Lymphocytes and lymphokines

Gustavo Nobre*

Abstract

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Lymphocytes are the major group of cells taking part in the immune response.

Lymphocytes have T-cell antigen receptors (TCR) on their membranes.

The antigens are processed by the macrophages or macrophage-like cells and are presented to T-cells, in the context of major histocompatibility complex (MHC) molecules.

The superantigens, including various bacterial products, do not require processing by antigen-presenting cells, interact directly with invariant regions of the MHC molecules and stimulate T-cells nonspecifically.

The hybridoma technology producing monoclonal antibodies has increased enormously the scope of Immunology research.

Subsets of lymphocytes and other leukocytes can be differentiated by the antigens on their surface. The antigens individualized by two or more monoclonal antibodies are called clusters of differentiation (CD).

Flow cytometry has advanced Clinical Immunology a lot, enabling the description of lymphocytes subsets.

The immune system function is modulated by a number of substances which act as intermediaries between lymphocytes and other cells (lymphokines, interleukins, stimulating growth factors, etc.) named in general as cytokines.

Cytokines are involved in the pathogenesis of certain diseases and are useful to treat cancer and immunodeficiency diseases.

Key words: Lymphokines, interleukins, cytokines, lymphocytes, immune response.

The concept of cell immunity was extremely useful, but revealed to include several defensive mechanisms, when facing microorganisms and foreign substances. In it, there are so diversified phenomena, as: 1. Phagocytosis non-specific processes made by macrophages, 2. Non-specific cytotoxicity made by NK natural killer lymphocytes, 3. Antigens processed by macrophages that fragment them into peptides of low molecular weight and present them to lymphocytes, 4. Hypersensitivity specific reactions or lymphocytes mediated by immunity, 5. Specific reactions to cytotoxicity also mediated by lymphocytes.

Several mechanisms are involved in these processes leading to the destruction of microorganisms, from oxidation phenomena mediated by superoxide radicals, cytokines release with the most diversified actions, that will call to the field several other cells, up to the substance production with a direct cytotoxic action, as perforin, common to NK cells, cytotoxic lymphocytes and eosinophiles, that open real holes in the target cells membrane and whose mechanism is closer from the one seen with the complement.

Mackaness's experiences in the 60ties were elucidative on the role played by macrophages and lymphocytes on protecting from infections as tuberculosis where the humoral immunity has a secondary role.

Mackaness observed that lymphocytes of immune animals had no protecting effect, when transferred to receptors irradiated by X Ray. He has admitted that in order to perform their immune action lymphocytes needed another cell which has been destroyed by radiations and that cell was the macrophage-monocyte. Besides, immune donors' lymphocytes by BCG, were unable to protect their normal receivers against Listeria, in spite of the donors being themselves highly resistant to these microorganisms, unless the receivers were also infected with BCG. This result made him thinking that the host macrophage activation of immune lymphocytes depends on a humoral factor, released by immune lymphocytes in the presence of a specific antigen.

This factor made by lymphocytes after a reaction with a specific antigen, came to be called lymphokine or in a wider sense, by cytokine, and in the case in question it is the gamma interferon.

The fundamental role of lymphocytes in the cell immune response was therefore established.

*Microbiology Professor in Lisbon Medical School. Head of the Clinical Analysis Laboratory in Amadora Health Center

Lymphocyte in the immune response

There are two main classes of lymphocytes. B lymphocytes, called this way, because in birds they are differentiated in the Bursa of Fabricius producing the humoral antibodies (several classes of immunoglobulins), after a differentiation in plasmocytes and T lymphocytes that are differentiated in the thymus and are implicated in the hypersensitivity of a delayed kind, cell immunity, graft rejection and cytotoxic actions. However, for a long time, it was not known which was the receptor for the antigen in the T lymphocytes and the way as these reacted as the first.

Antigen molecules seem not to react directly with the matching T cells. The antigen, surely, should be processed in adequate fragments to be presented by appropriate accessory cells. Antigens linked to T cells, were rarely demonstrated.

Meuer et al, in the USA gave us the first conclusive information about the receptor biochemistry structure for the T cells antigen (TCR). They have developed cytolytic T lymphocyte clones, alloreactive and showed that such clones, carrying superficial T4 structures (this is the way lymphocytes CD4+ were called) reacted with MHC class II antigens, while clones with T8 structures (CD8+) reacted with MHC Class I antigens. At present, it is known that the receivers for T cells antigens (TCR) are glycoproteins made up by heterodymers of designated sub-units by alpha, beta, gamma and theta which are codified by 4 distinct genes.

It was at the beginning of the 70ties, that a suspicion started that MHC transplant antigens were also implied in the general immune response. In 1974, two Canberra immunologists, Zinkernagal and Doherty mixed mice T killer lymphocytes with infected cells of the same animal and verified that the former only destroyed the latter when originated from mice with the same MHC antigen type. It seemed, therefore that MHC molecules were needed for the T lymphocytes to work properly.

Only in 1985 it was clarified the role played by MHC molecules. Emil Unanue in Saint Louis (USA) has purified these molecules proving that when linked to antigens fragments, were recognized by T helper lymphocytes. They work as a key opening the door to the immune response.

In 1987, Srominger and Willey, Harvard University biochemists, got MHC molecule crystals and assessed their shape and way of reflecting the light. They came to the conclusion that the molecule is made up by two chains of atoms, called alpha and beta, forming a slit with the exact dimensions to enable it to work as a protein fragment support to the antigen to be present to the T cell.

At present, it is known that MHC (named in humans as HLA complex) is crucial in the immune response. Its genes are located in the 6 chromosome.

In MHC there are at least 6 molecules, 3 of Class I and 3 of Class II. Genes modifying these molecules are 3 of Class I, HLA – A, B and C and 3 of Class II, HLA – DR, DQ and DP. There is a Class III matching the genes codifying complement proteins C2, C4 and B.

The Class I antigens are at the surface of all cells in our bodies. Class II molecules are found in macrophages and B lymphocytes. Both of them are on the surface of the presenting cells (macrophages and other) and serve to introduce the antigen to T lymphocytes.

HLA complex genes vary from one person to another. Each MHC molecule has about 20 different shapes and then, each person has a MHC molecule combination, which is unique and exclusive to him/her. The HLA system is a known antigenic system, more polymorph. Hundreds of alleles have been identified.

Lymphocytes clones with specificity for a certain antigen (according to the clonal selection theory) must, therefore, to recognize not only the respective antigen, but MHC molecules of the presenting cells.

The knowledge of receptors structures in lymphocytes T and the way as these react with the respective antigens, led to the demonstration of the existence of substances produced by several microorganisms, escaping the rules and were called superantigens.

These antigens were capable, in relatively low concentrations, to stimulate a great percentage of T cells. Different from conventional antigens, superantigens do not need to be processed by presenting cells, but react directly with regions relatively constant of MHC Class II molecules and out of the slit serving as support to the conventional antigens. This way, superantigens can be linked to a wide variety of MHC Class I molecules. The MHC-superantigen complex reacts with the T cell receptor (TCR), also out of the slit which is the area of the conventional antigen recognition. Therefore, superantigen interaction with T cells is non-specific. There is however a certain

specificity involved, because the superantigen reacts as a variable (V) part of TCR beta chain. Thus all T cells carrying a certain V beta region will be stimulated by superantigen.

Superantigens are scarlet fever streptococcus toxin, enterotoxins and staphylococcus exfoliating toxin, streptococcus M protein and pyrogenic toxins of certain streptococcus responsible by the toxic syndrome with shock.

The symptoms caused by superantigens emerge from the release of cytokines, which may be involved in diseases as diverse as rheumatic fever, atopic eczema and psoriasis guttata.

Leukocytes differentiation antigens

The technique to obtain the monoclonal antibodies, is common knowledge, since Kohler and Milstein, from the Medical Research Council Laboratory of Molecular Biology, in Cambridge, published in Nature, in 1975, the hybridoma technique.

In its original approach, a mouse is repeatedly immunized with a desired antigen and the spleen containing B lymphocytes proliferating, is removed. B lymphocytes, usually die in culture, but can be kept alive in fusion with nonsecretory myeloma cells.

The resulting hybridoma, can then segregate huge amounts of codified antibodies by B lymphocytes and the most adequate clones are selected.

Monoclonal antibodies open wide research and diagnosis perspectives in the Immunology field. Conjugated with fluorochrome (fluorescein isotiocianate or phycoerythrin) may be used in immunofluorescence, immunoenzymatic and radioimmunology techniques.

Thousands of monoclonal antibodies were produced against hematopoietic cells since the hybridoma invention. In an attempt to classify antigens found in the surface of human leukocytes and to achieve a uniform nomenclature for these structures, it was held in Paris in 1982, an international meeting on human leukocytes differentiation antigens. Laboratories all over the world, have exchanged their monoclonal antibodies to be compared through immune and biochemical methods. Monoclonal antibodies groups who have shown a similar linkage and tissue allocation capacity were called CD (Cluster of Differentiation).

CDs are effectively the expression of well defined antigens (most proteins and glycoproteins) present in the cell surface.

To these antigens there are matching genes which have been cloned and put in sequence and many were located in specific chromosomes. These genes nucleic acid transfection into adequate host cells, confirms these markers individuality.

These antigens are not, however, mere differentiation markers but they play important roles to the cell:

- They recognize antigens promoting the activation and maturation of cells, before adequate receptors (TCR), described previously;
- It intervenes in the immune regulation as cytokine receptors;
- Work as membrane enzymes;
- They mediate the cell adhesion to other cells and to extra cellular matrix compounds;
- They are receptors for essential growth factors;
- They are receptors for serial proteins.

In a meeting held in Wien in 1989, 78 CD were identified. We highlight some of these more common markers:

CD2 – it is found in T lymphocytes and in most NK cells.

CD3 – It is the main T lymphocytes marker. Receptor for antigens (TCR).

CD4 – It is found in a T lymphocytes and monocytes subclass. Receptor for antigens in the context of MHC Class II.

It is a HIV receptor

CD5 – It is found in most T cells and in a B lymphocytes subclass, which is involved in producing antibodies.

CD7 – It is found in many T lymphocytes, platelets and NK cells.

CD8 – It is found in a T lymphocytes subclass and NK cells.

Receptor to antigens in the context of MHC Class I antigens.

CD19 – It is found in all cells of the B lymphocytes line

CD20 – It is also found in B lymphocytes and it is usually the marker used to be counted.

CD29 – Memory B lymphocytes marker

CD56 – It is found in NK cells and it is one of the markers used to its count.

Quantitative flow cytometry for the count of the different lymphocytes, is at present, a valuable working tool for the diagnosis and prognosis of se-

TABLE I

Most important cytokines

Cytokines	Origin	Some functions
Alpha interferon	B Lymphocytes and macrophages	Viral replication and cellular proliferation inhibition
Beta interferon	Fibroblasts, epithelial cells and macrophages	Viral replication and cellular proliferation inhibition
Gamma interferon	T lymphocytes	Macrophages activation
IL-1	Macrophages, keratinocytes	B and T lymphocytes activation
IL-2	T lymphocytes	T, B and NK lymphocytes proliferation
IL-3	T lymphocytes	Hematopoietic cells proliferation
IL-4	T lymphocytes and mastocytes	B lymphocytes differentiation
IL-5	T lymphocytes and mastocytes	Eosinophiles proliferation and differentiation
IL-6	Macrophages, T lymphocytes, fibroblasts	Multifunctional
IL-7	Bone marrow stromal cells	Regulating T lymphocyte T proliferation
IL-8	Macrophages, keratinocytes and fibroblasts	Neutrophils chemotaxis
IL-9	T lymphocytes	T lymphocytes, thymocytes and mastocytes proliferation
IL-10	T lymphocytes and mastocytes	Cytokines synthesis inhibition
G-CSF GM – CSF M – CSF	T lymphocytes	Hematopoiesis stimulation
TNF alpha, beta	Macrophage, neutrophiles, T and NK lymphocytes	Multifunctional

veral clinical situations, including organ transplant and bone marrow, leukemia and lymphomas diagnosis and evaluation of immunodeficiency situations. The technique allows, with monoclonal antibodies, driven against cell surface antigens and fluorochrome conjugates, to distinguish: T lymphocytes, B lymphocytes, NK cells and several subclasses within these populations.

Cytokines

The expression cytokine is used to a great number of substances produced mainly by leukocytes, which serve as intermediaries to regulate the immune system. Interferons are the longer known among these substances.

Those produced by lymphocytes, monocytes and macrophage are called lymphokines and those produced in a general way by leukocytes are called interleukins.

There are other cytokines whose designations are based on their biologic properties as CSF (Colony Stimulating Factor), TGF (Transforming Growth Factor) and TNF (Tumor Necrosis Factor).

Cytokines are low molecular weight glycoproteins (10,000 to 60,000 Da) many of them well characterized from a chemical point of view. One of the better known, interleukin-2 (IL-2) is a 133 aminoacid protein with a molecular weight of around 15,000 Da. The respective genes were cloned in 1983 and their production was achieved through genetic engineering in bacterial cells (Escherichia coli) or eukaryotic cells. At present, 20 cytokines were cloned and obtained through genetic engineering.

The WHO Nomenclature Committee for Interleukins recommends the use of the expression interleukin to be applied only to substances whose molecules have already been purified and its genes cloned and expressed. They must be naturally segregated by the immune system cells, and should intervene as mediators in processes potentially important in the immune response.

Cytokines have local and systemic effects. They are needed for a normal immune response. They act together, according to a complex scheme and

in "cascade" in such way that one will induce the production of another. The regulation of cytokines themselves is utterly complex and only now we start have a glimpse of how it works.

The most important cytokines are mentioned in Table I.

Interferons are distinct antigenic proteins which might be induced in most cells by different stimuli.

Initially it were seen cell cultures infected by virus, produced a protein reacting with other cells and giving resilience to the infection for many virus. At present, it is known that interferons do affect several vital organs, whether at cell level, whether at the body in general, as the metabolism, cellular proliferation, hormonal stimulation, immunity and tumor development.

Interferons are made up of three molecules families alpha, beta and gamma. Alpha and beta interferons reduce viral replication having a anti-proliferative action on a number of cell types. They do not act upon virus, but induce many protein in cells (more than two dozen) interfering with the viral genome translation.

Gamma interferon is the one with most interest for Immunology. Its production is induced in T lymphocytes, by antigens for which, T cells are sensitized and it is the main macrophage activator.

Interferon interaction with other cytokines is very complex. For instances, the production of gamma interferon is often followed by the production of other cytokines. The activated macrophages by the gamma interferon produced TNF. IL-1 can induce the IL-2 production that on its turn induces gamma or beta interferon.

There are ten interleukins (IL). IL-1 which is also called LAF (Lymphocyte Activating Factor) is produced by macrophages and other cells involved in activating B and T lymphocytes, in response to antigens and mitogens. It does affect a wide range of other cell types, but the main role is the T lymphocyte activation, resulting on the IL-2 production. In the central nervous system it works as endogenous pyrogenium.

IL-2 is also called as T cells growth factor. It is produced by T helper lymphocytes after antigenic stimulation. It helps the proliferation of other T lymphocytes, B lymphocytes and NK lymphocytes (natural killer).

IL-3 stimulates the proliferation and differentiation of bone marrow reticular cells.

IL-4 is a stimulation factor and a B lymphocytes differentiation. It seems to have an important role modulating the immune and inflammatory response.

IL-5 is an important factor for eosinophile differentiation and proliferation. It is also involved in the production of IgA.

IL-6 is multifunctional, produced by several cell categories, in response to several stimuli. The diversity of IL-6 biological actions suggests an important role mediating the immune and inflammatory response to infections and other sorts of aggressions.

IL-7 is a factor of regulation in the proliferation of T lymphocytes. It induces the formation of K(killer) lymphocytes activated from T lymphocytes (CD4+ and CD8+). It has an immunoregulator role.

IL-8 is a powerful chemotaxis factor to neutrophiles, produced by monocytes, keranocytes and fibroblasts.

IL-9 is a factor produced by T lymphocytes promoting the proliferation of the very own T cells, thymocytes and mastocytes.

IL-10 is an inhibitor of cytokines synthesis in several cell types. It is a negative regulating factor. Its properties suggest they can ease hipersensitivity reaction of a delayed kind.

There are several CSF (Colony Stimulating Factor) stimulating macrophages function, maturation and proliferation (M-CSF), granulocytes (G-CSF) and granulocytes and macrophages (GM-CSF). All of them are produced by T lymphocytes and establish the connection between the lymphatic and hematopoietic systems.

There are two TNFs (Tumor Necrosis Factors): alpha also known by cachetin and beta, known as lymphotoxin. They are produced by several cells, neutrophiles, lymphocytes, macrophages, NK cells and others. They are potent pleiotropy factors, due to its receptors ubiquity, important in inflammation, tumoral defense and cellular proliferation including wounds cicatrisation.

TGFs (Transforming Growth Factors) are a family of polypeptide growth factors, multifunctional, whose receptors are present in almost all sorts of cells in mammals.

We can come to the conclusion that cytokines has, usually several functions and that often there have an overlapping role.

In a general way, IL-1, IL-6 and IL-8 are pro-

inflammatory, IL-2 and IL-9 are potent factors of lymphocyte growth and IL-4 and IL-5 are involved in the production of several immunoglobulins by B lymphocytes.

Changes on the cytokines productions and in the ability of cell response where they act, it has been implied in cancer, infectious diseases, auto-immune diseases and allergies pathogenesis.

We have already seen that the symptoms caused by superantigens emerge from cytokines release. At least, IL-4 and IL-5 are implicated in allergic inflammatory reactions.

Its specific inflammatory effects may be blocked by anti-cytokines or antibodies against cytokines receptors. It was then established that IL-4 is necessary to produce IgE antibodies and the eosinophile infiltrate is blocked by an anti-IL-5.

On the other hand, it is verified that high levels of IL-6 are associated to auto-immune diseases, proliferative glomerular-nephrite, psoriasis and some malignant diseases (plasmacytoma, myeloma).

The cytokines produced at present by genetic engineering methods, at industrial scale, have many therapy applications.

Alpha and gamma interferon have been approved in the USA by the Food and Drug Administration. Alpha interferon is used to treat condyloma acuminata, C hepatitis, leukemia, AIDS and Kaposi sarcoma.

Gamma interferon was approved as immunomodulator in the treatment of chronic granulomatous diseases. It is promising the outcome of basal and spinocellular cancer, B hepatitis, laryngeal papillomatosis and chronic myeloid leukemia.

Several interleukins have been used in therapy or have therapy indications, although the most used is the IL-2. IL-7 can be beneficial in the treatment of immune deficiencies and cancer. IL-10 can be useful in the treatment of sepsis, rheumatoid arthritis, psoriasis, certain autoimmune diseases as, type I diabetes and multiple sclerosis as anti-inflammatory agent to extend the graft survival.

GM-CSF has been used in medulla transplant and the M-CSF can be potentially useful to treat cancer and mycosis.

TNF has been tried in clinic practice as anti-tumoral agent.

Immunotherapy with IL-2 has developed from the observation that tumor cells could be destroyed "in vitro" by lymphocytes stimulated by IL-2. It has been used in the therapy of kidney, melanoma, non-Hodgkin lymphoma and colon-rectum metastatic cancer. It is the most active medicine in kidney cancer and a rare efficient medicine in the melanoma. The use of this kind of therapy is, however, limited by its toxicity. It gives way to a capillary leak syndrome of clinical severity.

Although IL-2 does not have a direct action in the vascular endothelium, it stimulates the release of other cytokines, including IL-1, TNF and gamma interferon. The activation of endotelial cells leads blood vessels to be permeable to macromolecules. The passage of fluid to the tissues, leads to hypovolemia, hypotension and it is responsible for an increase in weight, edemas, dyspnea, pulmonary edema and in serious cases, psychic changes as, disorientation, confusion, personality changes, hallucinations and somnolence.

Finding the IL-2 specific receptors, enables to anticipate a second generation immunotherapy, based on antagonistic substances or cytokines inhibitors. ■

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