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The biological and clinical role of the C-reactive protein in the diagnosis of trauma in a literature review

The C-reactive protein (CRP) is produced in the organism as a result of an acute phase reaction (APR) in response to an infection, trauma, anoxia, necrosis and sudden tumour growth as well as thermal and chemical damages. Its plasma concentration rises in the case of infectious diseases, inflammations and tissue necrosis (3, 4, 10).

CRP is one of the most prominent acute phase proteins, a permanent component of the organism, which is present in trace amounts in the plasma of healthy people and animals in physiological conditions. It is present in both the vertebrate and invertebrate world (5, 9). In the course of the evolution the primary CRP structure was not modified, which suggests an important role of this substance in fulfilling life functions of organisms (2, 4, 7, 11).

The gene responsible for the synthesis of the C-reactive protein is found on the chromosome 1 (6, 8, 10). Its expression activates the production process in the liver, at first in periportal hepatocytes and as the acute phase reaction intensifies, in all hepatocytes (6). The description stimulation of macrophages caused by tissue damage is an indispensible condition for the synthesis induction (4, 6, 10). These cells produce cytokines, which induce the expression of the human acute phase protein gene. Interleukin 6 (IL-6) is the most important substance stimulating CRP synthesis and all other acute phase proteins (3, 6, 10). Others operate selectively and induce the production of only specific proteins. Interleukin 1 (IL-1), as well as the tumour necrosis factor alpha (TNF-α) reinforce indirectly the CRP occurrence, because they stimulate macrophages, monocytes, fibroblasts, endothelium cells and others to produce IL-6 (5, 6).

C-reactive protein belongs to the pentraxin group, named thus for an identical subunit structure. The CRP molecule is built from 5 identical protomers arranged on a singular plane in the form of a regular pentagon, connected with non-covalent bonds (1, 3, 6, 7, 9). Its structural properties are only maintained in the presence of Ca^{+2} ions, which can simultaneously block the bonding of polycations (3, 7, 9). CRP derived from different individuals does not exhibit genetic polymorphism, conditioned by a permanent sequence of 206 amino acids (1).

CRP was discovered by means of the x-ray crystallography method. Each protomer possesses β-antiparallel rolled sheets and flattened structures (1, 2). The ligand-binding side is found on the surface of the concave subunit and contains two calcium ions in the presence of which the reaction with phosphocholine (PC) occurs (Fig. 1). CRP also binds with substances containing PC, e.g. phospholipids and their analogues which are widespread in the animal world. It creates compounds with molecules that do not possess PC such as chromatin and histones (3, 4, 7, 11). These reactions lead to an aggregation and precipitation of cellular or molecular structures containing ligands (4, 7, 9). Research on CRP mutants shows that Phe^{66} and Glu^{81} amino acids are those that bring in their own essential part in binding CRP with PC, probably through mutual interaction with its choline

group in special hydrophobic pockets (1). On the opposite surface of each C-reactive protein subunit a second binding site with a C_{1q} complement element and possibly a Fcγ receptor IgG is located (1, 4, 11). The depression stretching from the middle of the protomer to the central point of the pentamer is significant in binding CRP and C_{1q}. Asp^{112} and Tyr^{175}, which take part in this binding, were discovered on its edges (2). A 3-dimentional structure of the globular region of C_{1q} head that explains the way of binding was proposed. C_{1q}-CRP binding model displays complementarity of the head to the depression, that is the globular C_{1q} head matches the central CRP pore and interacts with 2 out of 5 protomers of the pentamer. It is possible, as the upper part of the C_{1q} head which is charged positively interacts with the negatively charged central pore of the CRP pentamer (2).

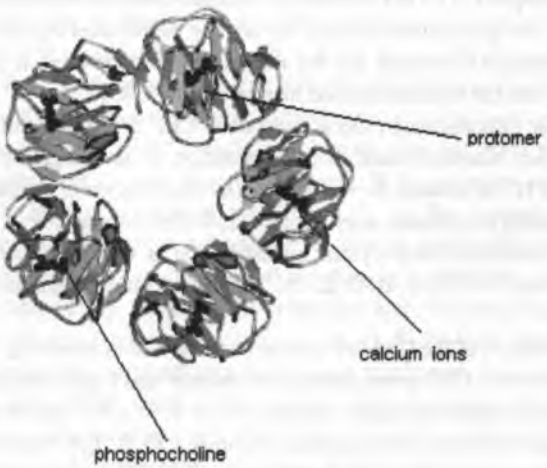

Fig. 1. Crystal structure of the C-reactive protein in a complex with phosphocholine and calcium ions

THE MEANING OF CRP IN HUMORAL AND CELLULAR NON-SPECIFIC IMMUNITY

The hitherto research of many years did not lead to a full explanation of the biological role of CRP in the human organism. Basing on numerous animal tests it can be said that it fulfils a defensive and reparative role (3, 4, 9, 7,10). The bacterial infections accompanying tissue necrosis are the cause of an infectious state in the place of injury, as well as an intensive multiplication of pathogens.

At this stage the specific immunological response consisting of the antibody production does not occur immediately and its appearance requires time. The humoral non-specific immunity, where an important part is played by acute phase proteins such as CRP, SAP, SAA and fibrinogen, as well as some complement components, does nonetheless work. Non-specific cellular mechanisms are activated first, the antigen is recognized by macrophages, monocytes, polymorphonuclear neutrophils and is attacked and destroyed after being coated by opsonins. The bound antigen undergoes lysis in the processes of agglutination, precipitation, the swelling of bacterial cell walls and phagocytosis by phagocytic cells. CRP, acting as an opsonin, accelerates the pathogen absorption (3, 4, 7, 9).

CRP binds with FcγR receptors of human monocytes, neutrophils and other leukocyte system cells by activating them (11). The cell stimulation results in various effector functions, e.g. in the cytotoxic effect, phagocytosis, oxidative burst or the release of inflammatory mediators, mainly

IL-1 and TNF (3, 11). In laboratory conditions in mice 3 types of receptors were described, the same for CRP and IgG. High affinity FcγRI binds with monomeric IgG. This receptor is induced by cytokines and INF-γ, contributing to phagocytosis of the IgG. FcγRII and FcγRIII are a low affinity IgG receptors that bind immune complexes. The process of phagocytosis is the result of integration, oxygen explosion and degranulation (4, 10, 11). At this stage the anti-inflammatory effect of CRP may be caused by binding phagocyte receptors (1, 11). Two basic classes of FcγRs are known today, stimulating (ITAM) and inhibiting (ITIM) receptors. The stimulation of ITAM containing FcγRs initiates immunological response – phagocytosis, cytokine and reactive oxygen forms secretion. ITIM containing FcγRs block ITAM activity. CRP through its direct influence on macrophages and through the complement activation increases leukocyte phagocytosis probably by binding with FcγRs receptors (1). The C-reactive protein decreases the number of pathogens and their toxicity by beginning the degradation reaction. By binding the released components of cell nucleus, including the defragmented chromatin and the nucleosome particle core, it protects the organism from absorbing them into the bloodstream and immunization (3, 8, 9).

Similarly to other inflammatory process mediators, CRP displays pleiotropic behaviour. The anti-inflammatory effect manifests itself in the stimulation of an antagonistic receptor IL-1. The increased secretion of IL-10 induces the maturation and proliferation of thymocytes and peripheral lymphocytes, diminishing the inflammatory reaction (1, 4). Pro-inflammatory CRP functions include the complement activation and phagocytosis intensification as well as the heightened secretion of pro-inflammatory kinins IL-1, IL-6, IL-8, IL-18, TNF-α and plasminogen inhibitor-1 activator (1, 3, 4, 10, 11).

The defensive function of the C-reactive protein and its role in removing foreign material from the organism is known best. CRP in the presence of calcium ions binds with polysaccharides of the pathogen organisms and other cell ligand structures (3, 4, 7, 9). CRP deposition on apoptotic cells mobilizes macrophages to phagocytosis and later elimination (3, 8, 9). C-reactive protein complexes with adequate antigens and ligands cause a complement cascade mainly on the classical pathway (1, 3, 9). CRP interaction with the element of the C_{1q} complement, which initiates the reaction, has precise spatial requirements and is possible due to a minimal deformation in CRP structure. These conformations vary, depending on the ligand type with which the protein is associated and on the localization in the ligand binding site (1, 4, 7, 9).

The binding of an immunological complex (antigen-antibody), as well as the ligand-CRP bindings to C_{1q} initiate a complement cascade in a similar way. It has been proved in experimental research that the role of CRP in the complement activation is limited for the main part to the initial phase C_1-C_4 and to a small degree to C_5-C_9. The process takes a different course in the case of complement initiation by immunological complexes, which activate mainly the later proteins. The antigen-CRP complex decreases the passing to the environment and creation of C_5-C_9 on an alternative complement pathway. The decrease of C_{3b} formation and lysis processes of the target antigen cell are consequences of this reaction (1). These C-reactive protein activities are probably associated with the reaction possibility of CRP with H factor, as a result of which the activation pathway runs by the C_5-convertase formation. CRP limits in this way the number of active complement proteins bound with C_{5a} and C_5-C_9 forms, displaying cytotoxic activity (1, 3). Taking part in the organism's defensiveness it simultaneously limits and controls the inflammatory response. CRP stimulates also endothelial cells to secrete three complement system inhibitors – the decay-accelerating factor (DAF), the membrane co-factor protein (MCP) and the CD59 (Li), and thus decreases the number of active complement proteins (6). The C-reactive protein may help in diminishing the inflammatory response by activating the complement and by assuring opsonization and minimal C_{5a} and C_{5b}-C_9 complements formation, which are responsible for the 'attack' of the cellular membrane of the antigen (1, 3, 10).

According to James (4) the C-reactive protein possesses some biological properties similar to antibodies. By binding with the antigen it enables its phagocytosis and subsequently precipitation and destruction. CRP-antigen complexes activate the complement cascade, analogically to the antibody-antigen complexes. It displays opsonin abilities. Liver is the production site, which determines the autonomy of this substance from immunoglobulins. CRP may be considered as a form of a primitive antibody, which has the specificity for compounds contained in the cell wall of pathogenic organisms.

CRP IN A SPECIFIC IMMUNOLOGICAL RESPONSE.

The influence of CRP on the specific immunological response is a disputable matter. *In vitro* research did not demonstrate an unambiguous activity. Most researchers believe that CRP binds lymphocytes and stops their proliferation (3, 7), others are of the opinion that it does not display such an influence or contrarily, that as a result of binding with lymphocytes it may come to their stimulation (7). It is known that in the presence of inflammatory factors or antigens in the organism CRP in a complex with phosphocholine (CRP-PC) binds with lymphocytes (9). As a result of this reaction the number of T lymphocytes (mainly suppressive ones), B lymphocytes and zero lymphocytes undergo a decrease. The binding mechanism is not known (7, 10). The C-reactive protein also exerts an immunosuppressive influence on the antibody production by stimulating immunosuppressive T lymphocytes (7). The influence on immunological reactions is a debatable question. CRP releases specific immunity indirectly, by activating macrophages and the complement cascade, which intensifies the inflammation in the area of the tissue injury (3, 4). Antigen coating contributes to its phagocytosis and subsequently fragmentation enabling its binding by immunological cells. These are antigen presenting cells (APC), which posses on their outer cellular membrane surface a binding site for the defragmented antigen (an epitope). It is bound with the class II histocompatibility antigen HLA. It can be presented only in this form to T-helper lymphocytes, which being stimulated begin differentiating into various lymphocyte populations (4).

CRP BLOOD PLASMA CONCENTRATION AND ITS CLINICAL SIGNIFICANCE IN TRAUMA

Skeleton trauma is one of the reasons of acute phase response (APR) advancement in the organism. The early defensive response is aimed to limit the work of the destructive factor and mobilize the subsequent tissue repair. After injuries to the bone many systemic and local factors partake in the process of healing. During the first phase of bone tissue regeneration the main role is assigned to platelets, macrophages and monocytes, which are the source of mediators and active factors that provoke inflammation of the damaged tissue (3). As a result of the activity of the secreted by them IL-1, IL-6, TNF, an acute phase response is stimulated and the production of defensive proteins is induced in the liver (3, 10). The C-reactive protein is both the earliest and strongest of the APR substances, for it occurs already 2 hours after the injury and reaches its maximum concentration in 48–72 hours. In the cases without complications it returns to the norm after 6–7 days (4, 8, 9, 10). As a consequence of soft and hard tissue injury, local inflammatory conditions develop in the organism, with all their symptoms and effects. CRP is a protein that responds very dynamically. A rapid, high growth and return to the norm may be indicators of success of the treatment method or the evaluation of the symptom regression after operative treatment. Persistent or sudden CRP value growth is, however, an early laboratory symptom of a threatening infection (8, 9). Heightened value occurs already several hours before the appearance of the first clinical symptoms (10). Reaching values 100, 1,000 times higher (25% in 24 hours) depicts a severe trauma or the course of the sickness (8).

Therefore, the acute phase protein is present in the blood serum after the initial trauma as well as the succeeding one, caused by the surgical intervention. The mobilization of defensive mechanisms and the receding of the inflammation are depicted in protein normalization (8). The bone union is a natural process which is necessary to restore regular bone function. In its course, filling in of the fracture fissure with bone tissue occurs and the recreation of the damaged anatomic structure continuity follows. This is a process of regeneration, for a proper bone is created as the end result. However, often despite using appropriate treatment techniques, healing problems occur caused by inflammatory complications (5, 8). CRP may be useful and helpful as an early, sensitive indicator of inflammation, available in the laboratory diagnosis of the hospitals. During the last years the usefulness of this parameter in diagnoses of infections has significantly increased.

The Department of Maxillofacial and Oral Surgery, Medical University of Lublin performed an investigation of CRP in facial trauma. The investigation included fifty-one patients with zygomatico-maxillo-orbital and single mandibular fractures. CRP reactions were routinely measured in the beginning, when the injured patient was admitted to the hospital and a number of times during the hospitalization and recuperation. In the course of the acute phase response in patients with maxillofacial fractures, it could be observed that CRP reached a value of 26.0 mg/l as a result of the trauma. The operative procedure of osteosynthesis triggered another peak in the protein value, equal to 23.3 mg/l. The normalization of CRP took 6.6 days in 81.6% of patients. The post-operative CRP value depended on the timing of the conduction of the surgical treatment and reached its maximum concentration in own examinations when the patient registered for hospital treatment up to 2 days after the injury. Hence, the procedure could have been conducted earlier, which influenced the obtaining of a higher value after the osteosynthesis.

Nowadays, new C-reactive protein measurement methods, which involve the highly specific CRP antibodies, are used. The result of the examination, depending on the type of the test, is obtained in 2 to 15 minutes (4, 8). For these reasons a renewed increase in the interest in the C-reactive protein can be discerned in Europe and around the world. This test is also used in Poland increasingly often, especially in cardiology and gynaecology. The presented literature has aimed at systematizing the known information and evaluating the usefulness of measuring C-reactive protein in trauma practice.

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SUMMARY

The C-reactive protein (CRP) is one of the most important defensive proteins that take part in the nonspecific immunity mechanism as a result of the acute phase response (APR). The presented literature aims at systematizing the known information and evaluating the usefulness of C-reactive protein measurements in clinical practice, especially after tissue trauma. The author also demonstrates own researches connected with CRP levels in patients with facial fractures.

Biologiczna i kliniczna rola białka C-reaktywnego w diagnostyce urazów w przeglądzie piśmiennictwa

Białko C-reaktywne jest jednym z najważniejszych obronnych białek biorących udział w niespecyficznej odporności organizmu, produkowanym w następstwie reakcji ostrej fazy. Praca próbuje usystematyzować wiadomości i ocenić przydatność oznaczania białka C-reaktywnego w praktyce klinicznej, głównie w następstwie urazów. Autorka przedstawia także badania własne stężenia CRP u pacjentów ze złamaniami czaszki twarzowej.