Biological factors of inflammation and methods of their detection.

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ABSTRACT: Inflammation is a complicated process occurring after a harmful stimuli or infection. During inflammation a large number of biochemical reactions and pathophysiological changes take place. These procedures are all induced by active biological compounds called mediators or chemokines. These molecules are derived from plasma and cells and are capable of performing the appropriate changes both on the endothelium and cells during the inflammatory process. Moreover, some of them have the ability to interact with each other. Today there are different methods to detect and quantitate these compounds. These techniques keep on evolving and improving. Which one is the most suitable depends on the researcher's scientific aim.

Key Words: Inflammation, Mediators, Chemokines, Biological determination.

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants[1]. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. Additional causes of inflammation are burns, chemical irritants, frostbite, toxins, physical injury, blunt or penetrating, immune reactions due to hypersensitivity, ionizing radiation, and foreign bodies.

Inflammation can be classified as either acute or chronic, local or systemic. Acute-local inflammation lasts from few minutes up to some days. Its classic signs and symptoms are redness, swelling, heat, pain, loss of function. All the above signs may be observed in specific instances, but no single sign must, as a matter of course, be present. Apart from loss of function these are the original, or "cardinal signs" of inflammation. Loss of function is an apocryphal notion, as it is not unique to inflammation and is a characteristic of many disease states. On the contrary, chronic-systemic inflammation can endure a longer period of time and it can be the result of acute inflammation[2,3,4].

PATHOPHYSIOLOGICAL MECHANISM OF INFLAMMATION

Changes in vascular caliber and blood flow

As soon as injury occurs there is often a transient vasoconstriction that takes place. Nevertheless, this is rapidly followed by vasodilation of the local blood vessels with consequent excess local blood flow. Many previously unopened capillaries open and that can lead to increased blood flow up to tenfold. However, the rate of flow through the still dilated vessels gradually decreases[5]. The increase of local blood flow is of great importance because it regulates to a large extent the amount of exudates. If for example local blood flow is temporarily stopped exudates will be reduced or abolished[6].

Increased vascular permeability

Under normal circumstances, small blood vessels in most tissues are fully permeable to water and wa-

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ter soluble small molecules but not quite permeable to plasma proteins. During inflammation increase in vascular permeability practically means increase in the permeability of plasma proteins, such as albumin. This leakage after local injury can occur through two different distinct mechanisms: a) directly as a result of the harmful agent itself (heat, mechanical trauma) b) indirectly from the release of chemical mediators in the absence of tissue damage. Direct injury can affect all types of vessels whereas indirect injury shows a high degree specificity for the venules.

In order to understand the two mechanisms mentioned above, we must proceed to examine the cellular mechanisms of such leakage. It is established that the main permeability barrier is the vascular endothelium. In fact, there are different models which explain how the endothelial layer leaks. The simplest one is the so-called endothelial destruction after exposure with the harmful agent. Cells break up or die whereas the basement membrane remains. When this happens, platelets adhere to the damaged surface but do not manage to obliterate the vascular lumen. Another cellular mechanism has to do with the formation of gaps by unzipping of the endothelial junctions. It often takes place in mild, direct injuries, as if the intracellular junctions are particularly delicate. Finally, the main and most complicated mechanism is the formation of intercellular gaps by endothelial contraction. This contraction results from activation of muscular elements within endothelial cells, which pull away from each other[5,6]. The process lasts about 15-30 minutes, it is reversible and takes place in venules, but not capillaries or arterioles. Many different endogenous factors, called mediators, such as bradykinin, histamine, C3, C5a participate in this procedure (see further).

As far as the term transcellular canal is concerned it appears that such canals exist in normal endothelium and they could increase in size under pathologic conditions. However, scanning electron microscopic observations show that most of the openings in leaky venules are intercellular gaps, not transcellular holes. The formation and closure of gaps are likely to be energy-dependent. Nevertheless, the cellular mechanisms of gap opening and closure remain to be elucidated[7].

Cellular events (leukocytic infiltration)

The tissue macrophage is the first line of defense against the harmful stimuli. Right after the beginning of inflammation the tissue macrophages, whether histiocytes in the subcutaneous tissues begin their phagocytic actions. These cells usually enlarge their size in order to achieve their mission, when activated by the products of inflammation. They break loose from their attachments and become mobile. During this first mobilization, the number of macrophages taking part is not great.

Neutrophil invasion into the inflamed area is a second line of defense. Within the first hour after inflammation begins, neutrophils invade the inflamed area from the blood. Different products from the inflamed tissues alter the inside surface of the endothelium leading neutrophils to stick to the capillary walls. This is achieved through various adhesion molecules such as selectins and integrins. The phenomenon is called margination. On the other hand direct adhesion is extremely rare. Soon after the adhesion on the endothelium surface, leukocytes extend pseudopodia and pass through gaps between endothelial cells. This is called diapedesis. Other products of the inflammatory process participate to the activation and chemoattraction of neutrophils, for example IL-8,leukotrienes (see further)[8,9].

The third line of defense consists of the second invasion of the macrophages in the inflames tissue. This constitutes a very important phenomenon as macrophages have a much stronger phagocytic ability compared to neutrophils themselves. However, this second invasion requires a much more lengthy process, as monocytes require over 8 hours in the inflamed tissue to swell to larger sizes and express their phagocytic mission. In addition, the storage pool in the bone marrow is much less than that of neutrophils.

The fourth line of defense includes the production of granulocytes and monocytes by the bone marrow. However it can take up to 3 or 4 days until the newly formed cells are able to leave the bone marrow. This procedure is induced by a large variety of biological factors. Nevertheless, these which seem to play the most significant role are tumor necrosis factor (TNF),interleukin-1 (IL-1), GM-CSF, G-CSF and M-CSF. The combination of all factors seems to pro-

vide a powerful feedback mechanism which gradually leads to the removal of the cause of inflammation[9].

Biological factors and mediators during inflammation

There is a large number of mediators and chemoattractants during inflammation. Mediators can be divided in two basic categories: plasma derived mediators and cell derived mediators. During this article we will try to focus to those which seem to play the most important role in the inflammatory process.

Plasma derived mediators

Bradykinin

Bradykinin is a mediator produced by the kinin system. It is a peptide which has multiple functions and effects. There are two subtypes of bradykinin receptors, B1 and B2 (BDKRB1 and BDKRB2). The B1 receptor encoding is regulated following tissue injury and inflammation. The respective gene contains three exons separated by two introns[10]. Receptor binding increases the cytolytic calcium ion concentration resulting in acute and chronic inflammations[11]. B1 receptor seems to play an important role in the initiation and maintenance of pain. That's why bradykinin inhibitors are being developed as pain-reducing agents in animal models. Some studies show that these B1 receptor-antagonists have no analgesic effect for incision induced pain[12.] Moreover, B1 receptor plays a functional role in the processes regulating the initial steps of leukocyte extravasation and leukocyte trafficking. The B1 receptor activation induces interactions between circulating leukocytes and the venular endothelium leading to leukocyte emigration from postcapillary venules[13].

The B2 receptor is related with bradykinin's vasodilatory role. Studies in dogs showed that the B2 receptor activation increases the vascular permeability and blood flow. This seems to be achieved through the release of an endothelium-dependent relaxing factor that may be nitric oxide[14].

C3

C3 is a protein of the immune system which plays a central role in the complement system. C3 cleaves to produce C3a and C3b. Receptors to C3a are expressed in many cells such as leukocytes, platelets, mast cells and astrocytes. Only one form of C3aR has been detected in human. C3a induces more unique or different signaling events in eosinophils, and perhaps monocytes, than it does in neutrophils despite the presence of C3a receptors on each of these cell types. Earlier it was widely thought that C3a can induce degranulation ,aggregation and chemotaxis of human neutrophils. However, recent studies show that the eosinophils stimulated by C3a release factors are able to stimulate neutrophils. C3a also activates mast cells rendering them capable of releasing histamine which causes vasodilation during inflammation. However, studies show that the primary site for C3a on the mast cell is the enzyme chymase. In other words, mast cell activation by C3a appears to be C3aR-independent[15].

C3b interacts with complement receptor 1, (CR1) functioning as an opsonin for phagocytes. It is able to bind to bacterial cell walls and marks the invader as a target for phagocytosis[16]. C3b cleaves C5 into C5a and C5b.

C5a

C5a plays a very important role in the development of many inflammatory diseases such as rheumatoid arthritis, lupus, sepsis, lung injury etc. C5a was first described as an anaphylatoxin able to cause rapid release of histamine and tryptase from mast cells and chemoattract macrophages during inflammation. Nowadays it is thought to have multiple function. It is considered a pleiotropic molecule as all cells of the myeloid lineage can be stimulated by C5a. There are two receptors for C5a. In neutrophils C5a leads to activate the p21-activated kinases (PAK). The PAK family members are involved in altering the cell morphology and chemotaxis. Moreover, C5a activates the transcription factor cAMP response element binding protein (CREB). CREB activation has been proposed to be a part of the mechanism by which C5a can delay neutrophil apoptosis and prolong an inflammatory response. The development of effective small molecule antagonists for C5a receptors is an attractive alternative. The usage of such compounds has offered a lot of help in order to understand how C5a gets involved in the inflammatory process. Nevertheless, although

C5aR antagonists may be beneficial for some existing inflammatory conditions they can also have some side effects, for example despite their protective role in the sensitization phase of asthma, they can make patients become more easily sensitized to new pulmonary allergens[17].

Hageman Factor (Factor XII)

Among coagulation factors it seems to be playing the most important role during inflammation. The Hageman factor normally exists inactive in plasma and is encoded by the F12 gene. A mutation in this gene can lead to factor XII deficiency. However, it remains unclear why people with such a deficiency do not experience abnormal bleeding like those with deficiencies of other coagulation factors. Of course, such patients' blood takes longer to clot[18].

The activated, due to endothelium damage, platelets release inorganic polyphosphate that directly bind and activate the Hageman factor. The activated factor can stimulate the kinin system and lead to bradykinin production which causes vascular permeability and pain as mentioned above. Through the intrinsic pathway of coagulation the factor initiates fibrin formation which is important for thrombus formation. In addition, Hageman factor stimulates plasmin, able to break down fibrin clots[19]. Experiments conducted in mice show that cultured hepatocytes transcribe, synthesize and secrete authentic factor XII[20].

Thrombin

Thrombin is a serine protease that plays a significant role in the coagulation procedure. It cleaves fibrinogen to insoluble fibrin. Thrombin is considered a polyfunctional tool during inflammation. It induces the activation of factors V, VIII, XI and platelet aggregation. Many cells participating in tissue repair such as monocytes, T-lymphocytes mast and nervous cells are stimulated by thrombin. Thrombin has the ability to release nitric oxide and prostacyclin from endothelial cells and cause vasodilation. The PAR-1 receptors of thrombin play an important role to the inflammatory process. The activation of such receptors can trigger many various transducing systems. It also stimulates the expression of IL-8 in human monocyte cell culture and expression of IL-6 and G-CSF in fibroblasts. Thrombin can activate adhesion and aggregation of platelets, release of growth factors from cells, adhesion and recruitment of leukocytes. Various studies show that thrombin can somehow interact with mast cells. However, little is known about how this is achieved. Thrombin causes degranulation of mast cells during inflammation. This may be mediated by stimulation of mast cell PAR-1 receptor. The latter is not yet proved but requires further experimental studies. In conclusion, thrombin functions as a conductor of cell responses during inflammation and reparative processes in tissues[21].

Cell derived mediators

Histamine

Histamine is the most significant vasoactive amine during inflammation. It is secreted by mast cells, platelets and basophils. Histamine performs its action by combining with special histamine-receptors. There are four types of such receptors, H1-H4 receptors. Every cell type expresses at least one of these receptors. Former studies showed that the only exception had to do with mast cells as it was thought that such cells could not express any receptors. H1 receptors can trigger a number of intracellular events characterized by changes in free cytosolic calcium levels. Such functions take place during smooth muscle contraction and separation of endothelial cells. Stimulation of H2 receptors results to elevation of cAMP, leading to vasodilation[22]. H3 receptors are mostly located in the central nervous system. Their function leads to decreased neurotransmitter release such as serotonin and acetylcholine and final inhibition of cAMP[23]. In opposition to what was formerly believed, H4 receptors are expressed in mast cells. Through these receptors, histamine causes chemotaxis of mast cells. Their function is also related to decrease in cAMP[24]. As mentioned above, histamine is mainly released by mast cells and platelets. The pathophysiologic mechanism of this is immunologic. These cells are sensitized by IgE antibodies which are attached to their membranes and they degranulate. Histamine is finally released from mast cells' cytoplasmic granules[25].

Interferon-gamma (IFN-γ)

Interferon Gamma is also called immune or type II interferon. It has the ability to regulate nearly all phases if immune and inflammatory responses. In contrast to type I interferons IFN-γ is more important as an immunoregulator than as an antiviral agent. It increases the cytotoxic activity of T cells, macrophages and natural killers. It also seems to be enhancing the antigen-presenting function of macrophages[26]. IFN-γ is secreted from CD4+ and CD8+ T cells and Natural Killers. It is IL-2 and IL-12 which stimulate such cells to produce IFN-γ. The most necessary step of the IFN-γ biochemical pathway is the interaction with the receptors located on the surface of the cells. There is actually an IFN Receptor complex, consisting of two alpha chains and two beta chains. Signal transduction is mediated by Jak1, Jak2 and STAT1a which initiates the transcriptional events[27]. IFN-γ enhances the microbicidal function of macrophages through formation of nitric oxide. It promotes the differentiation of naïve helper T cells into Th1 cells. Interferons are used therapeutically by injection in the blood stream. The main priority of clinical research for the future is the combination of interferons with other drug therapies.

TNF-a

TNF-a is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. TNF-a has multiple roles such as regulation of immune cells, inflammation and apoptotic cell death. During the inflammatory process TNF-a is mainly secreted by macrophages or monocytes that can induce the necrotic cell death. TNF-a can proliferate and differentiate many types of cells. This cytokine can trigger many similar inflammatory reactions such as fever or activation of fibroblasts. It can function as a pyrogen through direct action or by stimulation of IL-1 secretion. All these actions are performed by TNF-a through specific members of the TNF receptor superfamily. Two receptors can be found by TNF-a. TNF-R1 is expressed in most tissues, whereas TNF-R2 is located only in cells of the immune system.

Once TNF-a binds with the correspondent receptor, the receptors form trimers. This binding enables an adaptor protein called TRADD and that's how three pathway can be initiated: activation of NF-kB, activation of the MAPK pathways, induction of death signaling. TNF-R1 is involved in death signaling. However, during the inflammatory process TNF-a plays only a minor role as far as the latter is concerned. TNF-a is also produced by lymphoid cells, mast cells and endothelial cells[28].

IL-1,IL-8

Interleukin-1 is a cytokine with a wide spectrum of metabolic activities. It binds to the IL-1 receptor. There are two types of IL-1, IL-1a and IL-1b. IL-1a is mostly known for its synergism with TNF-a as mentioned above. There are only few cases of its action where such a synergism has not been detected. IL-1a mainly induces TNF-a release by endothelial cells and causes proliferation of smooth muscle cells.

IL-8 is mainly secreted by macrophages and works as a chemotactic factor. IL-8 attracts neutrophils at the site of inflammation. Neutrophil infiltration into inflammatory sites is one of the hallmarks of acute inflammation[29]. In neutrophils, IL-8 can induce increase of intracellular calcium, exocytosis, respiratory burst. IL-8 can have a weak impact on monocytes and eosinophils. The molecular mechanism in which IL-8 is involved to mediate its signal is the induction of NF-kB through a unique pathway in a similar way as TNF-a[30].

Nitric oxide

Nitric oxide plays an important role in various biological systems. It is synthesized from the amino-acid L-arginine via the enzyme nitric oxide synthase. Such synthases are widely expressed in a large variety of cells such as immune cells but also from cells outside the classical immune system. The main stimuli for nitric oxide synthases induction include interferon-γ, TNF-a and other mediators of inflammation.

During inflammation nitric oxide is known as the endothelium-derived relaxing factor. It can oxidate the iron containing proteins and activate the enzyme leading to cyclic guanosine monophosphate formation, cGMP. High levels of cGMP are responsible for many cellular actions of NO, such as muscle relaxation, platelet aggregation and adherence. Induced macrophages can produce large amounts of NO and

this can have a direct impact on DNA of target cells, resulting to fragmentation.

Another important chemical reaction during inflammation is this between NO and O_2^-. As a result, peroxynitrite, $OONO^-$ is produced. $OONO^-$ is a potent oxidant and can lead to production of free radicals. This can cause a number of effects on cells such as modification of structural proteins and lipid peroxidation of membranes[31].

Nitric oxide is considered an antianginal drug. It causes vasodilation and decreases the amount of volume that the heart has to pump.

METHODS OF DETECTION OF BIOLOGICAL FACTORS

There Is a large number of methods to detect the biological factors mentioned above. However, we will try to focus to the most significant and the most widely accepted.

Bradykinin

A quite sensitive technique for detecting and quantitating fluorescamine-labeled kinins and their products is the high performance liquid chromatography. Bradykinin is labeled with fluorescamine subjected to the chromatography and was scanned for the fluorescence signal with excitation at 390 nm and emission at 476 nm. During this process a system of methanol-triethylammonium formate buffer is used, in order to separate the fluorescamine-labeled kinins. Labeled kinins are eluted in the following order: bradykinin, Lys-bradykinin, Met-Lys-bradykinin. At the same time, unlabeled kinins can be subjected to high performance chromatography and the order of elution is Lys-bradykinin, bradykinin and Met-Lys-bradykinin. These two different patterns provided with these two different methods is a way to identify purified bradykinins. The fluorescamine label is a much more sensitive method to detect bradykinin and its metabolic products[32].

There are of course various other methods for the quantitative determination of bradykinin in blood samples such as the ELISA, which has been developed as an enzyme linked immunosorbent assay. According to the latter, bradykinin in a sample and peroxidase labeled bradykinin are allowed to react competitively to anti-bradykinin antibody captured by anti-rabbit IgG antibody coated in microstrip wells. Bradykinins concentration is determined from the enzyme activity of peroxidase labeled bradykinin bound to anti-bradykinin antibody.

High levels of Bradykinin can be measured in various situations such as acquired and hereditary angiooedema.

C3

In the past, different methods were used to detect C3. C3 bound to sheep erythrocytes reacts with anti-C3 antibody. This complex can be detected quantitatively by the grade of lysis which occurs when whole complement is added to it. The C3 to be measured is added to a standard amount of anti-C3 antibody. The absorbed antiserum is infused to sheep erythrocytes and then the lysis takes place after adding pig complement. The results are compared with the curves acquired by known C3 antigen. This method can detect even very low concentrations of bound C3[33].

Uncleaved C3 and its fragments, C3a and C3b can be detected using monoclonal antibodies through the development of sandwhich-enzyme-linked immunosorbent assays. The ELISA method can be used to detect uncleaved C3 in human plasma, whereas the addition of EDTA in plasma is used to detect C3a and C3b fragment. This method has a high grade of sensitivity and specificity. The detection limits for both C3 and C3a are 1ng/ml[34].

C3b can be detected through real time techniques. A reaction between antigen and antibody takes place on a reflective surface within a flow cell. This immunoreaction occurs between mouse monoclonal antibody and known concentrations of purified C3b in human serum. The rate of the signal changes is monitored during the first 60 seconds of the reaction. Through this method the lowest detectable concentration of C3b is 20ng/ml[35].

Levels of C3 may be measured to support or refute a particular medical diagnosis. For example, low levels of C3 are associated with some types of kidney disease, such as post-infectious glomerulonephritis and shunt nephritis.

C5a

The usual method to detect C5a is the ELISA method using monoclonal antibodies, in the same way the method detects C3a and C3b as mentioned above.[34] However, the direct quantitation of C5a/C5a-desArg in human plasma was achieved through the ELISA method, using the specific monoclonal antibody C17/5. C5a-desArg is a metabolite of C5a which is a much less potent anaphylatoxin. The antibody C17/5 blocks the binding of C5a/C5a-desArg to its cellular receptors and that's why it can provide useful information as far as the action of C5a is concerned, in complex biologic systems. The lowest detection limit of C5a using this method is 20pg C5a/ml plasma[36].

In order to quantify C5a/C5a-desArg in bovine biological fluids using the ELISA method with monoclonal antibodies, zymosan can be used. Zymosan is a potent activator of the complement cascade, used to generate C5a/C5a-des Arg[37].

C5a is worth to be measured in cardiac anaphylaxis, psoriasis and atopic dermatitis.

Hageman factor (Factor XII)

The two main methods for the detection and the quantitation of the Hageman factor are the immunobinding assay and the ELISA method. For the quantitification of factor XII, an immunobinding assay on nitrocellulose has been developed. Plasma samples are dotted on nitrocellulose filters and the Hageman factor is detected using a radiolabelled antigen overlay. The radioactivity of the bound with I^{125} Hageman factor is used for its quantitification. Normal human plasma or purified factor in to factor XII deficient plasma are used as standards. This method can detect factor XII concentration corresponding to 1% of the level in normal human plasma. The advantages of the immunobinding assay are the fast quantitification of large number of samples and its high sensitivity[38].

The ELISA method employing 2/215 mouse monoclonal antibody has been used to detect the activated Hageman factor in patients with renal failure, pregnancy and diabetes compared to a control group[39].

The main indication of measuring factor XII is to diagnose the Hageman factor deficiency. This disorder is thought to be benign and usually presents no symptoms.

Thrombin

Thrombin can be detected through different nanomaterial and thrombin-aptamer based detection methods. Such methods usually use cerium oxide nanoparticles[40]. However, there is also a label-free, colorimetric assay, using gold nanoparticles and fibrinogen. When fibrinogen is added into a solution of gold nanoparticles electrostatic and hydrophobic interactions take place, leading to the formation of agglutinations. In the presence of excess fibrinogen, the addition of thrombin induces the formation of insoluble fibrillar fibrin-gold nanoparticles. This occurs through the polymerization of the conjugated and unconjugated fibrinogen. High concentrations of thrombin, decrease the absorbance of the supernatant after centrifugation and that's how the quantitative detection of thrombin is achieved. The last method can provide lower limits of detection comparing to the aptamer based detection methods[41].

The ELISA method has been used in order to detect thrombin-hirudin complex and thrombin-antithrombin complex in plasma and in purified systems[42,43].

Thrombin must be measured to detect any acquired or genetic thrombotic disorder or support the development of antithrombotic drugs.

Histamine

Histamine can be determined in plasma by liquid chromatography and electrochemistry. The method is based upon deproteination of plasma. Plasma proteins are precipitated by adding concentrated perchloric acid. After centrifugation, the deproteinized supernate is adjusted to ph 7.5. Standards containing histamine and 3-methylhistamine are used as standards and are prepared in the same way. The samples are then purified on cation-exchange resin. The eluate is evaporated and histamine in the sample is condensed with o-phthalaidehyde and 2-mercaptoethanol at ph 11.5. The adduct is then separated with liquid chromatography and detected electrochemically. This method provides high sensitivity in the quantitation of histamine. In contrast to radioenzymatic assay the technique requires no histamine N-methyltranferase. The method can determine histamine in 25 plasma samples once they have been prepared for injection in chromatography, within 8 hours[44].

The radioimmunoassay is another method used for detection of histamine. The double-antibody RIA can be used for studies of histamine release in both whole blood and cell supernatants. Nevertheless, it is not suitable for detection of histamine in plasma due to cross-reactivity with N-methyl-histamine. The latter is an enzymic product of histamine released from basophils. The radioimmunoassay using succinyl-glycine as a reagent is the most sensitive method for detecting histamine in plasma[45].

High histamine levels can be detected in allergic reactions, in mastocytosis and in various autoimmune disorders.

Interferon-gamma

The ELISA method is a very sensitive method to determine IFN-γ[46]. Its sensitivity is less than 2pg/ml and it does not cross-react with other human interleukins and mediators. IFN-γ is a selective marker for tuberculosis pleurisy. That's why the electrochemical detection of it was achieved, through a simple and direct electrochemical sensor. The method uses interferons RNA and DNA aptamers in order to detect it[47]. Moreover, immunohistochemical methods were used in order to detect IFN-γ and cells producing it. Such methods use a large panel of anti-IFNgamma antibodies to identify the IFNgamma-positive cells through immunoenzyme double staining. However, such method proves to be unreliable because of lack of specificity to stain T-cells in situ. Information provided by immunochemistry must be interpreted with great attention[48].

The detection of human IFN-γ soluble receptors can be achieved through a dot blot assay using human IFN-γ labeled with ^{32}P[49].

A quite interesting indication to detect IFN-γ is in pleural fluid, in order to detect tuberculosis.

TNF-a

The production of TNF-a by human peripheral blood mononuclear cells can be measured through a radioimmunoassay for human TNF-a. A rabbit antiserum against human recombinant TNF-a is used. The human TNF-a is labeled with Na^{125} . This method does not detect other interleukins and chemokines. Human mononuclear cells are exposed to different concentrations of lipopolysaccharide. Without an external stimuli, TNF concentrations were found below the detection limit, whereas as the lipopolysaccharide concentration increased, so did the concentration of TNF-a detected. The median concentration of TNF-a detected with this method was 2ng/ml[50].

TNF-a can of course be measured using the ELISA method. The traditional technique uses the spectroscopic detection of a chromogen such as tetramethylbenzidine. Horseradish peroxidase bound to the detection antibody catalyses the oxidation of tetramethylbenzidine to generate colored products. However, the traditional colorimetric detection can be substituted with resonance Raman spectroscopy. The achievement is 50 times lower detection limits and the potential for multiplexed analysis is higher[51].

Increased levels of TNF-a can be detected in patients with chronic obstructive pulmonary disease and with type 1 diabetes mellitus.

IL-1, IL-8

A very sensitive method for the quantitation of IL-1 is a solid-phase radioassay based. The method is based on the capability of IL-1 to bind the nitrocellulose-immobilized IL-1 receptor solubilized from plasma membranes of a subclone of the human B cell lymphoma Raji. The assay has the ability to detect only the biologically active IL-1 and can detect low levels of human IL-1. Moreover, the sensitivity for IL-1a and IL-1b is even higher. The method can test large numbers of samples simultaneously[52].

IL-8 can be quantitated through a sensitive sandwich ELISA. A polyclonal antibody to human IL-8 was raised in rabbits and the IgG was isolated from the antisera using a protein A column. This method can detect even picogram quantities of IL-8. The lower sensitivity limit was found to be 84 pg/ml[53].

IL-1 and IL-8 are found in high levels in a variety of chronic inflammatory diseases.

Nitric oxide

As well known, NO can be oxidated to NO_2^- and NO_3^-. Nitrite and nitrate has been used as an index of endothelial NO synthase activity. NO can be indirectly determinated through the spectrophotometric measurement of nitrite and nitrate. Nitrate must first be reduced to nitrite and then nitrite is measured by the

Griess reaction. N_2O_3 is generated from nitrite and nitric oxide and then reacts with sulfanilamide to yield a diazonium derivative. This reactive intermediate will interact with N-1-napthylethelene diamine to yield a colored diazo product that absorbs strongly at 540nm.

Various fluorometric methods have been used for the detection of nitric oxide. One interesting method uses 2, 3-diaminonapthalene as an indicator for NO formation. 2, 3-diaminonapthalene reacts with N_2O_3 to yield the highly fluorescent product 2, 3-napthotriazole. This assay has a high grade of specificity and sensitivity. It can detect very low levels of 2, 3-napthotriazole and may be used for the quantitation of NO in physiological fluids and tissue culture media.

In any case, the detection and quantitation of NO remains a controversial issue. The mechanisms of detection, as well as limitations of every methodology is very important to interprete the data given by every method[54].

Chronic expression of NO is associated with various carcinomas and inflammatory conditions such as multiple sclerosis, arthritis and ulcerative colitis.

CONCLUSION

All the factors mentioned above constitute very important tools for the clinical doctors, in order to diagnose rare diseases and understand the inflammatory processes that can take place in a pathophysiological level. However, the methods of their detection can be improved and provide useful information both in medical and research level.

Βιολογικοί παράγοντες φλεγμονής και μέθοδοι προσδιορισμού τους.

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ΠΕΡΙΛΗΨΗ: Η φλεγμονή είναι μια περίπλοκη διαδικασία που συμβαίνει μετά από κάποιο βλαπτικό ερέθισμα ή λοίμωξη. Κατά τη διάρκειά της, συμβαίνει μεγάλος αριθμός βιοχημικών αντιδράσεων και παθοφυσιολογικών αλλαγών. Αυτές οι διαδικασίες επάγονται από βιολογικά ενεργά μόρια που καλούνται μεσολαβητές ή χημιοκίνες. Τα μόρια αυτά παράγονται στο πλάσμα ή από κύτταρα κατά τη διάρκεια της φλεγμονώδους διαδικασίας. Κάποια από αυτά έχουν την ικανότητα να αλληλεπιδρούν μεταξύ τους. Σήμερα υπάρχουν διάφορες μέθοδοι ανίχνευσης και ποσοτικοποίησης τους. Οι τεχνικές αυτές εξελίσσονται και βελτιώνονται. Το ποια είναι η πιο κατάλληλη εξαρτάται από τον επιστημονικό σκοπό του κάθε ερευνητή.

Λέξεις Κλειδιά: Φλεγμονή, Μεσολαβητές, Χημιοκίνες, Βιολογικός προσδιορισμός.

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