Lecture 4 of Advanced Immunology
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In fact, the immune system has developed the genetic mechanisms for generating an extremely diverse set of antigen receptors from a limited number of inherited genes, allowing recognition of many millions of different antigens.

The variable domains of the BCR and TCR are encoded by gene segments which are often located far away from each other in the DNA. While conventional genes encode the constant domains of immunoglobulin chains (heavy- and light) and the TCR chains (α and β), the variable domains are encoded by gene sequences which are created by the combination of several small gene segments. The variable domains of the receptors are combined from 2 or 3 gene segments, during their development lymphocytes start rearranging their original DNA (germline DNA) to create unique DNA sequences. Only the rearranged gene sequence is translated into protein from the mature mRNA.

The diversity is further increased due to the variable domains of two chains form the antigen-binding site: in B cells the heavy and light chains, in T cells the α and β (or γ and δ) chains, and their combined random pattern together determines receptor diversity and specificity for both T- and B-cells. (The variable domains of the heavy chain of the BCR and the β-chain of the TCR are randomly assembled from three gene segments, while the light chain and the α-chain variable regions are assembled from 2 segments).

The diversity is further increased by several orders of the gene segments rearrangement through deletion or addition of some nucleotides randomly added at the junctions points using an appropriate polymerase and ligase enzymes), which lead to new sequences appear that were not present in the germline sequence.

However, these antigen recognition receptors are structurally the same in the constant domains not subject to the above assembly mechanisms.

1- 10 million-1 billion B and T cells are generated and mature daily in the bone marrow and in the thymus, replacing the dying cells in the periphery.

2- The antigen recognition receptors (BCR or TCR) are unique with different specificity and each lymphocyte expresses only one type of antigen recognition receptor for only one antigen.

3- Lymphocytes develop in the primary lymphoid organs in the absence of foreign antigens, those lymphocytes that recognize self-structures with high “intensity” die or become inactivated in the early stage of their development, and should not leave the primary lymphoid organs.

4-Mature lymphocytes leave the primary lymphoid organs through the blood circulation and they regularly enter the secondary lymphoid organs to check whether their specific antigen has appeared there. If they do not meet their specific antigen,

they continue their circulation in the blood and lymph to monitor the antigen repertoire of other secondary lymphoid organs.
5-The newly developed lymphocytes, for which specific antigen is not present in the body, spend only a few weeks in the circulation, then die by apoptosis.
6- The recognition of antigen leads to the activation and proliferation of the specific lymphocyte. After foreign antigens disappear from the body, thus the specific lymphocyte clones are not needed anymore. At the final stage of the immune response these effector cells die by apoptosis.
7- However some of the specific B and T cells differentiate into memory cells or long-lived effector cells, which ensure a quicker and more efficient immune response in case of reinfection.
Note: In case of B cells the strength of the antigen binding usually improves during the clonal proliferation due to point mutations introduced into the coding region of the antigen recognition receptor, and resulting in binding to the antigen with higher affinity will win the competition for the antigen available, thus survive and proliferate, this process called "affinity maturation" requires the contribution of helper T cells.

Communication between the innate and adaptive immune system
The innate and adaptive immune systems cooperate in total harmony.
1- The innate immune system cells especially macrophages and dendritic cells are essential for initiation of the adaptive immune response. T cells are unable to function without these antigen presenting cells. B cells require the signals coming from innate immune cells and / or T helper cells. Through their costimulatory, and adhesion molecules, as well as by secretion of cytokines they orchestrate the adaptive response (Th1, Th2, etc).
2- A- Cytokines produced by T cells help the activation of macrophages, elimination of the ingested microbes, maturation of dendritic cells, antigen presentation and the cytotoxicity of NK cells.
Cytokines control the action, the maturation and the localisation of innate cells. The same time, cytokines also play a key role in limiting the innate immune response.
B- Cytokines and chemokines produced by macrophages are the main regulators of neutrophils in the inflammatory process, and also have on effect on migration and the functional activity of NK cells.
C- Cytokines facilitate the extravasation of complements.

Ideally, **B and T cells recognize the same antigen during an adaptive immune response.** In this case, the cytokines produced by T cells contribute to the activation

and affinity maturation of B cells as well as to generation of memory cells. Antigen presentation by B cells may facilitate the survival of effector / memory T cells.
The antibodies produced by B cells have the effect through anchored to pathogens facilitate their recognition and phagocytosis and enhance the function of NK cells and the activity of the complement system.
Antibodies alter the function of mast cells and granulocytes.
Both the innate and the adaptive immune system have self-control mechanisms.

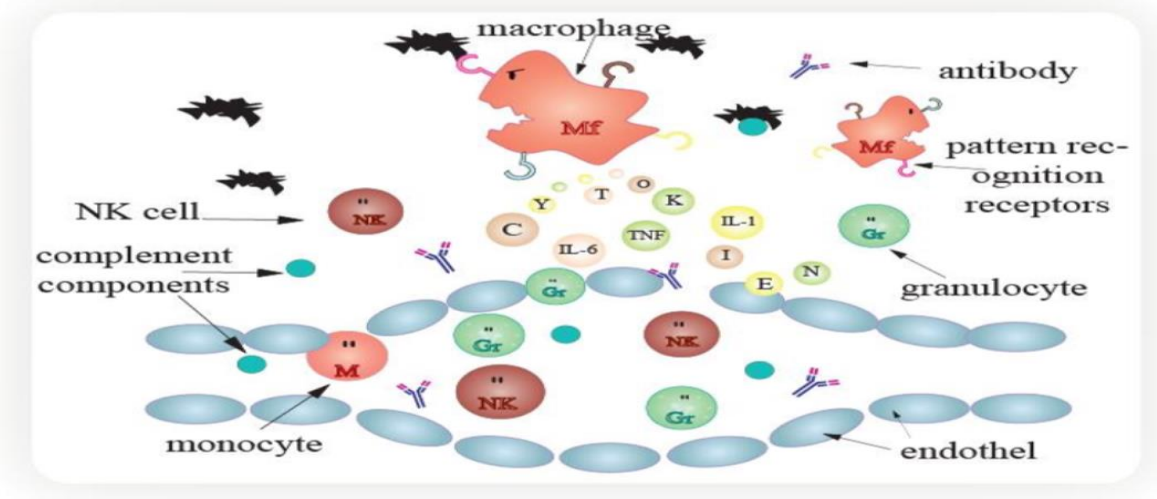
The process of the immune response

The invading pathogens are recognized first by the cells of the innate immune system (eg. macrophages and dendritic cells), expressing pattern recognition receptors. They immediately trigger the well-known reactions of the innate immune system (elimination of pathogens, recruitment of other immunocytes) leading to induction of inflammation in the infected tissue. The induced production and flow of lymph will carry some of the pathogens into the regional (draining) lymph nodes. Activated dendritic cells also reach the lymph nodes through the lymph, where they present processed phagocytosed antigens to the T lymphocytes. Thus B and T lymphocytes, which recognize the pathogen-specific antigens, get activated, proliferate and differentiate into effector or memory cells.
Depending on the amount and the growth rate of the invading pathogen we can distinguish between three potential courses of the immune response.

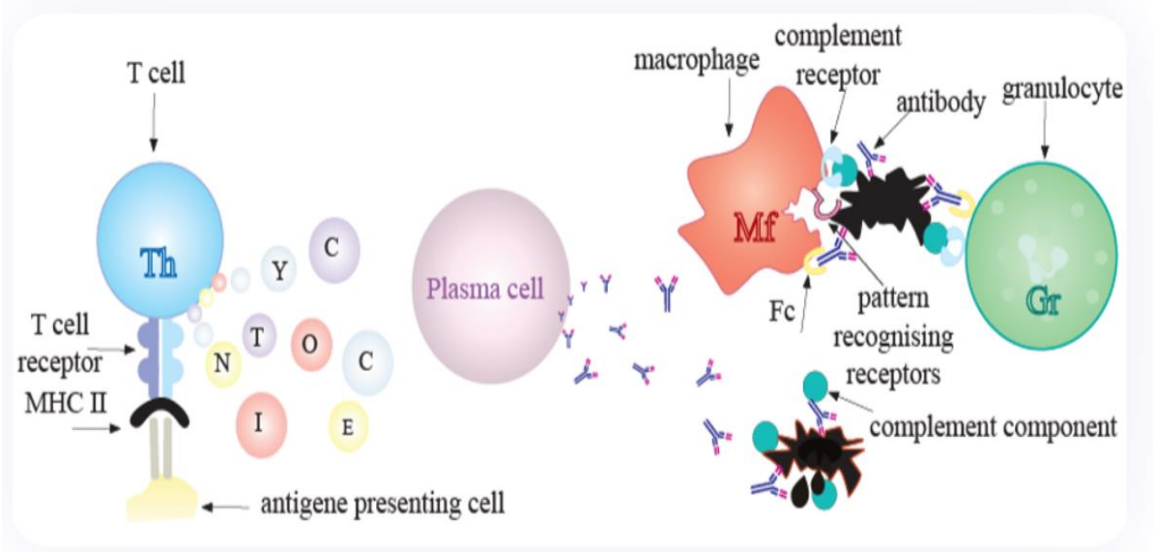
1- Small amounts of pathogens are immediately recognized at the site of infection and eliminated by macrophages found almost everywhere in our body.
2- If this mechanism is insufficient for clearing the pathogen more participants of the innate immune system will be recruited to help macrophages.
3- If the pathogen survives for an extended period, the adaptive immune system will also be activated, which finally eliminates the pathogens either by its own mechanisms, or more often by enhancing the innate response.

If the human has already met the pathogen, the effector mechanisms of the adaptive immune response can provide immediate protection.

Pathogen-specific antibodies produced by the long-lived plasma cells neutralize the pathogens and the effector T cells eliminate the infected cells in a few days or produce cytokines to enhance the efficiency of the other participants of the immune system. In parallel, memory cells in the lymph nodes get activated and begin to proliferate rapidly.



At the site of infection macrophages recognize the pathogens and subsequently produce inflammatory cytokines, inducing vasodilation and vascular permeability. Granulocytes, Monocytes, NK cells, complement components can exit from the circulation at this point and eliminate the pathogens in the infected tissues.



Elimination of extracellular pathogens Phagocytes (Mf) recognize pathogens via PRR or pathogens opsonised with antibodies and/or complement via Fc- and/or complement-receptors. Pathogens bound by these receptors get ingested and killed in the phagolysosomes by multiple mechanisms (right). Antibodies produced by plasma cells may neutralize the pathogen and/or activate antibodymediated effector functions (middle). (Complement components may directly eliminate pathogens) Type 2 cytokine-producing T helper cells augment immune response against extracellular pathogens primarily (left).

Most pathogenic bacteria and fungi entering the body live in the interstitial space between cells (extracellular pathogens), These are the main targets of antibodies and complement proteins diffusing out of the plasma during inflammation.

Pathogens opsonized by antibodies are efficiently recognised by phagocytes, mainly by macrophages and neutrophil granulocytes, and the complement system, via complement receptors and Fc-receptors phagocytes are induced to ingest and kill opsonized pathogens.

Dendritic cells activated by microbes start expressing costimulatory molecules at high levels and migrate to draining lymph nodes, where they activate naive T lymphocytes which maturated and activated through pathogens phagocytosed and presented with MHC II on its surface , and this activation occure by cell-cell contact and secretion of cytokines (e.g. IFNγ) will help macrophages to kill the ingested pathogens.

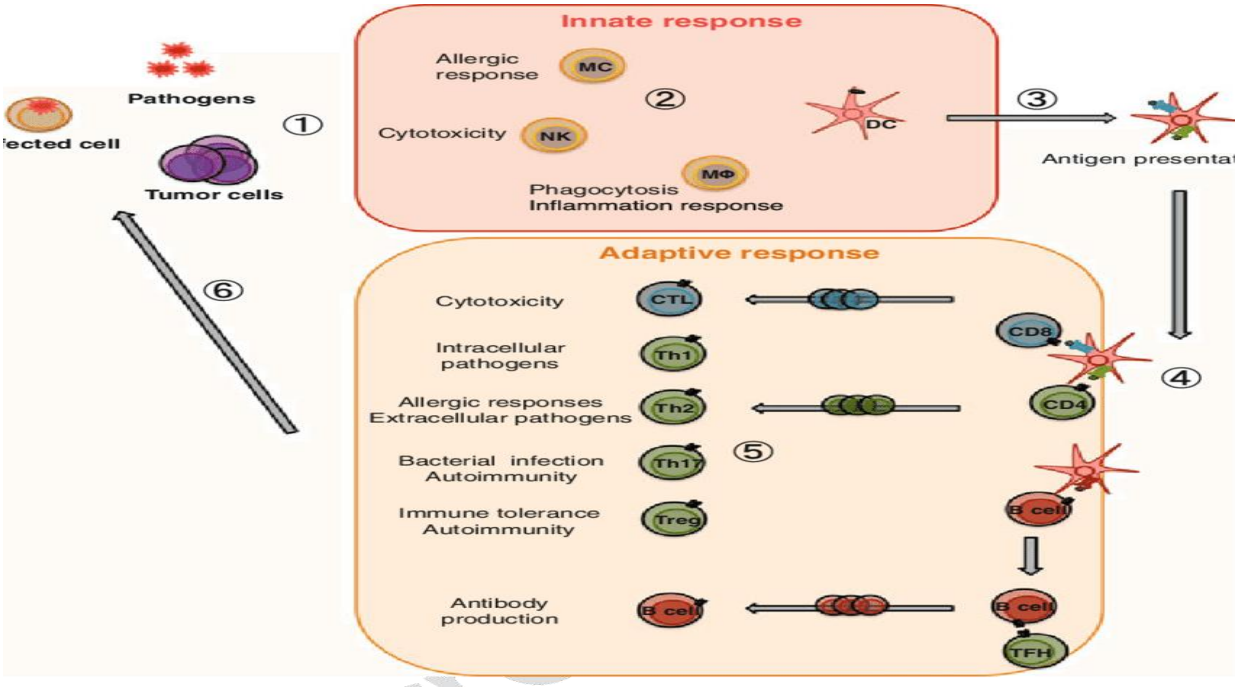
While intracellular infection, requires epitopes synthesized in infected cells and presented through MHC class I molecules at the cell surface of infected cells and recognized and eliminated by cytotoxic T cells (CTL)through the destruction of the infected host cells(as tumour cells as well are aberrant host cells). If intracellular pathogens down-regulate expression of MHC class I molecules, infected cells are recognized and eliminated by NK cells,

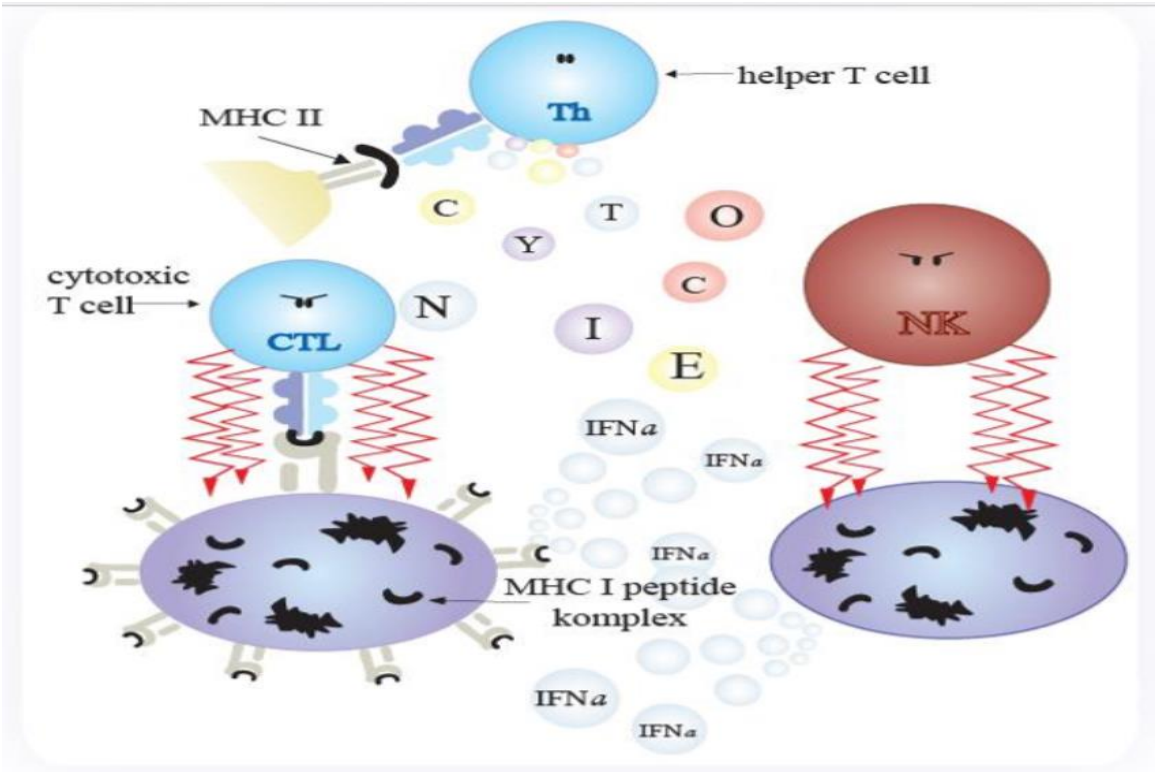
IFN-γ is produced mainly by T helper cells and cytotoxic cells (NK, Tc), and besides their direct antiviral effect, their main function is to it promote macrophage functions. Due to its effect macrophages kill engulfed microorganisms more efficiently.

In addition, IFN-γ enhances the expression of MHC class I and II molecules on the surface.

IFN-α, IFN-β can be produced by almost any cell types that infected with a virus, which have autocrine or paracrine effects and interfere with viral replication by various mechanisms eg. inhibition of protein synthesis, increased expression of MHC class I molecules etc.

Antibodies may also partake in the defence against viruses, through neutralizing effect of antibodies prevents adherence of viruses on host cells.
Viral proteins may also be displayed on the membrane of infected cells. Antibodies recognizing these antigens will bind them, therefore mark the cell for antibody-dependent cellular cytotoxicity (ADCC). NK cells may also kill infected cells by ADCC via activation by their Fc receptors.
Some small, single-cell intracellular parasites such as Plasmodium species eukaryotic parasites induce similar T cell responses.
Elimination of large multicellular extracellular parasites often requires a much broader spectrum of immune response delivered by specialized cells.
Eosinophil and basophil granulocytes or mast cells are involved in inflammation triggered by parasites. These cells release special toxic agents and degrading enzymes from their vacuoles to destroy the parasites at the area of infection. Also many pro-inflammatory cytokines are secreted.
The parasite-specific IgE antibodies bound to high-affinity Fc receptors of mast cells and basophil granulocytes play important role inactivation of these cells and thereby in the immune response against parasites.





Elimination of intracellular pathogens Virus-infected cells are recognized via intracellular pattern recognition receptors followed by production of type I interferons. Concomitantly, MHC class I molecules present antigens of intracellular pathogens on the cell surface and activate cytotoxic T lymphocytes, which kill the infected cells (bottom left). In the absence of cell surface MHC I (indicating an aberrant cellular function) cells become targets for NK cells, which again lead to the elimination of the cell (right).

B cells

Similar to dendritic cells and macrophages, B cells are professional antigen-presenting cells.

The recognition of antigen by BCR not only activation, division and differentiation of the B cell, it also induces endocytosis of the bound antigen by the process of receptor-mediated endocytosis and presented on the surface of B-cells as peptide / MHC II complexes and could be seen by T cells will recognize the same antigen, but not necessarily the same part (epitope) of the antigen.

B-lymphocytes are responsible for the production of antibody molecules during adaptive immunity.

Antibodies are critical in removing extracellular microorganisms and toxins.

B-lymphocytes refer to lymphocytes that are produced in the bone marrow and require bone marrow stromal cells and their cytokines for maturation.

During its development, each B-lymphocyte becomes genetically programmed to produce an antibody molecule with a unique 3-dimensional shape capable of binding a specific epitope of an antigen, and puts molecules of that antibody on its surface that function as B-cell receptors or BCRs.

Naive B-lymphocytes can be activated by both T-dependent antigens and T-independent antigens.

In order for naive B-lymphocytes to proliferate, differentiate, and mount an antibody response against T-dependent antigens, such as most proteins, these B-lymphocytes must interact with effector T4-lymphocytes called TFH cells.

The first signal for the activation of a naive B-lymphocyte occurs when BCRs on the surface of the B-lymphocyte bind epitopes of antigens having a corresponding shape.

Once bound to the BCR, the antigen is engulfed, placed in a phagosome, and degraded with lysosomes. During this process, protein antigens are broken down into a series of peptide epitopes, bind to MHC-II molecules, and are transported to the surface of the B-lymphocyte.

The T-cell receptors and CD4 molecules on TFH cells bind to the MHC-II molecules with bound peptide epitope on the B-lymphocyte which enables the TFH cells to produce cytokines that collectively enable the B-lymphocytes to proliferate, synthesize and secrete antibodies, differentiate into antibody-secreting plasma cells, and switch the class of antibodies being produced.

By way of a mutation process called affinity maturation, activated B-lymphocytes are able over time to “fine-tune" the shape of the antibody for better fit with the original epitope.

During the proliferation and differentiation that follows lymphocyte activation, some of the B-lymphocytes stop replicating and become circulating, long-lived memory cells that will initiate a rapid, heightened secondary response against that antigen if it again enters the body.

T-independent (TI) antigens are usually large carbohydrate and lipid molecules with multiple, repeating subunits. B-lymphocytes mount an antibody response to T-independent antigens without the requirement of interaction with effector T4-lymphocytes, but the resulting antibody molecules are generally of the IgM isotype only and do not give rise to a memory response.

Memory cells

In the case of repeated exposure to the same pathogen, the immune system responds faster and more effectively compared to the first exposure. Memory response is characteristic only of B and T-cells, during clonal proliferation and differentiation, in the secondary lymphoid tissues differentiate into memory cells which have the same antigen receptor specificity that is they recognize the same antigen that exposure to

it, the majority of antigen-specific B and T cells becomes unnecessary and die by apoptosis. However, a relatively large number of lymphocytes survive and become memory cells which survive and preserve their antigen specificity for decades, even in the absence of their specific antigen. B cells can differentiate into long-lived plasma cells in the secondary lymph nodes and may produce antibodies throughout the entire lifetime of an individual.

What are the differences between cytotoxic T cells and natural killer cells?

Both of them are cytotoxic immune cells that are capable of killing foreign pathogens and also cancer cells. However, they belong to different systems within the immune system.

Natural killer (NK) cells belong to the innate immune system. They generally serve as the first line of defence against foreign pathogens. NK cells are not specific in their immune response, any microorganism will activate them.

1- Natural killer cells, also known as NK cells or large granular lymphocytes (LGL), are a type of cytotoxic lymphocyte critical to the innate immune system.

2- Belonging to the group of innate lymphoid cells.

3- NK cells are unique, however, as they have the ability to recognize stressed cells in the absence of antibodies and MHC, without require activation to kill cells that are missing "self" markers of MHC class I. This role is especially important because harmful cells that are missing MHC I markers cannot be detected and destroyed by other immune cells, such as T lymphocyte cells, so for this character they named "natural killers"

4- NK cells can be identified by the presence of CD56 and the absence of CD3 (CD56+, CD3−).

5- NKT cells, NK cells do not express T-cell antigen receptors (TCR) or Pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD56 in humans.

6- NK cell will first use perforin to create pores in a target cell, allowing it to inject granzymes through an aqueous channel. The granzymes then break down the target cell, inducing death by either apoptosis or osmotic cell lysis.

CD8+ cytotoxic T cells

1- these belong to the adaptive immune system. Their effects are late and highly specific.

2- CTLs have cytoplasmic granules that contain the proteins perforin and granzymes.

3- Most of these CTLs will die (apoptosis) when they have done their job, but some will become memory cells. 4-belong to the CD8+ subset of T cells, have the αβ T-cell receptor for antigen (TCR) thus recognize antigens nestled in the groove of class I histocompatibility (MHC) molecules.

Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems, that mediate and regulate immunity, inflammation, and hematopoiesis. They act by binding to specific cell surface receptors, which then signal the cell via kinase cascades, often tyrosine kinases, to modulate gene expression. They also help to boost anti-cancer activity by sending signals that can help make abnormal cells die and normal cells live longer.

Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action).

Cytokines are usually produced in a cascade, as one cytokine can stimulate its target cells to produce additional cytokines. Two or three cytokines can act together to show enhanced activity (synergistic), or one cytokine can attenuate the activity of the others (antagonistic).

Cytokines can be produced by many cells types and act on many cell types (pleiotropic). Cytokines are redundant, meaning that a number of different cytokines are able to carry out the same function.

Cytokines are multifunctional, meaning that the same cytokine is able to regulate a number of different functions.

Different cytokines usually exhibit similar biological functions, a property termed redundancy.

Redundancy has been considered an important feature for cytokines in that the immune response to an antigen(s) can be signalled to different effector cell types promptly.

Cytokines grouped by structures into families

- interferons (IFN): are chemicals that help the body resist virus infections and cancers. Type I interferons, produced abundantly by plasmacytoid dendritic cells, by virtually any virus-infected cell, and by other defence cells provide an early innate immune response against viruses by inducing uninfected cells to produce enzymes capable of degrading viral mRNA and blocking translation in eukaryotic cells. They also enhance the activities of CTLs, macrophages, dendritic cells, NK cells, and antibody-producing cells and induce chemokine production to attract leukocytes to the area.
- Interleukin: interaction by chemical signals between leukocytes. e.g. IL-1, IL-2
- chemokine: chemotactic activity, that means make immune cells move toward a target. There are different kinds of chemokines, including interleukins, interferons, tumour necrosis factors, and growth factors.
- hematopoietin: erythropoietin (EPO), colony- stimulation factors (CSF)
- Lymphokine: made by activated lymphocytes, especially TH cells, e.g. IL-2
- Monokine: made by mononuclear phagocytes, e.g. Mig/CXCL9