OSTEOARTHRITIS AND IMMUNITY

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Abstract. *Low-grade inflammation is part of the pathogenesis of osteoarthritis (OA) from its earliest stages and contributes to the acceleration of the degenerative process. Innate immunity has a leading role in it. Activation of the innate immune response is initiated by stimulation of the receptors on the cell membrane to recognize the secreted PAMPs (pathogen-associated molecular patterns). However, PAMPs can also be activated by endogenous damage-related molecular patterns (DAMPs). The group of DAMPs also includes toll-like receptors (TLRs). The disruption of matrix homeostasis in the course of OA is an example of activation of these receptors in chronic damage. The complement system is a key element of the innate immune system. It is one of the serum enzyme systems whose function is to opsonize antigens. The complement receptors on the surface of the cell membranes adhere to the targets for phagocytosis. The C3R fraction activates the complement cascade itself, as well as the oxygen metabolism of the cell, which is essential for the phagocytosis. The cartilage damage products released during joint damage are a separate class of potent complement modulators. Complement fractions bind to complement receptors on the surface of the chondrocyte and the synoviocyte cell membranes by TLR. The complement system is involved in many processes in the course of osteoarthritis: chondrocyte degeneration, ECM degradation, low-grade inflammation in the osteoarthritis, cell lysis, unbalanced bone remodeling, osteophyte formation, and neoangiogenesis. Whether drug control of complement activation may be a future therapeutic strategy in the treatment of OA and prevent its progression is a subject of future studies.*

Key words: *osteoarthritis, immunity, receptors, complement system, phagocytosis*

Low-grade inflammation is part of the pathogenesis of osteoarthritis (OA) from its earliest stages and contributes to the acceleration of the degenerative process. Innate immunity has a leading role in it. This fact is supported by multiple studies [1]. It is not connected to the expressed adaptive immune response.

Circulating macrophages, together with the other phagocytizing cells (polynuclei, Mo, Eo, OK, Ma in the spleen, lymph nodes, skin; Kupffer cells in the liver; mesangial phagocytes; microglial cells in the central nervous system, type-A synoviocytes in the synovium, histiocytes in the connective tissue) are the main cellular representatives of non-specific immunity. NK cells (neither B nor T, destroying viral-invaded or tumour-changed cells) as well as physical barriers (skin and mucous membranes) [2] also participate. Here is the place to note the role of dendritic cells as immune system coordinators as well. They are macrophages specialized as antigen-presenting cells and are a separate branch of myeloid order. They migrate from the bone marrow into different organs and tissues (usually contacting with the external environment) and they play the role of an immunological sensor. Being present in most tissues,

expressing TLR on their cell membrane, they active or suppress T-cells and are an important link between innate and adaptive immunity [3].

Activation of the innate immune response is initiated by stimulation of the receptors on the cell membrane to recognize the secreted pathogen-associated molecules – classical microbial ligands. These receptors are called PAMPs – pathogen-associated molecular patterns. Apart from the cases of infection, those receptors are recognized and activated with damaged cells and extracellular matrix [4]. In the conditions of degenerative joint changes, ECM components, fibronectin isoforms, fragments of hyaluronic acid are supposed ligands for PAMPs. In these cases, PAMPs can also be activated by endogenous damage-related molecular patterns (DAMPs), and not by microbial ligands. The group of DAMPs also includes toll-like receptors (TLRs), constructively expressed on the cell membrane of different cells, including macrophages – through TLR-4 [5, 6]. A number of the PAMPs-receptors of mammals are members of the TLRs family. A human-being has at least 10 TLRs. Different ligands active different TLRs. The disruption of matrix homeostasis in the course of OA is an example of activation of these recep-

tors in cases of chronic damage. TLRs 1-7 and 9 are found in the synovium in cases of OA and PA, and in cases of in-vitro studies synovial fibroblasts react to their activation [7, 8, 9, 10]. The cellular response which follows is through activating specific transcription factors, among which nuclear-factor κ B (NF- κ B) plays the main role. TLR activation in SM is a stimulus for an NF κ B activation, followed by production of chemokines (IL-8 and CCL5) and cytokines (IL-1, IL-6 and TNF), with association and activation of Ma, granulocytes, and lymphocytes [11]. Chondrocytes can also serve as targets for TLR-activation. A number of matrix metalloproteinases, produced by the cartilage in cases of OA, depend on the activation of NF- κ B [12, 13]. Despite the incompletely clarified role of TLRs in cases of OA, their targeting, as well as the ways of their activation are to be analyzed as potential therapeutic approaches in cases of OA.

The humoral components of innate immunity are the CRP and the complement system.

The complement system is a key element of innate immune system [14, 15]. It is one of the serum enzyme systems whose function is to opsonize antigens. This means easier antigen-immunocyte and antigen-antigen relations; preparation of the antigen for phagocytosis, increased membrane permeability, and mediation of the inflammation. In the past decade, the complement system has been reviewed as a bridge between innate and acquired immunity. It consists of more than 50 proteins and glycoproteins, including serum proteins and cell-membrane receptors, which are basically synthesized by hepatocytes [16] and in less amounts by tissue macrophages, by blood monocytes, by epithelial cells of gastrointestinal and urogenital tract, and by type-A synoviocytes. They represent around 5% of the globulin fraction of blood serum and circulate in blood as inactive precursors. Class-III HLA-gene molecules code some of the complement components: C2, C4, and C3 proactivators [3]. When an exogenic or an endogenic trigger activates the complement system, proteases cut complement proteins, activate them, and actuate the system's cascade. Activation is a chain reaction based on proteolysis, as system activity increases on each step of the cascade. The three ways of complement activation (classical, alternative, and lectin) have a common key stage of activation – C3-convertase protease. It cuts and activates the C3 component, creating C3a and C3b [17, 18]. The products from C3 decomposition may serve for assessing complement system activation. In difference to the specified C3 fragments, C3c

does not connect to other structures like pathogens, cellular receptors or plasma proteins, which is why it is a reliably stable biomarker for assessing C3 activation. C3-conversion in C3c is done for around an hour, under body temperature and, in difference to other C3-fractions, which are with a short semi-life, it is stable and used for assessment of the C3 levels in laboratory tests [19, 20]. The surface of many cells has complement receptors – CR. Their function is to adhere objects targeted for phagocytosis. The C3R fraction activates the complement cascade itself, as well as the oxygen metabolism of the cell, which is essential for the phagocytosis. Defects in C and CR (genetically determined) lead to a defect in phagocytosis, respectively, infections, autoimmunity, etc. [3].

C4 participates in the classical (properdin) way of complement activation (it is activated by specific antibodies in difference to the alternative one, which is activated without the presence of such). If, in case of inflammation, C4 is at a normal level, the probable way of activation is the alternative one. C4 is reduced in case of activated C3CT, autoimmune thyroiditis, bacterial or viral meningitis, streptococcus and staphylococcal sepsis, etc. It is increased in cases of chronic inflammation conditions, pregnancy, etc. [21, 22]. There are activation triggers for each way of complement cascade [23, 24].

The alternative way does not have a specific activation and is a major C3 component; the classical one is activated by a specific antibody and C4 is a main participant in it; while the lectin way is activated through carbohydrate recognition on the microbial surface (mannose-binding lectin – MBL and mannose-binding protein – MBP) [25, 26]. Complement activation is based on proteolysis and generation of small soluble fragments. The most common methods of quantitative specification of C3 and C4 measure intact proteins, as well as the major soluble fragments formed with activation [27]. The last unit of complement cascade activation is binding C5b with C6 and C7 in a membrane attack complex (MAC). MAC is a cytolytic end-product of the complement cascade, which, through C5b, adheres to the cell membrane and forms a transmembrane channel, which leads to an osmotic lysis to the target cell. Membrane lysis is accelerated by C9 (perforin) and a membrane channel, through which calcium ions, NaCl and water [3, 17, 18] penetrate, is formed. The final result of complement activation is lysis of target cells, opsonization, followed by chemotaxis of macrophages, and transportation of immune

complexes to the Kupffer cells in the liver and to the spleen, in order to purify them [28, 29].

The complement system, as a part of innate immunity, is one of the first lines of protection against external pathogens. Apart from this major, evolutionary determined protective function, it also plays a role in the removal of cellular remains and immune complexes, opsonization, and B- and T-lymphocyte stimulation [30]. The cartilage damage products released during joint damage are a separate class of potent complement modulators [31, 32, 33, 34]. Different ECM components and their fragments in the degenerative joints can also activate the complement [35, 36]. Fibromodulin [37], cartilage oligomeric matrix protein (COMP) [38] and osteoadherin [39] can activate complement cascade, both in the classical and in the alternative way. Complement activation in the joint can also be directly activated by a mechanical stress and with a local production of complement factors of type-A synoviocytes at the activation of arthritis. The data from analyses of joint traumas in the first several weeks after the trauma supports this thesis. [40, 41]. Other matrix components, such as NC4 domain of type-IX collagen, act like complement inhibitors [42, 43]. Lots of data from the last studies supports the thesis that low-grade inflammation in cases of OA includes TLR involvement and complement cascade activation, through the degradation products of joint destruction [44, 45, 46, 47]. The inflammatory synovial reaction which follows leads to a synthesis and release of a variety of cytokines and chemokines [48, 49]. Humoral factors of inflammation are a potent hepatostimulating factor for a CRP synthesis and other acute-phase proteins. CRP in the serum and undergoes calcium-dependent binding with protein molecules like phosphorylcholine, nuclear chromatin, and plasma lipoproteins with very low density and forms aggregates with them. These aggregates also activate a complement cascade, which activity grows for each next step of the cascade, until the formation of a MAC. This is followed by phagocytosis, transport to Kupffer cells and spleen and, as a final result – removal of foreign, damaged, and apoptotic cells. The maintenance of low-grade inflammation in an OA joint increases the metabolic activity of chondrocytes, including production of MMP, which, on their side, have a catabolic effect on the cartilage.

The major OA cells in the joint fluid are chondrocyte and synovocytes. Complement fractions bind to complement receptors on the surface of their cell membranes by TLR [50]. Sandeep Sila-

wal asks the question whether the maintenance of balanced complement activation can represent a future therapeutic strategy of OA therapy and prevent its progression [51]. A team of Bulgarian immunologists have also contributed about that. In 2018, they published their observation on mice with collagen-induced monoarthritis as a model of activated OA. They prove a C5aR expression on mouse chondrocytes and C5aR and C3aR in the synoviocytes, as well as influence on the joint inflammation with them, through application of a C5aR antagonist [52].

The complement system is involved in many processes in the course of osteoarthritis: chondrocyte degeneration, ECM degradation, low-grade inflammation in the osteoarthritis, cell lysis, unbalanced bone remodeling, osteophyte formation, and reparative processes like neoangiogenesis. Science has not still solved the problem whether the complement cascade plays a role of the purification system only or is a leading pathogenic factor in OA activation.

Targeted studies regarding the participation of humoral and cellular factors of innate immunity would contribute for their therapeutic targeting as a future pathogenetic therapy of the disease.

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