

Inflammation

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Inflammation

- Inflammation is the response of living tissue to damage. The acute inflammatory response has 3 main functions.
- The affected area is occupied by a transient material called the acute inflammatory exudate. The exudate carries proteins, fluid and cells from local blood vessels into the damaged area to mediate local defenses.
- If an infective causative agent (e.g. bacteria) is present in the damaged area, it can be destroyed and eliminated by components of the exudate.
- The damaged tissue can be broken down and partially liquefied, and the debris removed from the site of damage.

Etiology

- The cause of acute inflammation may be due to physical damage, chemical substances, micro-organisms or other agents. The inflammatory response consist of changes in blood flow, increased permeability of blood vessels and escape of cells from the blood into the tissues. The changes are essentially the same whatever the cause and wherever the site.
- Acute inflammation is short-lasting, lasting only a few days.

Inflammation

- In all these situations, the inflammatory stimulus will be met by a series of changes in the human body; it will induce production of certain cytokines and hormones which in turn will regulate haematopoiesis, protein synthesis and metabolism.
- Most inflammatory stimuli are controlled by a normal immune system. The human immune system is divided into two parts which constantly and closely collaborate - the innate and the adaptive immune system.

Inflammation

- **The innate system** reacts promptly without specificity and memory. Phagocytic cells are important contributors in innate reactivity together with enzymes, complement activation and acute phase proteins. When phagocytic cells are activated, the synthesis of different cytokines is triggered. These cytokines are not only important in regulation of the innate reaction, but also for induction of the adaptive immune system. There, specificity and memory are the two main characteristics.
- In order to induce a strong **adaptive immune response**, some lymphocytes must have been educated to recognise the specific antigen on the antigen-presenting cell (APC) in context of self major histocompatibility molecules. The initial recognition will mediate a cellular immune reaction, production of antigen-specific antibodies or a combination of both. Some of the cells which have been educated to recognise a specific antigen will survive for a long time with the memory of the specific antigen intact, rendering the host "immune" to the antigen.

Systemic manifestation of inflammation

- 1. Increase of body temperature (fever)
- 2. Acute phase reaction

Systemic effects of acute inflammation

- **Pyrexia**
- Polymorphs and macrophages produce compounds known as endogenous pyrogens which act on the hypothalamus to set the thermoregulatory mechanisms at a higher temperature. Release of endogenous pyrogen is stimulated by phagocytosis, endotoxins and immune complexes.
- **Constitutional symptoms**
- Constitutional symptoms include malaise, anorexia and nausea. Weight loss is common when there is extensive chronic inflammation. For this reason, tuberculosis used to be called 'consumption'.
- **Reactive hyperplasia of the reticulo-endothelial system**
- Local or systemic lymph node enlargement commonly accompanies inflammation, while splenomegaly is found in certain specific infections (e.g. malaria, infectious mononucleosis).

Systemic effects of acute inflammation

- **Haematological changes**
- **Increased erythrocyte sedimentation rate.** An increased erythrocyte sedimentation rate is a non-specific finding in many types of inflammation.
- **Leukocytosis.** Neutrophilia occurs in pyogenic infections and tissue destruction; eosinophilia in allergic disorders and parasitic infection; lymphocytosis in chronic infection (e.g. tuberculosis), many viral infections and in whooping cough; and monocytosis occurs in infectious mononucleosis and certain bacterial infections (e.g. tuberculosis, typhoid). **Anaemia.** This may result from blood-loss in the inflammatory exudate (e.g. in ulcerative colitis), haemolysis (due to bacterial toxins), and 'the anaemia of chronic disorders' due to toxic depression of the bone marrow.
- **Amyloidosis**
- Longstanding chronic inflammation (for example, in rheumatoid arthritis, tuberculosis and bronchiectasis), by elevating serum amyloid A protein (SAA), may cause amyloid to be deposited in various tissues resulting in secondary (reactive) amyloidosis

Macroscopic appearance of acute inflammation

- The cardinal signs of acute inflammation are modified according to the tissue involved and the type of agent provoking the inflammation. Several descriptive terms are used for the appearances.
- **Serous inflammation.**
- **Catarrhal inflammation**
- **Fibrinous inflammation**
- **Haemorrhagic inflammation**
- **Suppurative (purulent) inflammation**
- **Membranous inflammation**
- **Pseudomembranous inflammation**
- **Necrotising (gangrenous) inflammation.**

Acute inflammation

- can be caused by microbial agents such as viruses, bacteria, fungi and parasites
- by non-infectious inflammatory stimuli, as in rheumatoid arthritis and graft-versus-host disease
- by tissue necrosis as in cancer
- by burns and toxic influences caused by drugs or radiation

Early Stages of Acute Inflammation

The acute inflammatory response involves three processes:

- changes in vessel calibre and, consequently, flow
- increased vascular permeability and formation of the fluid exudate
- formation of the cellular exudate by emigration of the neutrophil polymorphs into the extravascular space.

Early Stages of Acute Inflammation

The steps involved in the acute inflammatory response are:

- Small blood vessels adjacent to the area of tissue damage initially become **dilated** with increased blood flow, then flow along them slows down.
- Endothelial cells **swell** and partially **retract** so that they no longer form a completely intact internal lining.
- The vessels become **leaky**, permitting the passage of water, salts, and some small proteins from the plasma into the damaged area (**exudation**). One of the main proteins to leak out is the small soluble molecule, fibrinogen.
- Circulating neutrophil polymorphs initially adhere to the swollen endothelial cells (**margination**), then actively migrate through the vessel basement membrane (**emigration**), passing into the area of tissue damage.
- Later, small numbers of blood monocytes (macrophages) migrate in a similar way, as do lymphocytes.

The acute phase reaction

- In the acute phase reaction, several biochemical, *metabolic, hormonal and cellular changes* take place in order to fight the stimulus and re-establish a normal functional state in the body.
- An **increase in the number of granulocytes** will *increase the phagocytotic capacity, an increase in scavengers* will potentiate the capability to neutralise free oxygen radicals, and *an increase in metabolic rate* will increase the energy available for cellular activities, despite a reduced food intake.
- Some of these changes can explain the symptoms of an acute phase reaction, which are typically fever, tiredness, loss of appetite and general sickness, in addition to local symptoms from the inducer of the acute phase.

General and local clinical symptoms of the acute phase reaction

General symptoms	Local symptoms
fever	calor
increased heart rate	rubor
hyperventilation	dolor
tiredness	tumor
loss of appetite	functio laesa

Biochemistry and physiology of the acute phase reaction

- The acute phase reaction is the body's first-line inflammatory defence system, functioning without specificity and memory, and in front of, and in parallel with, the adaptive immune system. CRP is a major acute phase protein acting mainly through Ca²⁺-dependent binding to, and clearance of, different target molecules in microbes, cell debris and cell nuclear material.
- In an acute phase reaction there may be a more than 1000-fold increase in the serum concentration of CRP. CRP is regarded as an important member of the family of acute phase proteins, having evolved almost unchanged from primitive to advanced species.

Changes compared with normal state	Increase	Decrease
Cellular	phagocytotic cells (in circulation and at the site of inflammation)	erythrocytes
Metabolic	acute phase proteins serum Cu protein catabolism gluconeogenesis	serum Fe serum Zn albumin synthesis transferrin
Endocrinological	glucagon insulin ACTH GH T4 cortisol aldosterone vasopressin	T3 TSH

The acute phase proteins

- Induction of the acute phase reaction means changes in the synthesis of many proteins which can be measured in plasma.
- Regulation of protein synthesis takes place at the level of both transcription (DNA, RNA) and translation to protein.
- The cells have intricate systems for up- and down-regulation of protein synthesis, initiated by a complex system of signals induced in the acute phase reaction.

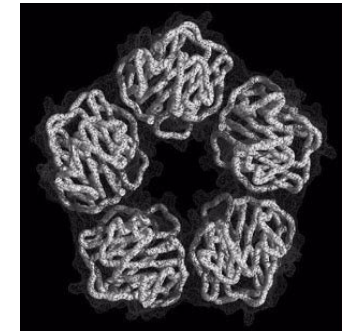
The acute phase proteins

Most of the proteins with increased serum concentrations have functions which are easily related to limiting the negative effects of the acute phase stimulus or to the repair of inflammatory induced damage. Examples are enzyme inhibitors limiting the negative effect of enzymes released from neutrophils, scavengers of free oxygen radicals, increase in some transport proteins and increased synthesis and activity of the cascade proteins such as coagulation and complement factors. The synthesis may be upregulated even if plasma levels are normal, due to increased consumption of acute phase proteins.

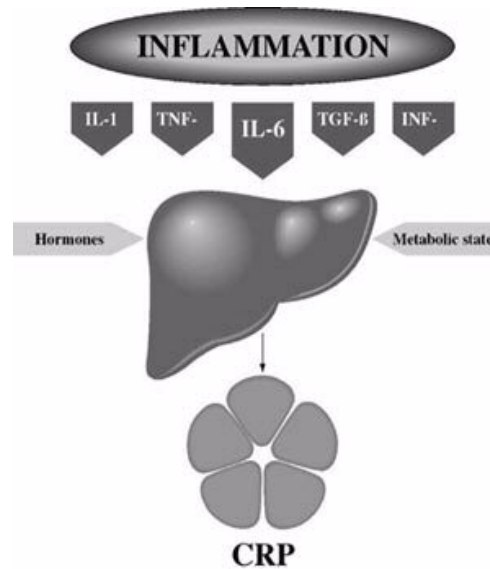
Function	Acute phase protein	Increase up to
Protease inhibitors	" ₁ -antitrypsin "α ₁ -antichymotrypsin	4 fold 6 fold
Coagulation proteins	fibrinogen prothrombin factor VIII plasminogen	8 fold
Complement factors	C1s C2b C3, C4, C5 C9 C5b	2 fold
Transport proteins	haptoglobin haemopexin ferritin	8 fold 2 fold 4 fold
Scavenger proteins	ceruloplasmin	4 fold
Miscellaneous	" ₁ -acid glycoprotein (orosomucoid) serum amyloid A protein C-reactive protein	4 fold 1000 fold 1000

C-reactive protein-structure and function

- CRP is a cyclic pentamer composed of five non-covalently bound, identical 23.5 kDa subunits.
- The main function of this pentamer is related to the ability to bind biologically significant ligands in vivo.
- CRP is found in primitive species like the horse-shoe crab, and evolutionary changes in higher vertebrates like man. This may indicate that CRP has an important function in the host defence system.



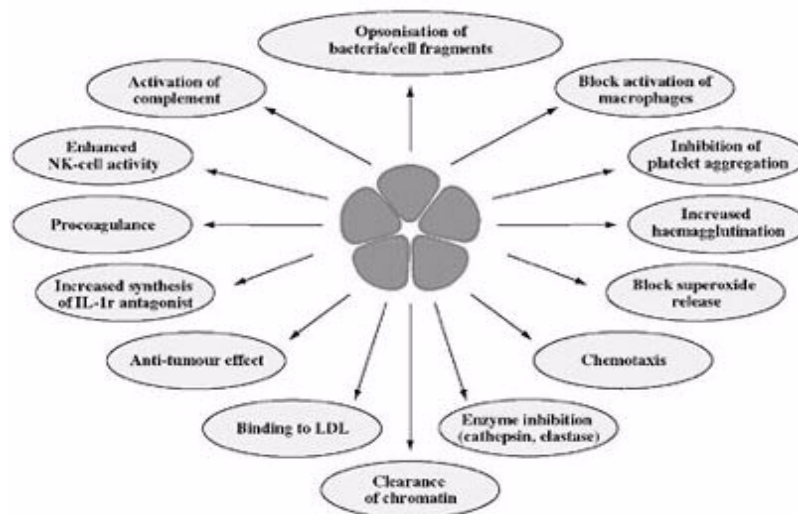
Induction and synthesis of CRP in hepatocytes.



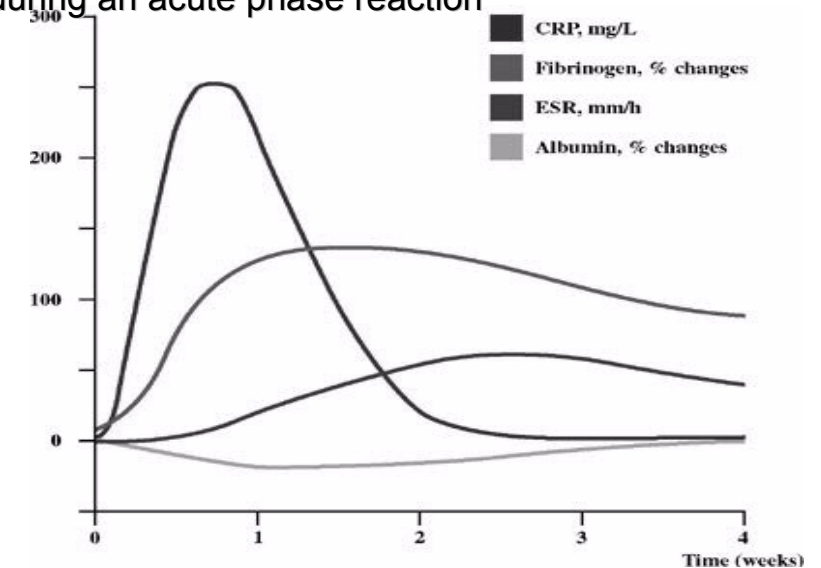
CRP functions

- Most functions of CRP are easily understood in the context of the body's defences against infective agents. The bacteria are **opsonised** by CRP and **increased phagocytosis** is induced. CRP **activates complement** with the split product being **chemotactic**, increasing the number of phagocytes at the site of infection. Enzyme inhibitors protect surrounding tissue from the damage of enzymes released from the phagocytes. CRP **binds to chromatin from dead cells and to cell debris** which are cleared from the circulation by phagocytosis, either directly or by binding to Fc, C3b - or CRP-specific receptors. **Platelet aggregation is inhibited**, decreasing the possibility of thrombosis. CRP **binds to low density lipoprotein (LDL)** and may clear LDL from the site of atherosclerotic plaques by binding to cell surface receptors on phagocytic cells.

Documented and proposed CRP functions.



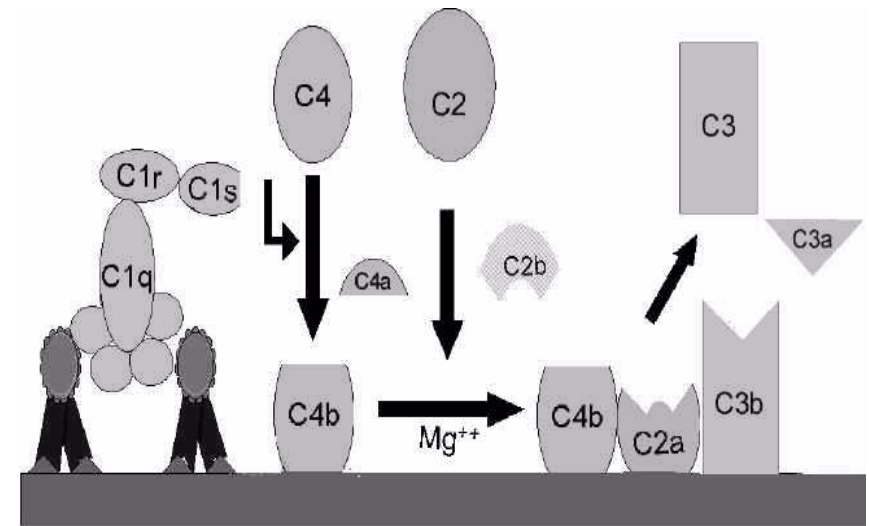
Typical changes of CRP, fibrinogen, ESR and albumin during an acute phase reaction



Classical pathway of complement activation

- normally requires a suitable Ab bound to antigen (Ag), complement components 1, 4, 2 and 3 and Ca^{++} and Mg^{++} cations.
- C1 activation**
Binding of C1qrs (a calcium-dependent complex), present in normal serum, to Ag-Ab complexes results in autocatalysis of C1r. The altered C1r cleaves C1s and this cleaved C1s becomes an enzyme (C4-C2 convertase) capable of cleaving both C4 and C2.
- C4 and C2 activation (generation of C3 convertase)**
Activated C1s enzymatically cleaves C4 into C4a and C4b. C4b binds to the Ag-bearing particle or cell membrane while C4a remains a biologically active peptide at the reaction site. C4b binds C2 which becomes susceptible to C1s and is cleaved into C2a and C2b. C2a remains complexed with C4b whereas C2b is released in the micro environment. C4b2a complex, is known as C3 convertase in which C2a is the enzymatic moiety.
- C3 activation (generation of C5 convertase)**
C3 convertase, in the presence of Mg^{++} , cleaves C3 into C3a and C3b. C3b binds to the membrane to form C4b2a3b complex whereas C3a remains in the micro environment. C4b2a3b complex functions as C5 convertase which cleaves C5 into C5a and C5b. Generation of C5 convertase marks the end of the classical pathway.

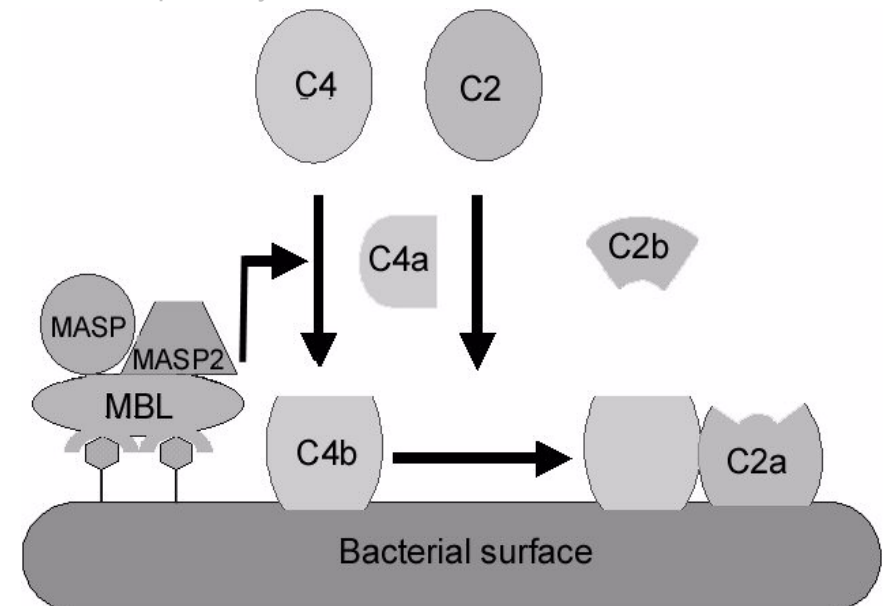
Classical pathway activation



Lectin pathway activation

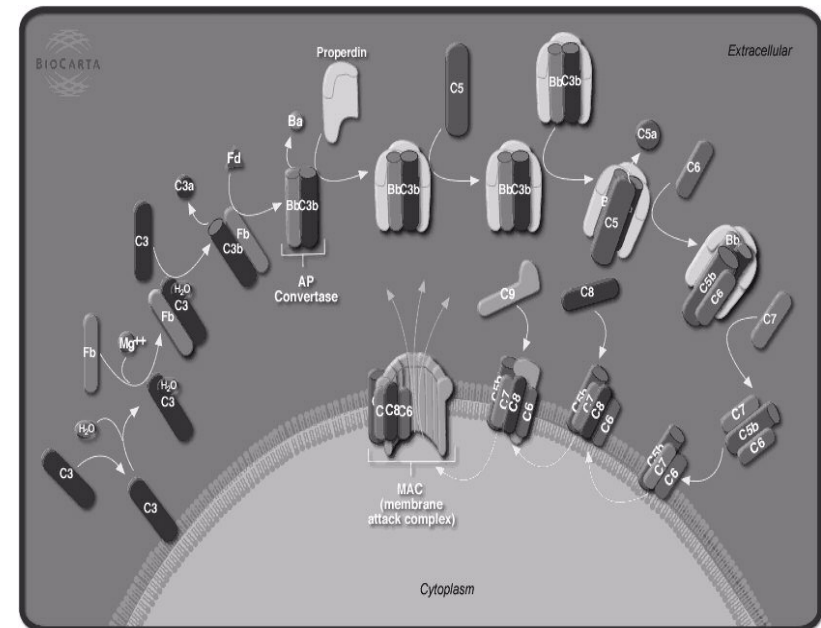
- C4 activation can be achieved without antibody and C1 participation by the lectin pathway. This pathway is initiated by three proteins: a mannan-binding lectin (MBL), also known as mannan-binding protein (MBP) which interacts with two mannan-binding lectin-associated serine proteases (MASP and MASP2), analogous to C1r and C1s. This interaction generates a complex analogous to C1qrs and leads to antibody-independent activation of the classical pathway.

Lectin pathway activation



Alternative pathway activation

Alternative pathway begins with the activation of C3 and requires Factors B and D and Mg^{++} cation, all present in normal serum. The alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents

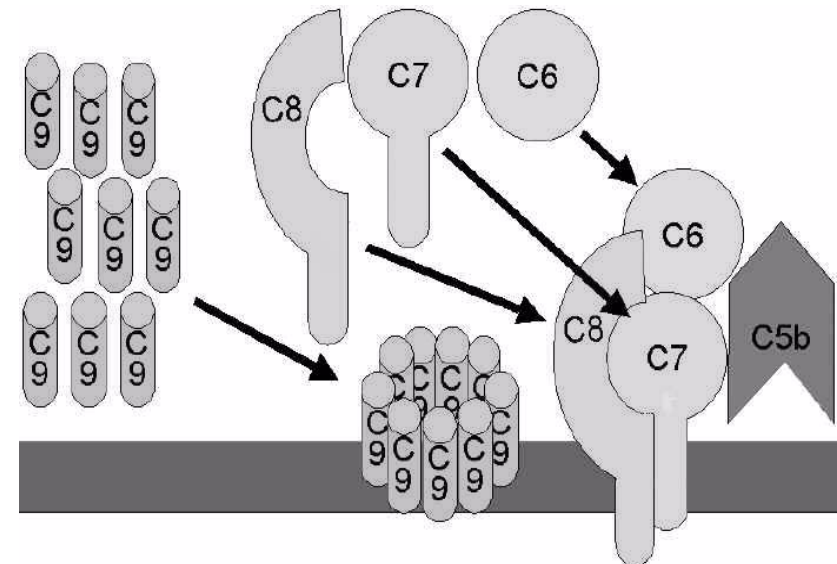


Alternative pathway of complement activation

Lytic pathway

- The lytic (membrane attack) pathway involves the C5-9 components. C5 convertase generated by the classical or alternative pathway cleaves C5 into C5a and C5b. C5b binds C6 and subsequently C7 to yield a hydrophobic C5b67 complex which attaches quickly to the plasma membrane. Subsequently, C8 binds to this complex and causes the insertion of several C9 molecules. bind to this complex and lead to formation of a hole in the membrane resulting in cell lysis. The lysis of target cell by C5b6789 complex is nonenzymatic and is believed to be due to a physical change in the plasma membrane. C5b67 can bind indiscriminately to any cell membrane leading to cell lysis. Such an indiscriminate damage to by-standing cells is prevented by protein S (vitronectin) which binds to C5b67 complex and blocks its indiscriminate binding to cells other than the primary target

The lytic pathway



Biologically active products of complement activation

- **Chemotactic factors**
C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells.
- **Opsonins**
C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis.
- **Other biologically active products of C activation**
Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their function.

Biologically active products of complement activation

- Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.
- **Kinin production**
C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin.
- **Anaphylotoxins**
C4a, C3a and C5a (in increasing order of activity) are all anaphylotoxins which cause basophil/mast cell degranulation and smooth muscle contraction.

Chemotaxis

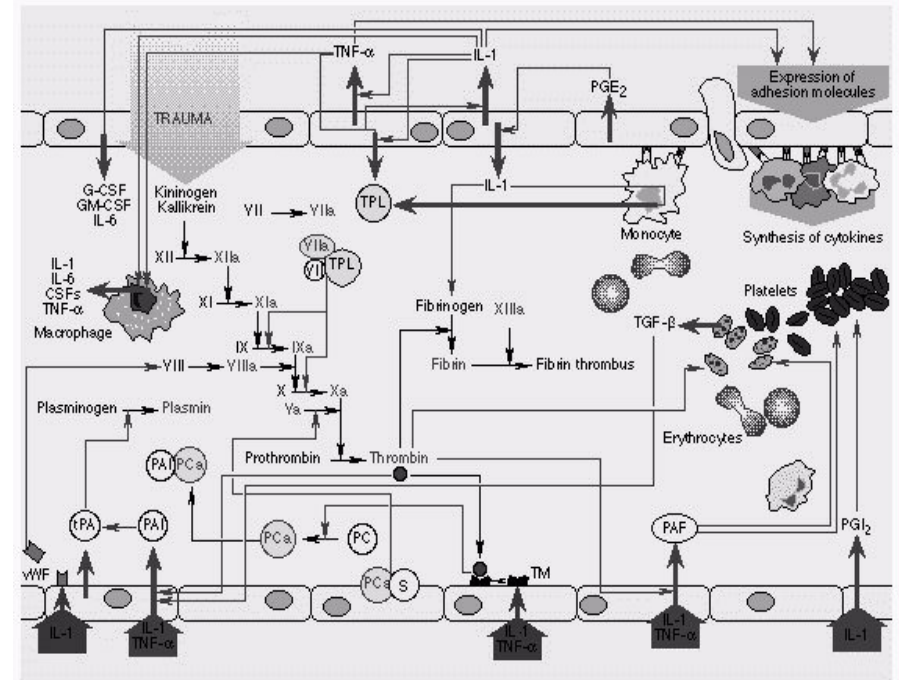
- is directed movement of cells in concentration gradient of soluble extracellular components.
- Chemotaxis factors, **chemotaxins** or **chemoattractants**
- **Positive chemotaxis** = cells move to the places with higher concentrations of chemotactic factors.
- **Negative chemotaxis** = cells move from the places with higher concentrations of chemotactic factors
- **Chemoinvasion** = cells move through basal membrane

Cytokines

- The term **cytokine** is used as a generic name for a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to picomolar concentrations and which, either under normal or pathological conditions, modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment.

Cytokine network

- This term essentially refers to the extremely complex interactions of **cytokines** by which they induce or suppress their own synthesis or that of other **cytokines** or their receptors, and antagonize or synergise with each other in many different and often redundant ways.
- These interactions often resemble **Cytokine cascades** with one cytokine initially triggering the expression of one or more other **cytokines** that, in turn, trigger the expression of further factors and create complicated feedback regulatory circuits.
- Mutually interdependent pleiotropic **cytokines** usually interact with a variety of cells, tissues and organs and produce various regulatory effects, both local and systemic.



Cytokines

- In many respects the biological activities of cytokines resemble those of classical hormones produced in specialized glandular tissues. Some cytokines also behave like classical hormones in that they act at a systemic level, affecting, for example, biological phenomena such as **inflammation**, **systemic inflammatory response syndrome**, and **acute phase reaction**, **wound healing**, and the **neuroimmune network**.
- In general, cytokines act on a wider spectrum of target cells than hormones. Perhaps the major feature distinguishing cytokines from mediators regarded generally as hormones is the fact that, unlike hormones, **cytokines are not produced by specialized cells** which are organized in specialized glands, i. e. there is not a single organ source for these mediators.
- The fact that cytokines are secreted proteins also means that the sites of their expression does not necessarily predict the sites at which they exert their biological function.

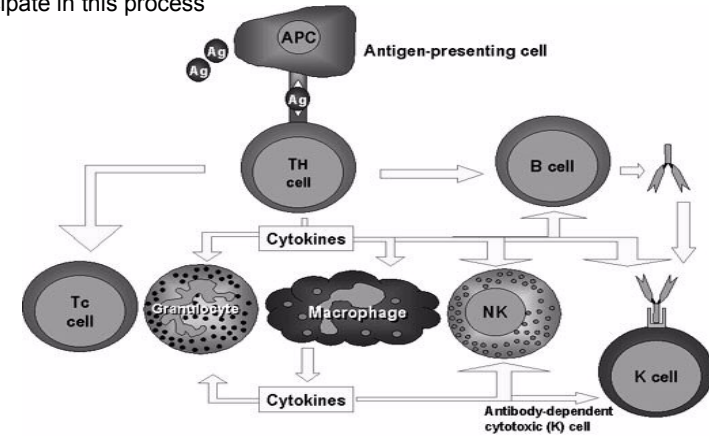
Th1/Th2 cytokines

- Th-1 (=cytokines type 1) and Th-2 (cytokines type 2) are secreted by different subpopulations of T-lymphocytes, monocytes, natural killers, B-lymphocytes, eosinophiles, basophiles, mastocytes.
- Th-1-helps cellular immunity response [IL-2, IFN γ (IL-18), TNF β]
- Th-2-helps B-cell development and antibody secretion (IgE) (IL-4, IL-5, IL-6, IL-10, IL-13)

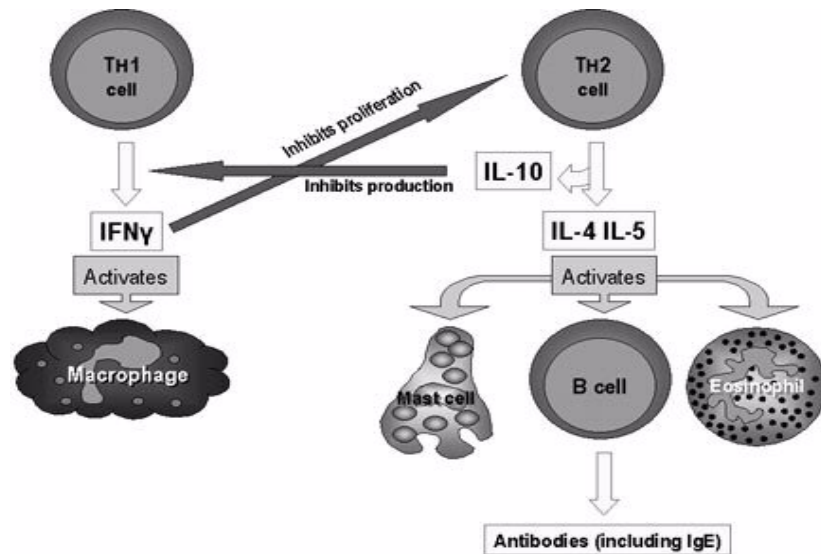
Subpopulations of helper T cells: Th1 and Th2

- When a naive CD4+ T cell (Th cell) responds to antigen in secondary lymphoid tissues, it is capable of differentiating into an inflammatory Th1 cell or a helper Th2 cell, which release distinctive patterns of cytokines.
- Functionally these subpopulations, when activated, affect different cells.

Th cells are at the center of cell-mediated immunity. The antigen-presenting cells present antigen to the T helper (Th) cell. The Th cell recognises specific epitopes which are selected as target epitopes. Appropriate effector mechanisms are now determined. For example, Th cells help the B cells to make antibody and also activate other cells. The activation signals produced by Th cells are cytokines (lymphokines) but similar cytokines made by macrophages and other cells also participate in this process



Selection of effector mechanisms by Th1 and Th2 cells.
In addition to determining various effector pathways by virtue of their lymphokine production, Th1 cells switch off Th2 cells and vice versa.



Differences between innate (non-specific) and specific (adaptive) immunologic reaction of organism

- | | |
|--|--|
| ✓ Non-specific Immunity | ✓ Specific Immunity |
| ✓ Response is antigen-independent | ✓ Response is antigen-dependent |
| ✓ There is immediate maximal response | ✓ There is a lag time between exposure and maximal response |
| ✓ Not antigen specific | ✓ Antigen-specific |
| ✓ Exposure results in no immunologic memory | ✓ Exposure results in immunologic memory |

Collaboration between the innate and acquired immune response

- The APCs produce cytokines, which stimulate the synthesis of acute phase proteins (i.e. CRP) by the hepatocytes. CRP bound to the antigen, increases the phagocytosis of the antigen either by binding to specific CRP receptors on phagocytic cells or via complement receptors when complement is attached to the CRP-antigen complex. APCs process and present antigens in the context of HLA class II for T-cell receptors (TcR) on T-lymphocytes. Cytokines from activated T-cells stimulate B-lymphocytes. Clonal expansion is induced for both cell types. B-lymphocytes are also activated via antigen binding to B-cell receptors, which are immunoglobulins on the cell surface. Activation of B-lymphocytes induces maturation of B-cells to plasma cells and synthesis of large amounts of soluble antigen-specific immunoglobulins. Free antigens are covered with antibodies. Antibody-covered antigens bind to Fc receptors or complement (C3b) receptors on phagocytic cells. APCs also produce cytokines responsible for stimulation of leukopoiesis, increasing the number of cells available for innate and acquired immune responses.
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