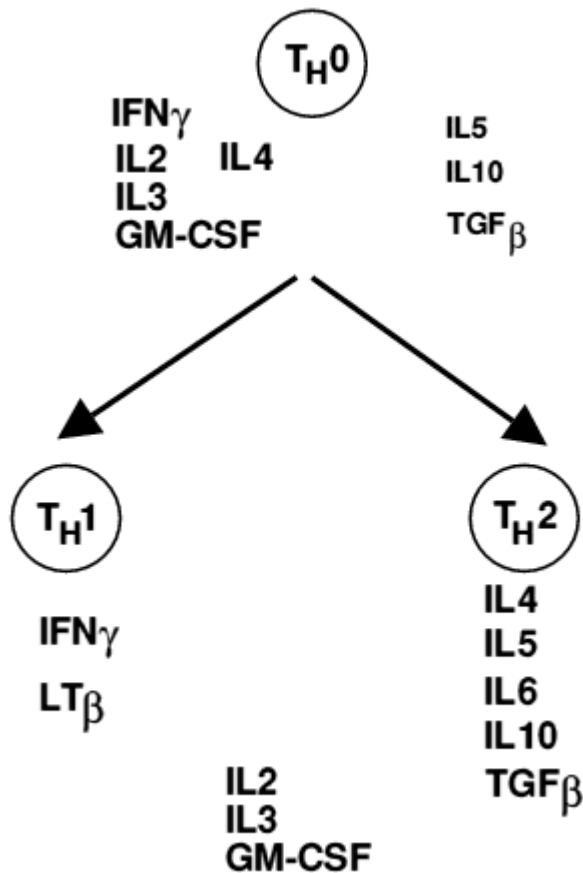
These tissue dendritic cells then differentiate so that their antigen uptake ability is reduced but their expression of B7.1 (CD80), CD40 and MHC (both classes) is markedly upregulated. They are now optimum antigen presenting cells. They encounter T cells, generally in lymph nodes, and activate those with specific receptors for the antigen-MHC complexes displayed on the dendritic cell surface.

Meanwhile, antigen specific B cells have acquired their relevant antigens by endocytosis of antigen bound via the surface Ig receptor. This antigen is processed (ie digested) and appropriate peptide fragment(s) represented in association with MHC class II molecules on the B cell surface. This process partially activates the B cell and one of the responses is to up regulate B7.2 (CD86) on the B cell. The whole process of antigen acquisition and B cell activation is much more efficient if the antigen has bound C3d due to the B cell CR2 receptor, which amplifies B cell signaling. The MHCII-peptide presenting B cell can now interact with the activated T cell and receive help via ligation of MHC, CD40 and B7.2 as well as cytokines such as IL4. The T-B interaction is obligatory for triggering B cell differentiation to induce class switching, somatic hyper-mutation and plasma cell formation.

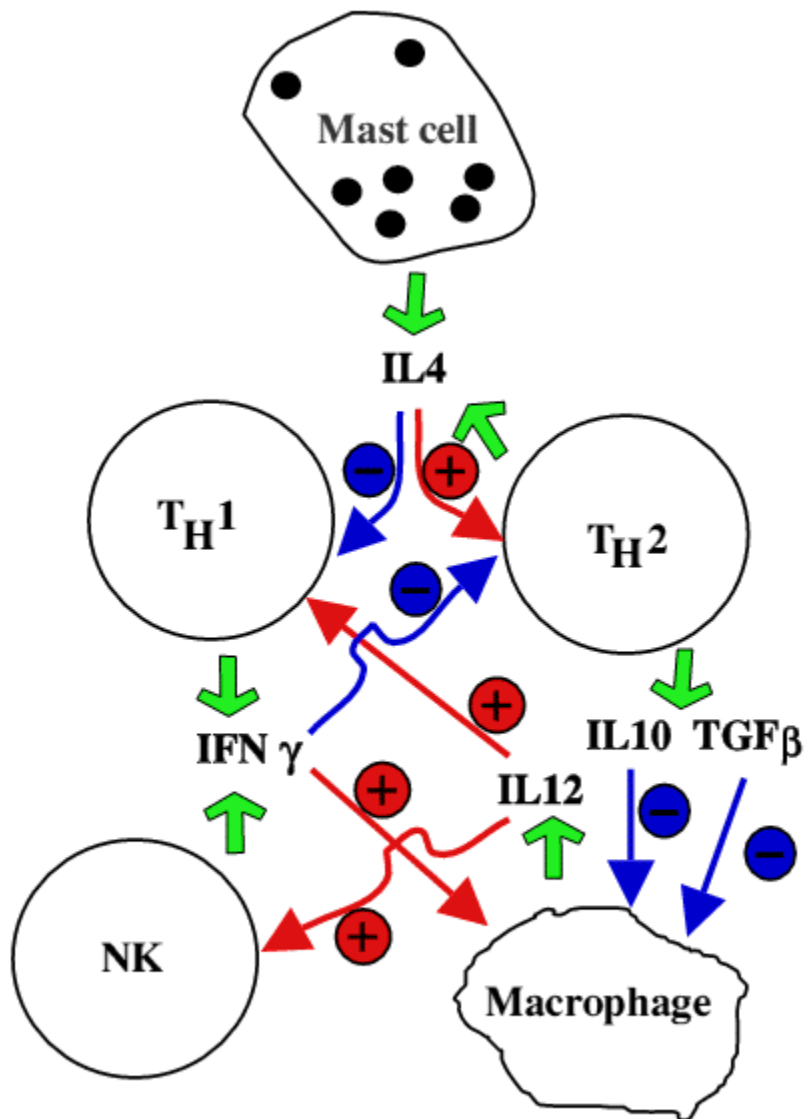
Finally activated B cells migrate into follicles to form germinal centres where class switching and somatic hyper-mutation take place and where Follicular Dendritic Cells display captured antigen-antibody-C3d complexes. The FDC bound antigen is very long lived and forms a depot of native antigen with which B cells can interact and become selected for plasma cell or memory cell differentiation. Factors which determine the pathway a B cell follows have yet to be determined.

CYTOKINES, T CELL SUBSETS AND Ig ISOTYPE REGULATION

A major theme of recent research in Immunology has been trying to understand how the immune system regulates the type of response to a given challenge. Different types of challenges require very different protective mechanisms to be activated, the wrong response can fail to protect the host against an organism or even cause damage to the host directly. Although our understanding of immune regulation is incomplete we now have an overview of the system. CD4⁺ helper T cells are capable of differentiating from an initial common state (T_H0) into 2 apparently distinct types called T_H1 and T_H2. These subtypes differ in their cytokine secretion as shown in the figure below.



The balance between T_H1 and T_H2 represents a sort of switch which can be used to bias immune response in one or other direction. The commitment of T_H0 cells to become T_H1 or T_H2 is influenced by cytokines secreted by the 2 subtypes themselves and by macrophages, NK cells and mast cells as shown in the following diagram.



The outcome of this differentiation switch is to activate 2 different pathways of immunity which are associated with different antibody isotypes.

The T_H1 pathway is essentially cell mediated immunity, with the activation of macrophages, NK cells, cytotoxic T cells and a prolonged inflammatory response. The cytokines secreted by T_H1 cells also boost production of IgG2a antibody production in mice.

The T_H2 pathway is essentially a humoral pathway, with the production cytokines which promote of B cell growth (like IL-4, IL-6) and production of IgG1 (IL-4), IgA (IL-5) and IgE (IL-4) in mice. It also stimulates effectors which use these antibody isotypes; eosinphils (via IL-5) and mast cells (IL-4).

Cytokines in Allergic Disease

The immune system is unique in that it can very selectively discriminate between self and non-self, leaving self alone while rapidly processing and destroying non-self (foreign) antigens in a primary immune response. In addition, a functioning immune system remembers previous encounters with these foreign antigens, resulting in a more vigorous and rapid secondary response (called immunologic memory). These responses depend on both T cells (dependent on the thymus gland for function) and, when specific antibody is made, B cells (which ultimately differentiate into antibody secreting plasma cells). These cells are present in bone marrow, lymphoid organs (i.e. thymus, lymph nodes, tonsils, spleen, etc) and peripheral blood. Many of these lymphoid cells look alike when viewed under a microscope. They can be categorized by the presence of specific cell surface markers called clusters of differentiation (CD). Each CD marker is given a specific number and is found on certain cell types. Thus the function of a cell can often be predicted by the specific CD marker present. For example, all mature human T cells have CD3 on their cell surface (thus they are CD3+). In contrast, human B cells are CD19+ but CD3-. CD3+ T cells can further be divided by function. T cells capable of killing target cells (called cytotoxic T cells- CTL) and/or downregulating immune responses (called suppressor T cells) are CD8+. T cells that help B cells make

antibody or other T cells (such as CTL) become active are called helper T cells (TH) and are CD4+.

While it has been appreciated for many years that TH cells existed, it was unclear how helper cells worked until recent studies showed that soluble peptides secreted by activated TH could turn on selective portions of the immune response. Initially, these glycoproteins produced by lymphocytes were called lymphokines. It is now known that many different cell types can produce these immune mediators. The current, more accurate term now used is cytokine.

Cytokines

All cytokines have certain properties in common. They are all small molecular weight peptides or glycopeptides. Many are produced by multiple cell types such as lymphocytes, monocytes/macrophages, mast cells, eosinophils, even endothelial cells lining blood vessels. Each individual cytokine can have multiple functions depending upon the cell that produces it and the target cell(s) upon which it acts (called pleiotropism). Also, several different cytokines can have the same biologic function (called redundancy). Cytokines can exert their effect through the bloodstream on distant target cells (endocrine), on target cells adjacent to those that produce them (paracrine) or on the same cell that produces the cytokine (autocrine). Physiologically it appears that most cytokines exert their most important effects in a paracrine and/or autocrine fashion. Their major functions appear to involve host defense or maintenance and repair of the blood elements.

Cytokines are categorized by their major specific function(s). There are four major categories of cytokines (Table 2). Interferons are so named because they interfere with virus replication. There are three major types based upon the source of the interferon. Interferon alpha (IFN α) is produced by the buffy coat layer from

white blood cells and is used in treatment of a variety of malignant and immune disorders. Interferon beta (IFN β) is produced by fibroblasts and is currently being evaluated in the treatment of multiple sclerosis. Interferon gamma (IFN γ) is produced by activated T cells and is an important immuno-regulatory molecule, particularly in allergic diseases.

The colony stimulating factors are so named because they support the growth and differentiation of various elements of the bone marrow. Many are named by the specific element they support such as granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF). Other CSFs include Interleukin (IL) -3, which can stimulate a variety of hematopoietic precursors and is being evaluated as a therapy in aplastic anemia and bone marrow transplantation; and c-Kit ligand (stem cell factor) which has recently been demonstrated as a cytokine necessary to cause the differentiation of bone marrow stem cells into their various precursor elements for eventual differentiation into RBC, WBC and megakaryocytes (platelets).

The tumor necrosis factors (TNF) are so called because injecting them into animals causes a hemorrhagic necrosis of their tumors. TNF α is produced by activated macrophages and TNF β is produced by activated T cells (both TH and CTL). These molecules appear to be involved in the pathogenesis of septic shock and much research is aimed at trying to inhibit their activity in septic patients. Attempts have also been made to use the TNFs clinically to treat human tumors. Because of their extremely narrow therapeutic window (efficacy vs toxicity), few view this as a useful stand-alone cancer therapy.

The largest group is the interleukins, so named because their fundamental function appears to be communication between (inter-) various populations of white blood cells (leucocytes - leukin). Interleukins (IL) are given numbers. They are produced

by a variety of cell types such as monocytes/macrophages, T cells, B cells and even non-leucocytes. The major interleukins currently of greatest interest to allergists are IL-4, IL-5, IL-10 and IFN γ . IL-4 causes a switch to IgE production by differentiating B cells. IFN γ can inhibit that switch and prevent the production of specific IgE. IL-10 can actually inhibit the activity of IFN γ , allowing the original IL-4 to proceed in the IgE cascade. Thus, an allergic response can be viewed as an allergen-specific production of excess IL-4 and/or IL-10, lack of adequate IFN γ production or both. Eosinophilic inflammation, a major component of allergic reactions, is under control of IL-5 and TNF α

The Allergic Response

There are three fundamental components of allergic reactions:

1. formation of allergen-specific IgE ;
2. activity of mast cells caused by allergen re-exposure, which cross links IgE on the surface of mast cells, activating them to cause the signs and symptoms of an immediate hypersensitivity reaction; and
3. allergic inflammation, mediated primarily by recruitment and activation of eosinophils.

Each of these events involve cellular recruitment to the reaction site (called chemotaxis) along with activation of these cells to produce their products and altered cellular traffic to gather the cells together in an optimal fashion to promote the allergic reaction. Remember, the host mistakenly believes this is a protective reaction. A group of proteins called adhesion molecules can be stimulated on both inflammatory cell surfaces as well as target cells (i.e. endothelial lining of blood vessels, lung tissue, etc.). These molecules function to "localize" the inflammatory reaction at the site of tissue injury and/or antigen deposition. In addition, certain adhesion molecules likely have a role in inflammatory cell activation, further enhancing allergic

inflammation.

IgE is one of five isotypes of antibody formed in humans. The cell responsible (B cell) starts with an IgM molecule on its surface that is specific for the antigen (or in the case on an allergic response, an allergen). T helper (TH2) cells assist B cells in making antibody by producing cytokines. One particular cytokine (IL-4) is responsible for causing the isotype switch from IgM to IgE. Although necessary, IL-4 is not in and of itself sufficient to cause a switch to IgE. A second signal, which can come from a variety of sources, is needed to complete the switch. Once formed, IgE seeks to bind to either the inciting allergen or to IgE receptors located on a variety of cell types. Mast cells have high affinity IgE receptors.

Of note, other cytokines are active in the regulation of IgE production. IFN γ , produced by TH1 helper cells, can antagonize the ability of IL-4 to induce IgE production. Recent studies have shown that T cells from non-atopic patients, when stimulated *in vitro* by specific allergen, produces primarily IFN γ while T cells from atopic patients produce allergen-induced IL-4. Further, TH2 cells can produce IL-10, which can inhibit the production of cytokines such as IFN γ . Thus IgE could be the prevalent antibody if TH2 rather than TH1 helper cells are stimulated in an atopic individual.

Once allergen-specific IgE is generated and bound to mast cells, subsequent allergen exposure causes cross linking of mast cell-bound IgE resulting in deregulation. This process takes only a matter of minutes and releases a variety of mediators, including histamine. Histamine binds to target receptors in the nose, lung, skin, gastrointestinal tract and near blood vessels via specific histamine receptors, especially H1 receptors. This activates a series of events leading to increased vascular permeability and dilation, stimulation of nerve fibers and initiation of inflammatory cascades that are collectively responsible for the signs and symptoms of

immediate hypersensitivity - itching, sneezing, increased mucus secretion (i.e. rhinorrhea, etc.), bronchospasm and, if enough vascular tissue is involved, hypotension.

Mast cells themselves both respond to and produce cytokines. IL-3 is a mast cell growth factor. Mast cells make IL-4 when stimulated. This may be particularly important in the propagation of IgE-producing B cells as well as the differentiation of T helper cells to the TH2 pathway (both necessary for IgE production). In addition, IL-4 appears to be a secondary but important growth factor for mast cells. Mast cells also make and secrete TNF α . This cytokine has important inflammatory properties that are consistent with the known pro-inflammatory activities of mast cells.

Eosinophils both respond to and manufacture certain cytokines. IL-5 appears to be a major growth factor for eosinophils. IL-5 is also produced by TH2 cells, further supporting the developing allergic cascade. Eosinophils can secrete many cytokines such as IL-3, GM-CSF, TNF α and IL-1 when activated. Any or all of these cytokines serve to enhance and sustain the allergic inflammatory process by mast cell activation (IL-3), further eosinophil recruitment (TNF α), altering the target tissue (IL-1) and even direct tissue damage. The activated eosinophils also produce and secrete multiple basic proteins and lipid mediators associated with allergic inflammation.

Inflammation has three major components: recruitment, where the inflammatory cells are drawn from the circulation under direct chemical influence called chemotaxis; altered traffic, where the inflammatory cells are held at the site of developing inflammation; and activation, where the inflammatory cells exert their influence, e.g. producing cytokines, lytic enzymes, phagocytosis, etc. In allergic inflammation, a combination of TH2 cell and mast cell activity appear to be most responsible for the initiation of the

eosinophilic activities.

A major source of chemotactic molecules is the activated mast cell. TNF α is a major pro-inflammatory cytokine whose activities include chemotaxis. Activated mast cells secrete TNF α and therefore may directly influence recruitment of eosinophils. Once activated, eosinophils are themselves a source of secreted TNF α which may serve to continue the recruitment of new eosinophils to the site of inflammation.

Altered traffic also involves changing how the inflammatory cells migrate through tissue. Most leucocytes migrate into tissue from the circulation. This process involves sticking to the endothelial lining of the blood vessel and movement (called diapedesis) between adjacent cells of the blood capillary endothelium to the site of developing inflammation in the tissue. Fundamental to this process is the expression of adhesion molecules on both the leucocytes and endothelial target tissue. These adhesion molecules are necessary to keep the inflammatory cell at the target tissue site as well as, in certain cases, participate in the cellular activation process.

Cytokines play a fundamental role in adhesion molecule expression. IL-1 can act on the endothelial cell to increase the expression of several adhesion molecules such as ELAM-1 (endothelial leucocyte adhesion molecule), ICAM-1 (intercellular adhesion molecule) and VCAM-1 (vascular cell adhesion molecule). The specific biochemical properties of these molecules is a subject for future discussion. It appears that VCAM-1 expression may be most important in allergic (eosinophilic) inflammation. Another adhesion molecule of special importance in allergic reactions is VLA-4. This molecule is expressed on activated lymphocytes, mast cells and eosinophils. Thus, expression of VCAM-1 on endothelium (of, say, the nose or lung) and VLA-4 on activated mast cells and eosinophils are necessary steps for eosinophilic infiltration of these organs in allergic late phase reactions. This has become particularly significant with the discovery

that IL-4 induces the expression of both VCAM-1 on endothelial surfaces and VLA-4 on eosinophils. TNF α further enhances IL-4-induced VCAM-1 expression.

Thus, the cytokine influences on allergic inflammatory reactions can be summarized into three major components:

1. Induction of allergen-specific IgE
 - IL-4 up regulates IgE production ,
 - Gamma IFN down regulates IgE production,
 - IL-10 inhibits the production/activity of gamma IFN
2. Mast cell activation
 - IL-3, IL-4 - mast cell growth factors,
 - TNF alpha – pro-inflammatory, chemotactic,
 - IL-4
 - Th2 differentiation,
 - VCAM-1, VLA-4 induction
3. Eosinophil inflammation -
 - IL-5 - eosinophilic growth factor,
 - IL-3 - supports mast cell growth,
 - GM-CSF – pro-inflammatory effects

It follows that excess, allergen-specific TH2 activity could produce activation of each component of the allergic cascade. It is reasonable to hypothesize that allergic disease is characterized by an imbalance between allergen- specific TH1 and TH2 activities.

Therapeutic Potentials of Cytokines in Allergic Diseases

This knowledge provides possibilities of new targets for therapeutic activity. Many therapeutic modalities have been examined for their effects on these TH subpopulations . Several have been demonstrated to have significant activities on this arm of the immune response. Currently, the most extensively used

anti-inflammatory agents, in various topical and systemic formulations, are the corticosteroids. While steroids have direct activities on inflammatory cells such as mast cells and eosinophils, they also exert a regulatory effect on cytokine production.

Recall that the immune reaction that leads to IgE production involves antigen presenting cells (APC- usually macrophages), T cells and B cells. The APC produces IL-1, TNF α and IL-6, all pro-inflammatory cytokines. In addition, IL-1 activates helper T cells to produce IL-2 (and other cytokines) which activates the immune cascade. Corticosteroids have a fundamental effect on IL-1 secretion and, thus, the ultimate production of IL-2 and resulting T cell activity. Further, mast cells produce their own cytokines including pro-inflammatories such as TNF α . These are also down regulated in the presence of corticosteroids

Perhaps most exciting is recent data from several investigators that address the effects of allergen immunotherapy (AIT) on cytokine production. What has been appreciated for many years is the positive clinical effects of AIT on symptoms of allergic diseases such as rhinitis and asthma. Until recently, the mechanisms were largely unknown. Now we are beginning to understand that the process of AIT can selectively change allergen-specific cytokine profiles. That is, when an atopic patient's peripheral blood lymphocytes (PBL) are stimulated *in vitro* with allergen, they produce mostly IL-4. In contrast, PBL from patients who were highly atopic but treated with AIT did not produce IL-4 but rather produced large amounts of IFN γ . In other words, it appears that successful AIT is characterized by a switch from allergen-specific TH2 to TH1 expression. The effect appears to be durable and related to length of AIT. It correlates reasonably well with symptom improvement.

The fact that successful AIT takes several years to achieve and the still controversial positions about duration of therapy suggests that we are not yet at the definitive stage for allergy therapy that we

desire. This raises the possibility of new therapeutic modalities that can achieve clinical control of allergic inflammation with the rapidity of corticosteroids and specificity of AIT. Such modalities could include cytokines themselves or pharmacologic agents that can modulate specific cytokine profiles.

Recombinant cytokines are being studied in a variety of clinical conditions such as malignant, infectious, autoimmune and allergic/asthmatic diseases. What is apparent is the limited use of these recombinant cytokines for three reasons: (a) they are extremely expensive, something not viewed kindly in the new age of managed health care; (b) they are extremely toxic, causing various systemic symptoms such as fever, chills, and muscle aches and fatigue. In higher doses they are potentially life threatening by their effects on causing hypotension; and (c) since these immune-based diseases (particularly allergic/asthmatic) are most likely an imbalance of "good" and "bad" cytokines, giving large pharmacologic doses of recombinant cytokines may create other imbalances in the host that could create other disease entities.

With those concerns, several studies have provided promising results in diseases such as atopic dermatitis (AD). IFN γ has been shown to clinically improve children with severe AD and can be steroid-sparing. It is still extremely expensive and can make the children ill. IFN γ is also being evaluated in topical form in asthma.

Finally, there are new immuno modulators being studied that can selectively activate cytokine production in a positive way at the inflammatory site. While not of themselves antigen-specific, they tend to be more effective in networks that are already antigen (or allergen)-activated. This may in part explain the long known observation that, in certain patients, treatment with AIT that does not involve a specific allergen (i.e. animal dander) provides relief of documented symptoms after successful therapy for pollens. Micro environmental production of IFN γ to pollens may act on

the animal dander-specific TH2 clone to down regulate its activity. Such a hypothesis remains to be verified.

The understanding of the allergen-specific TH1/TH2 functional ratio and its importance in the pathophysiology of allergic diseases may also have utility in clinical monitoring situations. It is provocative to consider clinically useful laboratory assays that could establish the TH2 nature of a specific response and then monitor response to therapy such as AIT by watching for the change to a normal balance. This could also be useful in evaluating therapeutic agents to determine dose and duration of therapy. Such concepts are currently being investigated as research related to allergic inflammation and cytokines continues to move steadily from bench to bedside.

Cytokines

The immune system has many different types of cells acting together to take care of unwanted infections and altered cells. Cytokines are the chemicals produced by these cells in order to communicate and orchestrate the attack. Just as hormones in the endocrine system can produce an effect on other cells, so cytokines can act on other immune cells, especially cells that are close by.

Cytokines have several important characteristics:

- the same cytokine may be made by a number of different cells.
- the same cytokine may have different effects in different circumstances. (This is called ' pleotropy ')
- Different cytokines may have the same activity depending on the situation 'redundancy'.
- Cytokines often act together and increase the effects of one another ' synergy '. They may also act as antagonists.
- most cytokines have either paracrine or autocrine

effects. Paracrine means they act on cells near to them or that they are actually touching. The autocrine function of IL-2 is well known because, when a T cell is stimulated to make IL-2, it stimulates itself via the IL-2 receptor to proliferate. An example of an uncommon endocrine function for cytokines is IL-1 which can cause fever by stimulating the hypothalamus.

Originally, the cytokines were named according to their function (like T cell growth factor, now called IL-2) but then the pleiotropy of cytokines was observed, making function specific names confusing. After more and more cytokines were identified, and in order to avoid confusion, immunologists started naming some of the cytokines 'interleukins' (or IL for short) and numbering them as they were found. The first interleukin identified therefore was IL-1 and the most recent one is IL-16.

Some cytokines are more important than others in basic immunology. Take a look at the following table, only the cytokines important for this course have links to pages with more detail. The functions of cytokines are best studied within the context of an actual immune response and the other books in this manual will describe this in more detail.

Table of cytokines

Cytokine Principle Source Principle activities

<u>IL-1</u>	Macrophage	T,B cell activation; fever; inflammation
<u>IL-2</u>	T cells	T cell proliferation

<u>IL-4</u>	T cells	B cell growth and differentiation
<u>IL-5</u>	T cells	B cell, eosinophil growth
<u>IL-6</u>	macrophages, T cells	B cell stimulation, inflammation
IL-7	stromal cells	Early B and T cell differentiation
<u>IL-8</u>	macrophages	Neutrophil (PMN)attraction
IL-9	T cells	mitogen
<u>IL-10</u>	T cells	Inhibits Th1 cytokine production
IL-11	Bone marrow stroma	Hematopoiesis
<u>IL-12</u>	APC	Stimulates T, NK cells
IL-13	T cells	Similar to IL-4
IL-14	dendritic cells, T cells	B cell memory
IL-15	T cells	same as IL-2
IL-16	-	-
IFNa	Most cells	Anti-viral
IFNb	Most cells	Anti-viral
<u>IFNg</u>	T, NK cells	inflammation, activates macrophages
<u>TGFb</u>	macrophages, lymphocytes	depends on target
TNFa	Macrophage	Inflammation; tumor killing
TNFb	T cells	Inflammation; tumor killing;

