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International

Complement System Involvement in Osteoarthritis Pathology

*Lyudmila Belenska-Todorova[1]; Petya Ganova[2]; Valeriya Gyurkovska[2]; Nina Ivanovska[2]**

[1]*Medical Faculty, Sofia University, Bulgaria*

[2]*Department of Immunology, Institute of Microbiology, Bulgaria*

Corresponding Author: Nina Ivanovska,

Department of Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Email: nina@microbio.bas.bg

Published Online: May 15, 2018

eBook: Osteoarthritis

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Osteoarthritis; C3aR; C5aR; Sclerostin; Collagenase-induced arthritis

Abstract

Osteoarthritis is associated with gradually developing loss of cartilage, which in the stationary phase of disease progression leads to the formation of osteophytes and joint space narrowing, and in the last phase results in bone repair and remodeling. Multiple factors contribute to the degradation of cartilage in OA, by either directly or indirectly regulating the anabolic and catabolic pathways of the cartilage matrix. Complement system consists of more than 50 soluble and membrane-bound serum proteins that connects innate and acquired immunity. The activation of complement cascade is indispensable part of many autoimmune and inflammatory diseases, including Osteoarthritis (OA). The generation of anaphylatoxins C3a and C5a bind to its receptors C3aR and C5aR with high affinity and influence many cell activities. They contribute to maintenance of osteoarthritic joint inflammation leading to synovitis, cartilage destruction, osteoclast formation. Because current treatments for OA act only on symptoms and do not prevent or cure OA, complement system is an attractive target to modulate cartilage degeneration and help the development of new therapeutic approaches. Collagenase-induced Arthritis in mice (CIOA) is discussed as an appropriate model of OA. Except, this model might be used for studies on the role of complement system, sclerostin expression and cell senescence.

Introduction

Complement system is a complex network of about 50 soluble and membrane-associated serum proteins that bridges innate and adaptive immunity [1]. They form a highly regulated and exquisitely directed to ensure humoral and cellular immune responses to infectious organisms, including bacteria, viruses and parasites, to tissue damage by chemical, physical, radiation or neoplasia insults and to other foreign agents not recognized as 'self' [2]. These soluble or surface expressed proteins might be grouped in two cathegories: participating in complement activation and regulating it. Through its activation complement system mediates critical for the organism defence bioreactions [3]. Complement cascade can be initiated by three distinct pathways, Classical (CP), Lectin (LP), and Alternative (AP), each leading to a common terminal pathway. Classical complement pathway is activated through C1qrs by complexes of IgM and IgG antibodies bound to bacterial or other pathogen antigens [4,5]. It depends on the presence of Ca2+ and is inhibited by divalent cations of chelating agents such as EDTA. In addition, CP maight be activated independently by C-reactive protein, virus proteins, β-amyloid, polyanions, bacterial products, such as Lypopolysacharides (LPS), DNA or RNA, mitochondrial fragments, necrotic or apoptotic cells [6,7]. Alternative complement pathway is activated mainly by pathogen associated molecular motives such as LPS and zymosan. Four types of molecules are needed for its trigger: C3, Factor B, Factor D and properdine. The process is dependent on Mg2+. Unlike the CP and LP, alternative pathway is constantly active because of spontaneous hydrolysis of the serum component C3 to C3(H2O), a process known as a complement "tick-over" [8,9]. The LP is Ca2+ dependent and is activated when mannan-binding lectin binds to mannose containing proteins on the surface of the pathogens [10]. A fourth complement activation pathway has been described since the beginning of XXI century. It acts through the serine

proteases that participate in coagulation cascades [11,12]. After activation, all the complement pathways lead to formation of C3 and C5 convertases, and to a release of C3a and C5a anafilatoxins. C5b binds to C6 and facilitates binding of C7 and C8 to C9, thus forming the Membrane Attack Complex C5b-9 (MAC). C3a and C5a induce inflammation by binding to their receptors C3aR и C5aR, that transmit signals for blood vessels dilatation and production of chemokines and cytokines by the immune cells [13,14]. Excessive or improper complement activation can be dangerous for the organism, so a strict and fine regulation is required. Complement system is controlled by a variety of membrane bound (membrane cofactor protein, MCP, CD46 or complement receptor 1, CD35) and soluble regulatory proteins, like factor H (FH), which bind to C3b [15,16].

Complement system in the joint

It was initially known that the plasma complement components are mainly produced in the liver by the hepatocytes [17]. Thereafter, local production of complement components has been reported in various tissues and their importance in tissue homeostasis was suggested. Many of these components are produced in human joints, especially from C1 to C9 [18]. In the synovium are found constitutive complement components and regulators except C8 and properdin [19-21]. The human articular chondrocytes produce clusterin, C1s, C1q, C1r, C4, C2, C3, factor B and C1 inhibitor [22-24]. In hypertrophic zone of the cartilage C5 and C9 have been detected [25] and human fibroblasts produce all the terminal complement components [26]. As for the complement receptors, most studied are C5aR, expressed on human articular chondrocytes [27] and C3aR and C5aR in the synovial tissue [18]. Immune cells in the joint also produce a number of complement components. Monocytes and macrophages in the synovial membrane of patients with RA release functionally active C2, B, D, P, C3bINA and beta 1H [28]. These results show that in RA, complement components can be synthesized locally in the inflamed joints, as well as local factors in the joints enhance complement synthesis. Decreased concentrations of factor B and properdin, and increased levels of Ba showed that increased alternative pathway turnover occurred in RA patients [29]. Local complement synthesis and activation in the synovial fluid and synovial tissues is important in the development of arthritis rather than systemic complement levels, based on the results described by Mizuno at all. The authors showed that in inflammatory mono-arthritis in rat the systemic complement consumption did not affect the development of the disease but the local complement suppression by administration of soluble complement receptor 1 (sCR1) into the joint space ameliorated arthritis [30,31]. Complement system is activated in the joint by degenerative fragments released in a result of any kind of tissue damage. The interaction of complement fragments with their receptors is crucial for the elimination of foreign bodies and degenerative self fragments [32]. However, complement system can also become self reactive and can be deleterious for the host. The anaphylatoxins C3a and C5a are small polypeptides, which bind to C3aR and C5aR with high affinity and modulate many cell activities [33]. Overexpression of C5a is implicated in human and experimental models of inflammatory conditions, such as RA and OA [34,35]. It has been reported that spontaneous activation of C3 occurs more often in joints than in other tissues [36]. C5a fragments resulting from the complement activation are one of the factors responsible for attraction and activation of cells expressing C5aR, such as neutrophils, monocytes, macrophages, eosinophils, and lymphocytes in the synovium [37]). Since C3a and C5a can be generated locally by BM cells during OC differentiation, their receptors, C3aR and C5aR, which are expressed on all cells of the hematopoietic lineage, could contribute to arthritic process, together with C3 Receptors (CR1-4). BM cells locally produce functional factor B, factor D, and C5 in addition to C3 during differentiation. It should be noted that C3 locally produced by BM cells, but not systemic C3 produced by hepatocytes, plays a critical role in OC differentiation [38]. The use of C5aR antagonist in a rat model of immune-mediated monarticular arthritis reduces the severity of the disease. With blocking antibodies against C5a a new generation of therapeutic complement inhibitors has now been introduced in pre-clinical and clinical trials [39]. C5a antibodies completely prevented established K/BxN arthritis in mice, The physiological role of the other C5a receptor, C5L2 is less clear, and studies with blocking mAbs to human C5L2 have failed to demonstrate a clear functional role in signaling to C5a [40].

Complement system in osteoarthritis (OA)

OA is one of the most common chronic painful and disabling joint disorder [41]. OA involves all joint tissues and its main feature is cartilage breakdown [42]. OA is characterized with bony growths, named osteophytes in conjunction with articular cartilage degeneration. Osteophytes are so common as a radiographic feature that they have been used to confirm the presence of disease. TGF-beta potentiates their appearance most often at the margins of the joint, initially as outgrowths of cartilage and followed by endochondral ossification and increased risk of joint space loss, which suggests cartilage loss.

Usually, the diagnosis of OA is established late in the disease process, thus an effective application of disease-modifying drugs is delayed. Despite efforts to identify reliable markers of disease, still biochemical and immunochemical markers need to be improved and extended with more specific and sensitive methods. A central role of complement system in the development of OA was identified using different animal models of OA, mice deficient of different complement components, as though synovial fluid of patients with OA. Mice genetically deficient in complement components C5 and C6 or the complement regulatory protein CD59a showed the decisive role of MAC in OA pathology. The persistence of low-grade inflammation with synovitis even in the early stage of the disease is of particular interest. Proteomic analyses revealed increased levels of numerous cytokines and complement components in OA, compared with healthy synovial fluids [43,44]. Markedly higher expression of almost all the complement effectors and lower expression of complement inhibitors was detected in the synovial membrane of OA patients compared to healthy ones [45]. The presence of immunoglobulins and complement in osteoarthritic cartilage [46] and the deposition of terminal complement complexes in synovial tissue [47] suggest involvement of local immune mechanisms in cartilage degradation, especially in those patients with longer term involvement. Cultured human chondrocytes and fibroblasts from normal cartilage synthesized C1q, C1s, C4 and C2 components and this process was modulated by IL-1-beta, TNF-alpha and IFN-gamma, showing that cytokines can probably regulate complement synthesis in intact cartilage [22]. Changes in OA cartilage are hardly subjected to repair. Chondrocytes are the only cells that maintain the balance between anabolic and catabolic processes in the extracellular matrix. The excessive mechanical load activates the low metabolic chondrocytes and stimulates them to produce mediators of inflammation that normally are secreted in response to trauma or infection [48],

such as IL-1, IL-6, IL-8, IL-17, IL-18, reactive oxygen species like NO, superoxide and the lipid mediators prostaglandins and leukotrienes. All of them increase the catabolic activity of chondrocytes leading to a destruction of cartilage matrix. IL-1 and TNF-α produced by activated synoviocytes and monocytes increase the expression of matrix metaloproteinases and suppress the compensation synthesis pathways in chondrocytes. Membrane Attack Complexes (MAC) are formed in a response to the presence of matrix proteins that are formed after matrix degradation by the metaloproteinases. Sublytic levels of MAC induce Janus Kinase Signal Transducer and Activation of Transcription (JAK-STAT) and NF-κB [49]. At sublytic doses, MAC expressed wide-range of effects on many cell types leading to adherence, aggregation, chemotaxis and even cell division. Although, certain pathogens might take advantage from the absence of lytic complement and succeeded to survive. Histologic data showed that the deposition of complement increases in accute stage of OA. Complement in OA can be activated by binding to Damage Associated Molecular Patterns (DAMPs), such as calcium crystals, extracellular matrix proteins, and apoptotic debris, fibromodulin and NC4 doamin of collagen type 4 [50-53]. For example fibromodulin activates the classical complement pathway through binding to C1q and increases the amount of C5b-9 MAC.

In recent years, research has been carried out using transgenic mice which allows better define the important role of complement system in OA pathogenesis. Mice deficient in complement effectors C5 and C6 were protected in an experimental OA model, whereas degenerative changes were aggravated in mice deficient of the cell surface inhibitor of the membrane attack complex CD59a. C5 deficient mice exhibited substantially less cartilage loss, osteophyte formation, and synovitis than did wild type mice. These authors also reported that joint damage is alleviated when complement is blocked pharmacologically using CR2-fH, a protein obtained after the fusion of the complement receptor CR2 and its natural inhibitor factor. Another complement inhibitor carboxypeptidase B has also a protective role on OA [54]. In this study is reported that the levels of carboxypeptidase B correlate to these of MAC in the synovial fluid of patients with OA and its anti-inflammatory role in the joint is suggested. Also using in vitro model, they found that the serum treated with carboxypeptidase B decreased the formation of MAC and concluded that carboxypeptidase B has an anti-inflammatory role in OA by inhibiting MAC formation. These findings suggest that strategies that block complement activation have therapeutic potential for OA, but the adverse effects of such inhibition are still unclear. It still remains unknown whether complement deposition in OA joints is itself a triggering event or whether it is a consequence of the damage of cartilage or joint tissue or of low-grade persistent inflammation.

Collagenase induced osteoarthritis (CIOA)

Mouse models of OA are having increasingly important roles to study the molecular mechanisms responsible for the initiation and progression of the disease. The chronic nature of OA combined with the significant variability in the rate of disease progression in patients also presents challenges. The in vivo preclinical animal models allow accomplishment and other goal - to study the therapeutic efficacy of treatment modalities. Papain, sodium monoiodoacetate, quinolone, and collagenase are some of the agents used to induce OA in animals. Their ease of induction and reproducibility are advantageous in designing short-term studies in contrast to surgery models [55-57]. The release of collagenase in OA leads to the degradation of proteins in the articular cartilage. The intra-articular administration of collagenase breaks down type I collagen within the cartilage leading to a decrease of collagen matrix in the tendons and ligaments, causing joint instability [58,59].

In our previous investigations we have used a mouse model of OA induced by i.a injection of collagenase from Clostridium histolyticum (2 U/10 µl) (Sigma-Aldrich, Germany). The incidence of OA was approximately 90% with typical OA chracteristics [60-62]. In Figure 1 is shown cartilage erosion, joint space narrowing, chondrocyte proliferation, panus formation (hematoxylin eosin, HE staining) and Proteoglycan (PG) loss (safranin O staining).

This model appeared to be very convenient to study complement-mediated processes in OA. We observed that at the active phase of CIOA (day 18), C3aR was highly expressed in the synovium of arthritic mice but not in the cartilage and bone (Figure 2).

Therefore, C5aR expression was detected not only in the synovium but in the cartilage also (Figure 3).

We have found that CIOA development is attended with one of the remodeling factors sclerostin. The Wnt signaling pathway is involved in the development of cartilage and bone changes in osteoarthritis influencing on chondrocytes and osteoblasts [63,64]. The modulation of Wnt signaling might be perspective for the treatment of skeletal disorders such as osteoporosis [65,66]. Wnt signaling has been shown to be involved in cartilage and bone changes in animal models of osteoarthritis also [67]. The results from transgenic mice have shown that activation of Wnt/β-catenin signaling caused premature chondrocyte differentiation and an osteoarthritis-like phenotype [68], while other studies have demonstrated that the inhibition of Wnt signaling leads to an increase in apoptosis of articular chondrocytes and destruction of cartilage [69]. Evidently, Wnt pathways in cartilage homeostasis is still a matter of elucidation, because both hyperactivation and inhibition of Wnt/β-catenin signaling can result in increased cartilage damage [69]. In animal models the results also are not very consistent. Sclerostin plays an important role in the regulation of bone remodeling through inhibition of osteoblastic bone formation. Very low sclerostin levels in humans leads to rare diseases, such as sclerosteosis and van Buchem disease. Compounds capable to inhibit sclerostin shifted bone balance to increased bone formation and reduced bone resorption. Antisclerostin therapy with monoclonal antibodies to sclerostin, including romosozumab, blosozumab, and BPS804 in phase II clinical trials have shown reduction of fracture risk or improvement in patients with osteoarthritis. A significant increase in bone mineral density was reported in mice treated with an antisclerostin monoclonal antibody (Scl-AbI) [70]. In knockout mice the absence of sclerostin did not influence the developmnt of age-dependent osteoarthritis as well as no effect was noticed on articular cartilage remodeling in rat post-traumatic osteoarthritis [71]. Moreover, sclerostin acts through the inhibition of the canonical Wnt pathway as well as through the inhibition of the non-canonical JNK pathways in chondrocytes since, the lack of sclerostin aggravated the development of OA in mice submitted to joint instability [72]. Our results in CIOA showed that sclerostin is expressed in arthritic cartilage at active and late established phases (Figure 4). Thus, this model might be used to follow the role of sclerostin in cartilage maintenance during OA development.

Yet, another change was observed in relation to joint inflammation in CIOA. With increasing age, the prevalence of osteoarthritis increases and the efficacy of articular cartilage repair decreases. Chondrocyte senescence induced by a variety of harmful biomechanical factors induces stress and causes irreversible damage, leading to cell death. Thus, chodrocytes started to synthesize smaller, less uniform aggrecan molecules and less functional link proteins. That progressive cell senescence decreases their ability to maintain and to restore articular cartilage. In OA near the osteoarthritic lesions in the cartilage, senescent cells were observed. They secrete various inflammatory cytokines, growth factors and soluble and insoluble molecules underlying the inflammation [73,74]. In the late stage of OA, the cartilage becomes hypocellular with formation of lacunar emptying. Complement effectors, and specifically, the Membrane Attack Complex (MAC) plays an important role in the pathogenesis of OA in different mouse models [45]. MAC led to the production of degradative molecules in cultured chondrocytes and it is co-localized with MMP-13 in human OA cartilage. It might be expected that MAC expresses dual effect. It can help the elimination of products of cartilage destruction but it also can maintain the inflammation. Literature data reveals that there is a definite correlation between the degree of cartilage damage and chondrocyte apoptosis. Lipofuscin is related to many ageing processes. It is also known to accumulate in senescent cells, as a by-product of the senescent process, and should be considered as a reliable hallmark of senescence [75,76]. We used SBB staining in CIOA and the results showed that the presence of senescent cells was obvious both in inflamed synovium, even more exerted at late stage (Figure 5).

In Figure 6 was well seen the hypercellular zone in cartilage with clearly SBB stained layer at both, active and late stages.

Conclusion

In osteoarthritis, there is abundant evidence implicating complement activation in humans and animal models. At present, there are still no applicable agents for therapeutic regulation of excessive complement activation in chronic inflammation. Therefore, anti-complement agents might be beneficial as part of clinical treatment. Mouse models of the disease in OA research are widely used, owing to the advancements of microsurgical techniques and the use of genetically modified mice, as well as the development of novel assessment tools. CIOA is a suitable model to study osteoarthritic changes, including complement participation, involvement of sclerostin and cell scenscence.

Acknowledgement

This work was supported by a Grant DH13/6/15.12.2017, National Research Fund, Ministry of Education and Science, Bulgaria.

Figures

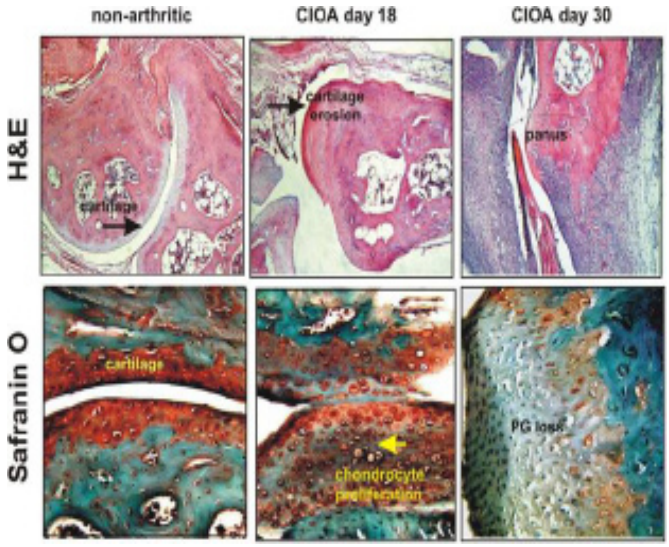

Figure 1: Main features of collagenase-induced osteoarthritis (CIOA).

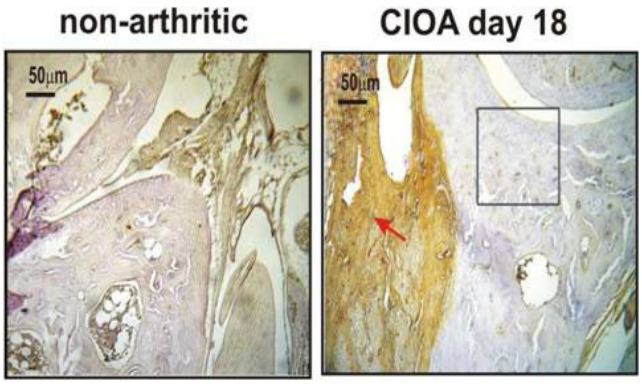

Figure 2: Immunohistochemical staining of C3a receptor. C3aR is expressed in the synovium of CIOA mice (red arrow) but not in the cartilage (black rectangle).

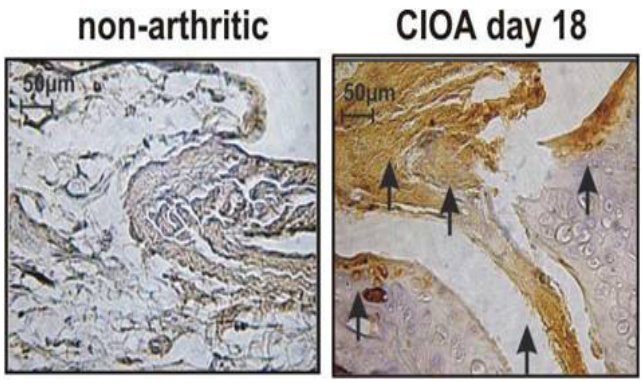

Figure 3: Immunohistochemical staining of C5a receptor. C5aR is expressed in the synovium and cartilage of CIOA mice (black arrows).

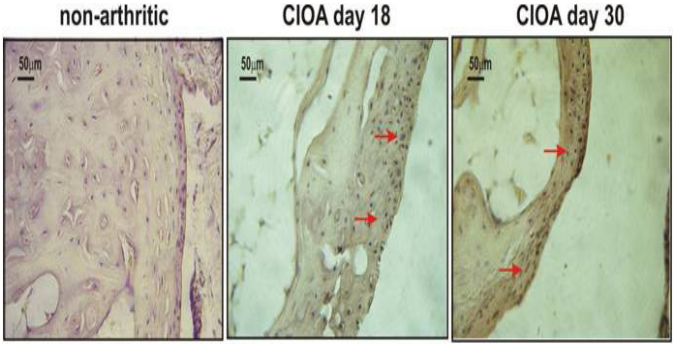

Figure 4: Immunohistochemically sclerostin is detected in the cartilage of CIOA mice, especially in the area of destruction at day 30 (red arrows).

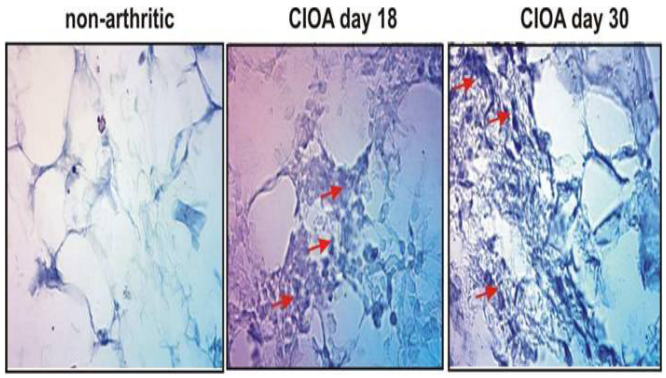

Figure 5: At day 18 and day 30 senescent cells were observed in synovium of CIOA mice by SBB staining (red arrows).

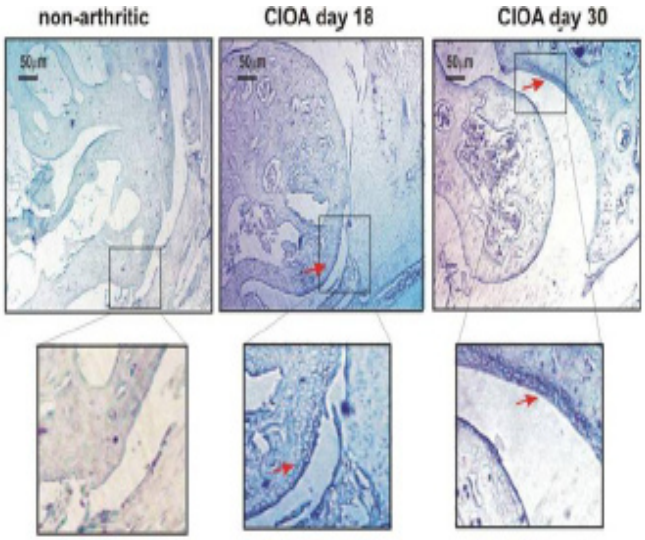

Figure 6: At day 18 and day 30 senescent cells were observed in cartilage of CIOA mice by SBB staining (red arrows).

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