Osteoarthritis and the Complement Cascade

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ABSTRACT: Accumulating evidence demonstrates that complement activation is involved in the pathogenesis of osteoarthritis (OA). However, the intimate complement regulation and cross talk with other signaling pathways in joint-associated tissues remain incompletely understood. Recent insights are summarized and discussed here, to put together a more comprehensive picture of complement involvement in OA pathogenesis. Complement is regulated by several catabolic and inflammatory mediators playing a key role in OA. It seems to be involved in many processes observed during OA development and progression, such as extracellular cartilage matrix (ECM) degradation, chondrocyte and synoviocyte inflammatory responses, cell lysis, synovitis, disbalanced bone remodeling, osteophyte formation, and stem cell recruitment, as well as cartilage angiogenesis. In reverse, complement can be activated by various ECM components and their cleavage products, which are released during OA-associated cartilage degradation. There are, however, some other cartilage ECM components that can inhibit complement, underlining the diverse effects of ECM on the complement activation. It is hypothesized that complement might also be directly activated by mechanical stress, thereby contributing to OA. The question arises whether keeping the complement activation in balance could represent a future therapeutic strategy in OA treatment and in the prevention of its progression.

KEYWORDS: Osteoarthritis, chondrocyte, complement, exercise

Introduction

Osteoarthritis (OA), the most common joint disease can develop as a sequela of a joint cartilage trauma. It is a “whole joint disease,” meaning that all joint-associated tissues are affected by OA and contribute to its pathogenesis.1,2 In the case of OA of the knee (gonarthrosis), in addition to the joint cartilage and the subchondral bone, the synovial membrane, even including the outer fibrous layer of the joint capsule, ligaments—especially intra-articular ligaments, such as anterior cruciate ligament (ACL) and posterior cruciate ligament—menisci,4 and the intra-articular fat pads, such as Hoffa fat pad, can be affected by the inflammatory processes. Synovitis, which used to be per definition primarily associated with rheumatoid arthritis (RA) is also defined as an important aspect of OA.2,5,6

A role of complement in the pathogenesis of OA was suggested 10 years ago7 and still remains a promising field for research.8 In a recent study, abnormally high complement expression was found in synovial fluid (SF) and membrane samples of human patients with OA.8 In the same study, significantly lower expression of inflammatory markers in mice deficient in the central complement component C5 could be shown compared with wild-type mice in an OA model.8

Furthermore, in a cartilage blunt trauma model in rabbits, the deposition of the terminal complement complex (TCC) on chondrocyte surface also known as membrane attack complex (MAC) could be demonstrated.9 Despite these novel results,8 the detailed pathogenetic pathway triggered in the joint by complement activation and its cross talk with other signaling routes contributing to OA still remains incompletely characterized.

Osteoarthritis

Traditionally, OA has usually been defined as a result of impulsive or repetitive overloading, leading to an excess of biomechanical stress of a joint. Also, excessive body weight or aberrations from the physiological axis of the articulating bones resulting in an overload of particular joint areas leading to degradation of the involved articular cartilage9,10 were identified as important causal factors. Even though repetitive physiological mechanical loading is an essential regulator for the metabolic activity of the chondrocytes,10 excess burden to the joint triggers cartilage inflammation with subsequent degradation, mediated by the release of inflammatory and extracellular cartilage matrix (ECM)-degrading factors such as interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and matrix metalloproteinase (MMP)-1, MMP-3, and MMP-9.11,12 In addition to overloading and joint instability, the modern concept of OA pathogenesis includes genetic, endocrinologic, metabolic, age-dependent, and other risk factors disturbing the homeostasis of the whole joint by the release of a multitude of factors from joint-associated tissues, which are catabolic, inflammatory, or impairing tissue integrity.13,14,15 Hence, the major contribution of IL-1β and TNF-α to OA pathogenesis, both representing key pro-inflammatory and catabolic cytokines, has been well...
accepted for the past decades.6,16–18 These factors initiate a low-grade inflammation and degradation of ECM components including collagen and proteoglycans (PG) by the induction of various MMPs through activated synovial macrophages, synovial fibroblasts, or the chondrocytes themselves.19,20 These cytokines directly facilitate persistent joint inflammation and joint cartilage destruction in OA. Obesity and diabetes mellitus, the last mentioned being one of the most common comorbidities in OA, are known to be associated with low-grade systemic inflammation.21,22 In addition, complement activation appears critical in OA pathogenesis and also affects the expression of inflammatory and degradative molecules in chondrocytes.8,14,23 Some complement components seem also to be dysregulated in obese and could contribute to insulin resistance.24,25 This possibly shared link could serve as a future potential therapeutic target for OA treatment. First, however, a better understanding of the interconnection between the complement system and various other inflammatory and noninflammatory factors in the OA scenario is necessary.

The Complement System

The complement system represents an important part of the innate immune system, which mediates multiple responses including the initiation of opsonization, phagocytosis of pathogens, inflammatory response, and terminating in cell lysis.26,27 Over the past decade, the complement system has been seen as a bridge between the innate and the acquired immunity.28 The 3 complement activation pathways (classical, alternative, and mannose-activated) have all been described as resulting in the cleavage of the C3 (complement component C3) component mediated by the C3 convertases27 and later in cleavage of the C5 molecule into C5a and C5b that subsequently forms the TCC (C5b–9) by recruiting downstream complement proteins29 (Figure 1). The TCC mediates lysis of affected or infected target cells, but sublytic TCC and soluble C5b–9 can also arise, which exerts a multitude of noncytolytic immune functions.29 Selection of examples of noncytolytic effects is summarized in Table 1, which suggests a cell type–dependent profile. Apparently, the complement system can be triggered in later stages of the cascade as a couple of other proteolytic factors, such as cathepsin D,45 thrombin,46 and plasmin,47 are known to activate the downstream part of the complement cascade. During complement activation, the inflammatory cleavage products, anaphylatoxins, C3a, C4a, and C5a, are released.48 They bind to their respective anaphylatoxin receptors, C3aR (C3a anaphylatoxin receptor), C4aR, and C5aR (C5a anaphylatoxin receptor),49 mediating various but mostly inflammatory effects such as increase in vascular permeability; induction of histamine release in mast cells; smooth muscle cell contraction; synthesis of angiogenetic factors by mesenchymal stromal cells (MSCs); release of chemoattractants by neutrophil, eosinophil, and basophil granulocytes; and regulation of apoptosis (Table 1). Various diseases have been associated with a disbalance in the activation or inhibition of the complement function.29

Figure 1. Simplified scheme of complement activation. Ab/Ag, antibody-antigen interaction; C1-INH, C1 inhibitor; C4bp, C4-binding protein; CSMD1, CUB and Sushi multiple domains 1; FH, factor H; Fl, factor I; MBL, mannos-binding lectin; SUSD4, Sushi domain–containing protein 4.
system, such as RA, systemic lupus erythematosus, hyperacute graft rejection after transplantation, and sepsis.\textsuperscript{50–53} To prevent damage of host cells, an efficient regulatory system is required to prevent excessive complement activation. The regulatory network includes soluble complement inhibitors, such as C1 inhibitor (C1-INH), C4b-binding protein (C4bp), factor H (FH), factor I (FI), and clusterin (Figure 1).\textsuperscript{23,54,55} In addition, cytoprotective complement regulatory proteins (CRPs) including CD35, CD46, CD55, and CD59 are localized within the cell membrane at the cell surface\textsuperscript{56} (Figure 1, Table 2). CD46, CD55, and CD59 have been detected on chondrocytes and found to be regulated by major inflammatory cytokines such as IL-1\textsubscript{β} and TNF-\textalpha.\textsuperscript{57,58} It has been demonstrated that the MAC inhibitory protein CD59 is most likely involved in protection against OA.\textsuperscript{8} Prominent expression of CD59 was seen on the synoviocytes, endothelial cells, and stromal cells in cases of OA.\textsuperscript{59} However, a thorough search for other CRPs in arthritic joints has yet to be undertaken.

### Sources for Complement in the Joint

Diarthrotic joints contain various tissues, which serve as source for complement proteins. The SF is a known source for complement factors\textsuperscript{8,60,61} and can also distribute complement within the joint. It is well known, that the complement cascade is activated in the SF in RA joints.\textsuperscript{62} Stuglics et al\textsuperscript{60} showed that the SF in OA and RA joints, pyrophosphate arthritis, and acute knee injuries contains substantial amounts of complement factors, such as C4d, C5 convertase of the alternative pathway (C3bBbP), and soluble TCC (as soluble end products of C4b, properdin-containing C3 convertase, and TCC, respectively), in comparison with the reference group. It was also suggested that the complement factors in the SF might originate from synoviocytes and chondrocytes, although all the tissues in the formation of a joint could similarly contribute to it. Another consideration is that the complement load in an affected joint can be increased through an elevated systemic concentration as plasma filtrate\textsuperscript{63} as a consequence of systemic inflammation as in RA and OA.

### Table 1. Nonlytic functions of main complement components.

| COMPLEMENT COMPONENTS | FUNCTIONS | INVOLVED TISSUE/CELLS | SOURCE |
|------------------------|-----------|-----------------------|--------|
| C5b-9                  | Release of pro-inflammatory cytokines such as IL-8 and chemokines | Umbilical vein endothelial cells | Kilgore et al\textsuperscript{50} |
|                        | Cell proliferation | Renal mesangial cells | Brandt et al\textsuperscript{51} |
|                        | Expression of growth factors such as PDGF, bFGF | Endothelial cells | Benzaquen et al\textsuperscript{54} |
|                        | Induces apoptosis | Renal mesangial cells | Nauta et al\textsuperscript{55} |
|                        | Inhibits apoptosis | Oligodendrocytes and Schwann cells | Rus et al\textsuperscript{56} |
| C5a                    | Inhibits apoptosis | Neutrophil granulocytes | Lee et al\textsuperscript{57} |
|                        | Induces apoptosis | Neuronal cell line | Farkas et al\textsuperscript{58} |
|                        | Early increase and later suppression in gene expression of CD46, CD55, CD59 | Tenocytes | Own unpublished work |
|                        | Chemotaxis | MSCs | Schraufstatter et al\textsuperscript{59} |
|                        | Chemotaxis | Neutrophil granulocytes | Ehrengruber et al\textsuperscript{60} |
|                        | Secretion and activation of MMP-9 | Neutrophil and eosinophil granulocytes | DiScipio et al\textsuperscript{61} |
| C3a                    | Inhibits apoptosis | Mesangial cells | van Beek et al\textsuperscript{62} |
|                        | Production of angiogenic factors, such as VEGF, CXCL8/IL-8, and IL-6 | MSCs | DiScipio et al\textsuperscript{63} |
| Hemotaxis              | Eosinophil granulocytes | Daffern et al\textsuperscript{64} |

Abbreviations: bFGF, basic fibroblast growth factor; IL-6, interleukin 6; IL-8, interleukin 8; MMP, matrix metalloproteinase; MSCs, mesenchymal stromal cells; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.
Cartilage, synovial membrane, and chondrocytes are important sources for the protein expression of components of the classical complement pathways such as C1q, C1s, C4, and C2. These proteins were detected immunohistologically in chondrocytes in situ in normal human articular cartilage and macroscopically unaffected articular cartilage from the femoral heads of patients with OA. Corresponding with these observations, the gene expression of these components was shown in articular cartilage. Articular chondrocytes cultured in vitro expressed C1r, C1s, C4, C2, C3, and C1 inhibitor but not C1q, C4bp, or FI. Hence, complement factor and CRP expression differed between cartilage and freshly isolated and cultured chondrocytes.

The complement components, C3aR and C5aR, and the CRPs, CD46, CD55, and CD59, were expressed not only in articular chondrocytes but also in nonarticular chondrocytes and in intervertebral disc–derived fibrochondrocytes. Interestingly, in synovial membrane, an extracellular deposition of CD55 produced by synovial fibroblasts and attached to the intimal collagen fiber network was shown. The expression of CD35 could not be demonstrated in cultured chondrocytes. As C4 is involved in the classical pathway, complement factor B (CFB) is represented only in the alternative pathway. Assirelli et al found expression of C3, C4, and CFB in osteoarthritic cartilage, synovial membrane, and cultured chondrocytes and synoviocytes. More CFB was expressed in chondrocytes and cartilage explants than C3 and C4, suggesting a substantial role for the alternative pathway in OA. There was a higher level of C5b-9 in supernatants of cartilage explant cultures compared with supernatants of synoviocyte cultures. In cultured chondrocytes, complement expression was regulated by the proinflammatory cytokines IL-1β, TNF-α, and IFN-γ. The complement component C1s (complement C1s subcomponent) was increased by TNF-α, which is a key factor in the OA. Both CFB and C3 were amplified by IL-1β stimulation in cultured chondrocytes and synoviocytes.

However, further analysis of the expression profiles regarding zone and OA grade dependency is required. The osteoblasts in the subchondral bone express the central complement components, C3 and C5, and the anaphylatoxin receptors, C3aR and C5aR, respectively. Based on the results from experimentally induced OA in C5 and C6 knockout mice models, C5 and C6 proteins have been suggested to contribute—in addition to cartilage loss—also to the formation of osteophytes, which represent a typical macroscopic feature in OA joints. The CRP CD59α is possibly a sex-dependent regulator of bone growth maintaining bone density and bone stability, as it is observed exclusively in

| REGULATOR | INTERACTION WITH COMPLEMENT |
|-----------|-----------------------------|
| SUSD4 (Sushi domain–containing protein 4) | Classical and lectin pathway inhibited via binding to C1q |
| CSMD1 (CUB and Sushi multiple domains 1) | C3 convertase inhibited, MAC (C7 incorporation inhibited) |
| CD59 | (Cell membrane anchored) MAC (C7 incorporation inhibited) |
| CD55 (DAF, decay-accelerating factor) | (Cell membrane anchored but also in soluble form, eg, in synovial fluid) C3 convertase inhibited, accelerating the decay of C3/C5 convertases |
| CD46 (MCP, membrane cofactor protein) | (Cell membrane anchored) C3 convertase inhibited |
| CD35 (CR1, complement receptor 1) | (Cell membrane anchored) Acts on the C3b and C4b inactivation, immune complex processing and cleaning |
| C4b-binding protein (C4bp) | Inhibits the classical and the lectin pathways (C4) Can bind C3b, facilitates decay of C3 convertase Serves as cofactor for factor I which cleaves C4b and C3b |
| Properdin | (Soluble) Regulates alternative pathways by C3 convertase stabilization |
| Factor H (FH) | (Soluble) Inhibits alternative pathway |
| Factor I (FI) | Inactivation of C3b and C4b to iC3b and iC4b (CD35 acts as a cofactor) |
| C1 inhibitor (C1INH) | Can inactivate C1s, C1r (and kallikrein, plasmin, and coagulation factors XI, XII) |
| Clusterin | Binds C7, C8, and C9b |
| Carboxypeptidase B | Inactivates C5a |

Abbreviations: C1r, complement C1r subcomponent; C1s, complement C1s subcomponent; C3, complement component C3; MAC, membrane attack complex.
The same authors also observed an increased osteoclastogenesis in vitro. During OA, multiple features of bone alterations can be observed, such as subchondral bone remodeling, sclerosis, and fractures, leading to cyst formation and osteophyte formations (Figure 2). Mesenchymal stromal cells, e.g., residing in the subchondral bone marrow contribute to remodeling and repair. The MSCs can produce complement inhibitory factor FH which could play a protective role in the joint against the complement activity.

There are no data available in the published literature concerning joint-associated fatty tissues and complement activation. Adipocytes can be found in the subintima of the synovial membrane or in Hoffa fat pad in the middle of the knee joint attached to the anterior part of the joint capsule. However, the Hoffa fat pad is directly covered by the synovial membrane and serves as a source of various mediators involved in OA. Mesenchymal cells that express fibrovascular tissue and adipose tissue exhibit complement activity. Complement plays an important role in fatty tissue, adipocytes produce complement proteins, and complement activation can lead to low-grade inflammation in adipose tissue. Metabolic disbalances in fat tissue are observed in metabolic diseases, such as diabetes mellitus, which represent a predisposition for OA. Moreover, C3aR and C5aR are involved in the development of adipocytes' insulin resistance through macrophage infiltration and the activation of adipose tissue. Taken together, fatty tissue synthesizes and regulates complement components, which present a source for the release of the anaphylatoxins C3a and C5a.

Ligaments and tendons express complement factors. The complement split fragment C3a, which is produced during the early phase of inflammation, induces gene expression of the anaphylatoxin receptors, C3aR and C5aR, as well as expression of the pro-inflammatory cytokines TNF-α and IL-1β. Furthermore, it impairs the gene expression of the cytoprotective CRPs, CD46 and CD55, in human tenocytes in vitro. The expression of C3aR and C5aR was also regulated (induced and suppressed) time dependently by mechanical cell injury in vitro, whereas the CRPs, CD55 and CD54, were induced under these conditions. Of note, it is well known that intra-articular ligaments, such as the ACL, are prone to OA and show various features of degeneration in osteoarthritic knee joints. Even in the fibrocartilaginous meniscus tissue, C4d deposits have been detected immunohistochemically in the ECM areas of mucoid degeneration or fibrillation after meniscectomy. In some of the samples, an association with the macrophage marker CD68 was found. However, the degeneration of the meniscus may not only go along with the onset of gonarthrosis but also might actively contribute to it.

Figure 2. Simplified scheme depicting complement involvement in OA. OA indicates osteoarthritis.
either leads to cell death or initiates them to produce matrix-degrading enzymes (such as MMPs), inflammatory mediators (eg, macrophage colony-stimulating factor, cyclooxygenases, C-C motif ligands 2 and 5), and further complement effectors—all of which promote joint pathology. The C5aR is expressed in cartilage of normal patients and patients with OA and RA and is induced by IL-1β in chondrocytes.85 The cell surface glycoproteins, CD35, CD46, CD55, and CD59, are widely distributed on normal tissue cells, protecting them from complement-mediated cell lysis.56 These CRPs are upregulated in arthritic joint diseases87; however, this mechanism appears to fail and cannot protect from tissue damage. Another role of complement activation in OA is indicated by its effect on pain, a characteristic clinical feature of OA. The anaphylatoxins, C5a and C3a, play a vital role in the onset of pain by activation and sensitization of nociceptors in vivo and in vitro. Inhibitors or antagonists of C5aR receptors might be key candidates for pain therapy in OA as well.88,89

Even though C5a and C3a are generally well known for their catabolic function, they also have a vital role in mobilizing, trafficking, and homing of bone marrow–derived hematopoietic stem cells and MSCs, which can contribute to OA-associated bone remodeling in vitro.90-95 This in return can promote the repair of the cartilage tissue as well (Figure 2). It indicates that the inhibition of C3aR and C5aR could likewise prevent the chemotactic response to MSC.39 Moreover, the interaction of C5a/C5aR has been implicated in osteogenic differentiation of MSCs66 which is important for subchondral bone remodeling.

**Activation of complement by ECM components and fragments**

Endogenous cartilage ECM components have been shown to be involved in OA pathogenesis.97 In OA, the damaged cartilage releases ECM components such as degradation products of collagen type II, fibromodulin, fibronectin, and hyaluronan (HA) into the SF. Other fragments and motifs of ECM proteins, the so-called damage-associated molecular patterns (DAMPs), which are exposed in OA cartilage by the dysregulated activity of various proteases, including MMPs, ADAM-TS, and others, can activate pro-inflammatory pathways.69 Many of these pathways feature interactions with complement factors (Table 2).

Complement activation can be mediated not only by various ECM components, such as fibromodulin,5,98 aggrecan,5,99 and cartilage oligomeric matrix protein (COMP)100 (Table 3), as described in detail below, but also by hydroxyapatite and calcium pyrophosphate dihydrate crystals, by apoptotic cells and the resulting cell debris.18

Hyaluronan is a major component of the ECM in cartilage and in various tissues. This ubiquitously found glycosaminoglycan (GAG) has a high turnover rate by natural degradation through hyaluronidase. Not only due to its viscoelastic property but also due to its anti-inflammatory functions,101 intra-articular HA injection shows pain-relieving and chondroprotective influence in clinical practice.102,103 However, there are reports suggesting that the chronic increment of HA fragments (<500 KDa) turns the benign inflammation into a chronic pro-inflammatory effect.104 One case reports a patient who received multiple intra-articular HA (Hylan G-F 20) injections which lead to complement activation, detectable by increased C5a and TCC release and accompanied by an induction of interleukin 6 (IL-6) and IL-1β.105 Possibly, this HA formulation could contain HA complexes or residual animal-derived antigens that induce immunologic responses in sensitive individuals. Therefore, it has to be considered that a chronic exposition of the joint cartilage to particular HA fragments in SF or joint-associated tissues could promote complement activation, which may contribute to development or progression of OA.

Mainly sulfated GAGs, such as chondroitin sulfate and keratan sulfate, with small portions of dermatan sulfate, bound to a core protein, build the cartilage-specific large PG aggrecan. Fragments of aggrecan, the most abundant large cartilage-specific PG, released in damaged cartilage can activate the complement system.99 The aggrecan C-type lectin domain (CLD), a part of the G3 domain, is seen released in articular diseases. They can activate the classical but to a lesser extent also the alternative complement pathway, via binding of C1q and C3, respectively. However, the observed complement activation is attenuated due to binding of complement inhibitor FH to CLD and CRP domains.99 This study therefore suggested aggrecan CLD as one factor involved in the sustained inflammation of the joint. GAG are generally described to have anti-complementary effects.106 Accordingly, chondroitin sulfate reduced inflammation directly by decreasing the expression of several complement components such as CFB, C1s, C3, and C1r (complement C1r subcomponent).107 Apart from the large aggregating PG aggrecan, small PGs, such as decorin, biglycan, and fibromodulin, are important components in articular cartilage.108 The homeostasis of these components may be altered in case of chronic inflammation.

Small leucine-rich repeat PGs (SLRPs), such as fibromodulin, were shown to bind directly to C1q and activate the classical pathway of complement.98 Other members of this family, namely, biglycan and decorin can also bind to C1q because they share significant sequence homology with fibromodulin,109 but the binding sites differ (stalk of the C1q molecule versus head) and both exert an inhibitory function on the classical pathway110,111 and do not activate complement.112 Although activators of C1q bind also the inhibitor FH, nonactivators, such as biglycan and decorin, do not interact with FH.112 This observation underlines regulatory interactions at multiple points of the cascade.

Other ECM components, such as osteoadherin in bones and chondroadherin in cartilage, tendon, bony growth plates, skeletal, and cardiac muscle, also tend to interact with C1q and activate the classical complement pathway. However, only moderate activation of the terminal pathway can be observed here.

Although with lower affinity than the above-mentioned SLRPs, lumican interacts with C1q component as well.
Lumican is a glycoprotein localized in the ECM of many connective tissues, including cartilage. Similar to fibromodulin, it binds to fibrillar collagens and limits their growth.\(^{113}\) The authors distinguished 2 different SLRP-binding sites on C1q, at the head and stalk of the molecule, respectively; the binding site to the head could activate the complement system.\(^{112}\) In contrast to the hypothesis of complement activation by ECM components, Struglics et al\(^{60}\) found that none of the complement factors (C4d, C3bBbP, and soluble TCC) measured after knee injury in the SF correlated with proteolytic fragments of aggrecan or COMP, a noncollagenous protein also called thrombospondin 5. However, 0 to 12 weeks after knee injury, the concentrations of C4d, C3bBbP, and soluble TCC in the SF correlated positively with levels of IL-1\(\beta\), IL-6, and TNF-\(\alpha\) (\(r_{\text{range}}: 0.232-0.547\)). Although C3bBbP and soluble TCC went down to reference levels after 3 to 12 weeks, C4d was still elevated several years after injury.\(^{60}\) Nevertheless, COMP has been reported as a biomarker in serum that correlates with the severity of OA.\(^{114-116}\) The cleavage of COMP by various proteases can result in a couple of neoepitopes.\(^{116}\) In another report, it was shown that the patients with OA had significantly higher COMP-C3b complex concentration in SF than in serum.\(^{100}\) How could COMP influence the complement activity in the cartilage turnover process? In the same report, it was shown that COMP inhibits the classical and the lectin pathways as it interacts with the stalk region of C1q and mannos-binding lectin. However, the complement activation still progresses via alternative complement pathway associated with a release and activation of the split fragments C3b and C9 mediated by an interaction of COMP and properdin\(^{100}\) suggesting no protective effect of COMP in OA. Whether COMP contributes to OA complement activation as reported for RA remains questionable.\(^{100}\)

Clark et al\(^{117}\) concluded that the glycomatrix in cartilage, which is prone to age-dependent or disease-dependent changes can recruit diverse positive and negative regulators of the complement system thereby possibly contributing to OA. In contrast to collagen type II which binds antibodies thereby facilitating complement activation via the classical pathway,\(^{118}\) a direct and indirect inhibition of complement was induced by the cartilage-specific collagen type IX (NC4 domain).\(^{119}\) The small number of chondrocytes residing in cartilage is surrounded by a dense matrix of the PG aggrecan bound to HA, which are embedded in a network of collagen fibrils stabilizing the ECM and providing protection for the cells. In the immediate pericellular environment of chondrocytes, particularly, type IX collagen exerts a protective role: the NC4 domain of the cartilage-specific collagen type IX binds both C4bp and FH indirectly inhibiting the complement activation and preventing directly the C9 polymerization and MAC formation.\(^{119}\) This result underlines the protective function of the cartilage-specific collagen type IX mediated by interacting with complement in the cartilage. Degradation or any congenital defect of this collagen type could facilitate the progression of OA increasing the vulnerability of the chondrocytes against the complement action.

**Complement and angiogenesis and anti-angiogenesis**

During OA, vascularization of the naturally avascular articular cartilage can be observed.\(^ {120,121}\) The expression of vascular endothelial growth factor (VEGF) has been strongly implicated in this process.\(^ {122}\) The anaphylatoxins, C5a and C3a, have been identified as pro-angiogenic factors inducing the expression of VEGF in the chorion tissue.\(^ {123}\) The maintenance of cartilage avascularity through control of angiogenesis in cartilage could function against cartilage deterioration in arthritis. As mentioned earlier, aspartate protease cathepsin D is able to cleave C5 in vitro, resulting in the generation of C5a.\(^ {45}\) At the same time, cathepsin D is able to cleave prolactin (PRL) to generate vasoinhibins, a family of antiangiogenic peptides which can also inhibit vasopermeability and vasodilation and can have pro-inflammatory effects.\(^ {124}\) Prolactin is present in SF.\(^ {125}\) In addition, PRL and vasoinhibins are produced in joint tissues, including cartilage,\(^ {126}\) synoviocytes,\(^ {127}\) vascular endothelial cells,\(^ {128,129}\) and immune cells.\(^ {127}\) Prolactin promotes cartilage survival and attenuates inflammation in inflammatory arthritis.\(^ {130}\) Moreover, PRL and vasoinhibins have been suggested to play a role in inhibition of angiogenesis in RA,\(^ {131}\) which may also be the case in OA. The relevance of the generation of C5a and vasoinhibins by cathepsin D in OA is yet unknown, but their partly antagonistic and partly synergistic profile of biological effects in terms of stimulating (C5a) or inhibiting (vasoinhibins) angiogenesis and promoting inflammation, both indicates that investigating their relative contribution to OA is justified and may provide novel insights into OA etiopathology.

However, in OA, cathepsin D levels were impaired in blood serum compared with healthy individuals.\(^ {132}\)

**Complement and exercise**

High levels of exercise can induce complement activity detectable by C5a release as already shown in 1990.\(^ {133,134}\) This observation might be explained by some tissue microdamage due to exercise. In agreement with this assumption, activation of C3 was correlated with an increase in creatinine kinase. It might be possible, therefore, to relate muscle damage and complement activation after strenuous exercise.\(^ {135}\) In response to high-intensity exercise, the expression of cathepsin D, a protease, which can cleave complement C5 component resulting in the generation of C5a, was significantly downregulated in the deep cartilage zone in horses and its expression differed also regionally in the joint, reflecting biomechanical differences.\(^ {136}\) On the contrary, it is known that OA-associated pain and disability can be inhibited by moderate exercise.\(^ {137}\) Exercise augmented the effect of medical therapy on OA, in general, showing a pain limitation and an improvement of mobility in the joint function in comparison...
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A meta-analysis showed that the strengthening exercises combined with other modalities, such as stretching and aerobic exercises, provide benefit in OA. However, the interrelation of direct mechanical stress or different degrees of mechanical strain and complement activity has not been described on the joint level and should be addressed in future.

### Table 3. Cartilage ECM components and chondrocyte cell surface proteins interacting with complement factors.

| ECM COMPONENT                  | NORMAL FUNCTION                                                                 | INTERACTION WITH COMPLEMENT                                                                 | REFERENCE |
|--------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------|
| Type II collagen               | Specific and major collagen type in cartilage ECM                               | (+) Formation of collagen-antibody immune complexes in cartilage and subsequent complement activation via classical pathway | Koobkkokruad et al118 |
| Type IX collagen               | Specific collagen type in cartilage ECM                                          | (-) NC4 domain of collagen IX inhibits complement directly due to attenuation of MAC formation and indirectly through binding and enhancing activity of complement inhibitors, C4B-binding protein, and factor H | Kalchishkova et al119 |
| Aggrecan                       | Specific and major proteoglycan in cartilage ECM                                | (+) The C-type lectin of the aggrecan G3 domain activates complement                         | Wang et al8, Furst et al90 |
| GAGs, eg, CS                   | Major component of aggrecan                                                     | (+) Factor H provides binding sites for GAGs                                                | Li et al140, Clark et al117, Calamia et al107 |
| Hyaluronan                     | Attached to aggrecan                                                            | (-) A case was reported where an induction of anaphylatoxin C5a and TCC led to joint inflammation in response to multiple intra-articular injections of hylan G-F 20 | Sofat97, Dragomir et al105 |
| COMP                           | Mediates collagen fibrillogenesis                                               | (+) COMP induces activation and deposition of C3b and C9 via the alternative pathway in RA | Blom23, Happonen et al100,141 |
| Fibronectin and its fragments  | Fibronectin regulates cell differentiation, adhesion, and migration             | (Effects not shown) binding to the C1q component of complement                               | Barilla et al142, Casons et al143 |
| Biglycan and decorin (SLRP)    | Decorin: limits collagen fiber formation, regulates TGF-β functions             | (-) Decorin and biglycan: bind to C1q, can inhibit classical pathway, biglycan: inhibits also lectin pathway | Groeneweld et al110, Krumdieck et al111 |
| Fibromodulin (SLRP)            | Keratan sulfate PG, bound to collagen fiber surface: limits collagen fiber formation | (+) Activates complement by binding to C1q, interaction with factor H                         | Wang et al8, Sofat97, Sjoberg et al108 |
| Chondroadherin and osteoadherin (SLRP) | Cell-matrix interaction binds to collagens and αβ integrin                       | (+) Activates complement by binding to C1q, interaction with factor H                         | Sjoberg et al112 |
| DAMP, damage-associated molecular patterns | Cartilage ECM or cellular fragments arising during OA-associated tissue disintegration | (+) A subgroup of DAMPs acts as neoantigens and exerts complement activation                  | Liu-Bryan6, Land144 |
| Integrins and TLR              | Integrins: important cell-ECM receptors regulating diverse cellular processes TLR: besides recognizing patterns of microbial origin and activating innate immunity, they can also bind to various DAMPs, fibronectin, hyaluronan, and biglycan fragments | (Effect not shown) αβ integrin Interacted with C1q Direct interaction between TLR and C5a/ C5aR signaling, shown in immune cells | Hajishengallis and Lambris145, Holst et al146, Sillat et al147, Zutter and Edelson148 |

**Abbreviations:** COMP, cartilage oligomeric protein; CS, chondroitin sulfate; DAMP, damage-associated molecular pattern; ECM, extracellular cartilage matrix; GAGs, glycosaminoglycans; OA, osteoarthritis; RA, rheumatoid arthritis; SLRP, small leucine-rich repeat protein; TGF-β, transforming growth factor β; TLR, toll-like receptor.

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**Inhibitors of complement in the joint**

To prevent uncontrolled complement activation and subsequent cell lysis, complement is controlled by several soluble and membrane-bound natural inhibitors23 (Table 2). CD59 is one of the membrane-bound proteins that inhibits the assembly of the downstream components, C5b-9, resulting in failure of TCC formation149 (Figure 1). The direct inhibitory role of
CD59 in the development of OA has been proven in a mice model.8

Sushi domain–containing protein 4 (SUSD4) inhibits the formation of the classical C3 convertase and can thereby interrupt the early phase of complement activation.150 However, its effects have not been shown in cartilage yet.

Synovial expression of complement inhibitors, such as the C5a-inactivating enzyme carboxypeptidase B (CPB), is suppressed in OA.18,151,152 Moreover, CPB also serves as a protective mediator against the development of OA by inhibiting the formation of TCC.151 Likewise, regulation of TCC formation was also observed through supplementation of frequently found GAGs in cartilage tissues and chondroitin sulfates, more specifically, low–molecular-weight chondroitin sulfates, which have been developed as therapeutic complement inhibitors, and revealed attenuation of OA in a mouse OA model.140 Furthermore, antiangiogenic, anti-inflammatory, and anti-catabolic properties of chondroitin sulfate decreasing the expression of several complement components, such as CFB, C1s, C3, and C1r, add to its OA attenuating potential.

As mentioned earlier, the cartilage–specific collagen type IX inhibits complement directly due to attenuation of TCC formation and indirectly through binding and enhancing activity of complement inhibitors C4bp and FH.139 CR2-fH is a synthetically assembled fusion protein that regulates the activation of the alternative pathway of the complement system inhibiting the activation of C3 and C5 in vitro and attenuating the development of collagen antibody–induced OA in a mouse in vivo model.8,153

There are very few therapeutics so far, such as ezulizumab, a C1-INH which has been approved for clinical use. However, various therapeutics and still plenty of remedies focusing on the complement systems are on trials.

Therapeutically, HA injection has shown promising effects on the attenuation of pain in patients with OA. Treatment of the affected joint with autologous platelet-rich plasma (PRP) injection,154 which usually contains the entire complement of plasma complement factors, has been compared with the synthetic HA injection, which in summary showed comparable beneficial effects, although with less risk of side effects. Platelet-rich plasma can inhibit the nuclear factor kB activation,155 which explains the absence of TNF-α explaining its anti-inflammatory character.156 However, the complement activation has also been shown through activated PRPs, which rather contradict this anti-inflammation.157 Even though the complement activation has been proven, the increased expression of CRPs, such as CD55 and CD59, in the platelet cell membrane,158,159 could provide protective effects against the complement attack. In addition, PRP generates via platelet stimulation thrombin activating the coagulation pathway. In fact, thrombin has recently been found to act directly as a C5 convertase and promotes terminal complement pathway activation46 underlining that the cross talk between the coagulation and complement pathways is intimate. Overall, we can conclude that there is an influence of the complement system in the PRP therapy, which has to be further defined in relation to OA.

Conclusions
Accumulated evidence demonstrates that complement activation contributes to OA pathogenesis and progression. Complement activation either via the 3 recognized pathways or direct activations through proteolytic enzymes could be involved in main features of OA, such as disbalanced bone remodeling, osteophyte formation, stem cell trafficking, synovitis, joint and meniscal cartilage–derived chondrocyte inflammatory response, and cell death and cartilage vascularization as well as ligament degeneration. Several intact or cleaved ECM components, released from degraded cartilage during OA, could present neoepitopes or DAMPs suspected to initiate and regulate complement activity. This so–called glycomatrix in the joint157 is not only affected by OA, initiating complement activation, but could also be modified by predisposing conditions for OA such as aging and underlying diseases including diabetes or joint calcinosis thereby facilitating OA progression. Understanding the role of the complement system in the joint and the involved tissues could present a future strategy for the treatment of OA.

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SS and GS-T wrote the first draft of the manuscript. JT and TB contributed to the writing of the manuscript.

REFERENCES
1. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. Arthritis Rheum. 2012;64:1697–1707.
2. Mathiesen A, Conaghan PG. Synovitis in osteoarthritis: current understanding with therapeutic implications. Arthritis Res Ther. 2017;19:88.
3. Ruschke K, Meier C, Ullah M, et al. Bone morphogenetic protein 2/SMAD signalling in human ligamentocytes of degenerated and aged anterior cruciate liga-
ments. Osteoarthritis Cartilage. 2016;24:1816–1825.
4. Pauli C, Grogan SP, Patil S, et al. Macroscopic and histopathologic analysis of human knee menisci in aging and osteoarthritis. Osteoarthritis Cartilage. 2011;19:1132–1141.
5. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51:249–257.
6. Liu-Bryan R. Synovium and the innate inflammatory network in osteoarthritis progression. Curr Rheumatol Rep. 2013;15:323.
7. John T, Stahel PF, Morgan SJ, Schulze-Tanzil G. Impact of the complement cascade on posttraumatic cartilage inflammation and degradation. Histol Histopathol. 2007;22:781–790.
8. Wang Q, Rozelle AL, Lepus CM, et al. Identification of a central role for complement in osteoarthritis. Nat Med. 2011;17:1674–1679.
9. Joos H, Leucht F, Riegger J, et al. Differential interactive effects of cartilage trauma and blood exposure in vitro and in vivo. Am J Sports Med. 2015;43:2822–2832.
10. Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. Lancet. 1972;1:519–522.
11. Urban JP. The chondrocyte: a cell under pressure. Br J Rheumatol. 1994;33:901–908.
12. Honda K, Ohno S, Tanimoto K, et al. The effects of high magnitude cyclic tensile load on cartilage matrix metabolism in cultured chondrocytes. Eur J Cell Biol. 2000;79:601–609.
73. Hugle T, Geurts J. What drives osteoarthritis—synovial versus subchondral bone
71. Huber-Lang M, Kovtun A, Ignatius A. The role of complement in trauma and
74. Findlay DM, Kuliwaba JS. Bone-cartilage crosstalk: a conversation for under -
75. Tu Z, Li Q, Bu H, Lin F. Mesenchymal stem cells inhibit complement activa -
68. Nakagawa K, Sakiyama H, Tsuchida T, et al. Complement C1s activation in
88. Quadros AU, Cunha TM. C5a and pain development: an old molecule, a new
89. Jang JH, Clark JD, Li X, Yorek MS, Usachev YM, Brennan TJ. Nociceptive sen -
80. Barbu A, Hamad OA, Lind L, Ekdahl KN, Nilsson B. The role of complement
85. Onuma H, Masuko-Hongo K, Yuan G, et al. Expression of the anaphylatoxin
86. Kim DD, Song WC. Membrane complement regulatory proteins.
94. Diekman BO, Rowland CR, Lennon DP, Caplan AI, Guilak F. Chondrogenesis
95. Hengartner NE, Fiedler J, Schrezenmeier H, Huber-Lang M, Brenner RE.
96. Huber-Lang M, Wegner R, Lampel L, Brenner RE. Mesenchymal stem cells after polytrauma: actor and target. Stem Cells Int. 2016;2016:6289825.
97. Sotn A. Analysing the role of endogenous matrix molecules in the development of osteoarthritis. Int J Exp Pathol. 2009;90:463–479.
98. Sjöberg A, Onnerford P, Mergelin M, Heinegard D, Blom AM. The extracel -
ular matrix and inflammation: fibromodulin activates the classical pathway of complement by directly binding C1q. J Biol Chem. 2005;280:32301–32308.
99. Fürst CM, Mergelin M, Vadinstrup K, Heinegard D, Aasberg A, Blom AM. The C-type lectin of the uroglycan G3 domain activates complement. PLoS ONE. 2013;8:e51407.
100. Happonen KE, Saxne T, Aasberg A, Mergelin M, Heinegard D, Blom AM. Regulation of complement by cartilage oligomeric matrix protein allows for a novel molecular diagnostic principle in rheumatoid arthritis. Arthritis Rheum. 2010;62:3574–3583.
101. Feinberg RN, Brede DC. Hyaluronate in vasculogenesis. Science. 1993;260:1177–1179.
102. Bhandari M, Bannuru RR, Babins EM, et al. Intra-articular hyaluronic acid in the treatment of knee osteoarthritis: a Canadian evidence-based perspective. Ther Adv Musculoskel Dis. 2017;9:231–246.
103. Wang CT, Lin J, Chang CJ, Lin YT, Hou SM. Therapeutic effects of hyaluronic acid in osteoarthritis of the knee: a meta-analysis of randomized controlled tri -
104. Petrey AC, de la Motte CA. Hyaluronan, a crucial regulator of inflammation. Front Immunol. 2014;5:101.
105. Dragomir CL, Scott JL, Ferrino G, Adler R, Fealy S, Goldring MB. Acute inflammation with induction of anaphylatoxin C5a and terminal complement complex C5b-9 associated with multiple intra-articular injections of hyaluronic acid and corticosteroids. South Med J. 2016;109:573–577.
106. Li L, Li Y, Iazz M, Shahbaz M, Lian Q, Wang F. Review on complement analy -
107. Groeneveld JTW, Orosdian M, Owen WT, et al. Interactions of the extracellular matrix proteins decorin and biglycan with C4q and collectins. J. Immunol. 2005;175:4715–4723.
108. Cs-Szabo G, Roughley PJ, Plaa AH, Glant TT. Large and small proteoglycans of cartilage and rheumatoid articular cartilage. Arthritis Rheum. 1995;38:660–668.
109. Antonsson P, Heinegard D, Oldberg A. Structure and deduced amino acid sequence of the human fibromodulin gene. Biochim Biophys Acta. 1993;1174:204–206.
110. Sato-Szabo G, Roughley PJ, Plaa AH, Glant TT. Small and large proteoglycans of cartilage and rheumatoid articular cartilage. Arthritis Rheum. 2005;56:860–868.
111. Krumdieck R, Hook M, Rosenberg LC, Volanakis JE. The proteoglycan decorin binds C4q and collectins. J. Immunol. 1995;155:490–507.
112. Calcina V, Lourido L, Fernandez-Puente P, et al. Secretome analysis of 94chon -
113. Kim DD, Song WC. Membrane complement regulatory proteins. Clin Immunol. 2016;159:127–136.
114. Davies ME, Horner A, Loveland BE, McKenzie IF. Uptregulation of comple -
115. Zivanovic S, Rackov LP, Zivanovic A, Jevtic M, Nikolic S, Kocic S. Cartilage oligomeric matrix protein: inflammation biomarker in knee osteoarthritis. J Basic Med Sci. 2011;11:27–32.
116. Ahirvan E, Lorenzo P, Holmgregen K, et al. Novel cartilage oligomeric matrix protein (COMP) neoepitopes identified in synovial fluids from patients with joint diseases using affinity chromatography and mass spectrometry. J Biol Chem. 2014;289:20908–20916.
117. Clark SJ, Bishop PN, Day AJ. The proteoglycan glycomatrix: a sugar microenvi -
118. Koolenkirk K, Tadokori T, Hutamakki P, Mizzutani N, Yoshino S. Arthro -
119. Kalchikovskina N, Furst CM, Heinegard D, Blom AM. NC4 domain of cartilage-specific collagen IX inhibits complement directly due to attenuation of membrane bridge attack function and indirectly through binding and enhancing activity of complement inhibitors, C1r/C1s, and factor H. J Biol Chem. 2011;286:27915–27926.
120. Mapp PJ, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol. 2012;8:390–398.
121. Ashraf S, Walsh DA. Angiogenesis in osteoarthritis. Curr Opin Rheumatol. 2008;20:573–580.
122. Murata M, Yodoh K, Masuko K. The potential role of vascular endothelial growth factor (VEGF) in cartilage: how the angiogenic factor could be involved in the pathogenesis of osteoarthritis? Osteoarthrits Cartil. 2008;16:279–286.
123. Nozaki M, Raisier BJ, Sakurai E, et al. Druen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A*. 2006;103:228–233.

124. Clapp C, Thebault S, Macotela Y, Fegan G, et al. Prolactin is a component of the human synovial liquid and modulates the growth and chondrogenic differentiation of bone marrow-derived mesenchymal stem cells. *Mol Cell Endocrinol*. 2002;190:51–63.

125. Ogueta S, Munoz J, Obregon E, Garcia-Ruiz JP. Prolactin is a component of the human synovial liquid and modulates the growth and chondrogenic differentiation of bone marrow-derived mesenchymal stem cells. *Mol Cell Endocrinol*. 2002;190:51–63.

126. Macotela Y, Aguilar MB, Guzman-Morales J, et al. Matrix metalloproteases from chondrocytes generate an antiangiogenic 16 kDa prolactin. *J Cell Sci*. 2006;119:1790–1800.

127. Nagafuchi H, Suzuki N, Kaneko A, Asai T, Sakane T. Prolactin locally produced by synovium infiltrating T lymphocytes induces excessive synovial cell functions in patients with rheumatoid arthritis. *J Rheumatol*. 1999;26:1890–1900.

128. Corbacho AM, Nava G, Eiserich JP, et al. Proteolytic cleavage confers nitric oxide synthase inducing activity upon prolactin. *J Biol Chem*. 2000;275:13813–13816.

129. Corbacho AM, Nava G, Eiserich JP, et al. Proteolytic cleavage confers nitric oxide synthase inducing activity upon prolactin. *J Biol Chem*. 2000;275:13813–13816.

130. Adan N, Guzman-Morales J, Ledesma-Colunga MG, et al. Prolactin promotes cartilage survival and attenuates inflammation in inflammatory arthritis. *J Clin Invest*. 2013;123:3902–3913.

131. Clapp C, Adan N, Ledesma-Colunga MG, Solis-Gutierrez M, Triebel J, de la Escalera GM. The role of the prolactin/vasoinhibin axis in rheumatoid arthritis: an integrative overview. *Cell Mol Life Sci*. 2016;73:2929–2948.

132. Xia H, Huang J, Mao F, Peng K, Hu X. Activity of cathepsin D and alpha-1 antitrypsin in patients with hip or knee osteoarthritis. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2014;39:1151–1156.

133. Smith JK, Chi DS, Krish G, Reynolds S, Cambron G. Effect of exercise on complement C5a anaphylatoxin C5a. *Anasthesiol*. 2012;67:60–64.

134. Barilla ML, Carsons SE. Fibronectin fragments and their role in inflammatory arthritis and osteoarthritis cartilage. *Acta Orthop*. 2013;84:585–592.

135. Zutter MM, Edelson BT. The alpha2beta1 integrin: a novel collectin/C1q receptor. *Immunobiology*. 2007;212:343–353.

136. Farkas I,  Endo K, Ishikawa Y, et al. CD59 blocks not only the insertion of C9 into MAC but inhibits ion channel formation by homologous C8b-9 as well as C8b-9. *J Physiol*. 2002;539:537–545.

137. Holmquist E, Okroj M, Nodin B, Jirstrom K, Blom AM. Sushi domain-containing protein 4 (SUSD4) inhibits complement by disrupting the formation of the classical C3 convertase. *FASEB J*. 2013;27:2355–2366.

138. Holst B, Raby AC, Hall JE, Labeta MO. Complement takes its toll: an inflammatory crosstalk between Toll-like receptor agonists and crosstalk in innate immunity and inflammation. *Immunol Rev*. 2016;262:233–244.

139. Holst B, Ruby AC, Hall JE, Labeta MO. Complement takes its toll: an inflammatory crosstalk between Toll-like receptor agonists and crosstalk in innate immunity and inflammation. *Immunol Rev*. 2016;262:233–244.

140. Li L, Li Y, Feng D, et al. Preparation of low molecular weight chondroitin sulfate and its biological activity studies in attenuating osteoarthritis. *Int J Mol Sci*. 2016;17:E1685.

141. Happonen KE, Heinigard D, Saxne T, Blom AM. Interactions of the complement system with molecules of extracellular matrix: relevance for joint diseases. *Immunobiology*. 2012;217:1088–1096.

142. Barilla ML, Carsons SE. Fibronectin fragments and their role in inflammatory arthritis. *Semin Arthritis Rheum*. 2000;29:252–265.

143. Carsons SE, Schwartzman S, Diamond HS, Berkowitz E. Interaction between fibronectin and C1q in rheumatoid synovial fluid and normal plasma. *Clin Exp Immunol*. 1988;72:37–42.

144. Lang WG. Emerging role of innate immunity in organ transplantation part II: potential of damage-associated molecular patterns to generate immunostimulatory dendritic cells. *Transplant Rev (Orlando)*. 2012;26:73–87.

145. Hajiisghallio G, Lambris JD. More than complementing Tolls: complement Toll-like receptor synergy and crosstalk in innate immunity and inflammation. *Immunol Rev*. 2016;262:233–244.

146. Bendinelli P, Matteucci E, Dogliotti G, et al. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-kB inhibition via HGF. *J Cell Physiol*. 2010;225:757–766.

147. Cavallo C, Ruffo A, Grigoilo B, et al. Platelet-rich plasma: the choice of activation method affects the release of bioactive molecules. *BioMed Res Int*. 2016;2016:6591717.

148. Del Conde I, Cruz MA, Zhang H, Lopez JA, Afchar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med*. 2005;201:871–879.

149. Yu HI, Holes YM, Sya T, Ballard L, Atkinson JP. Identification of a third component of complement-binding glycoprotein of human platelets. *J Clin Invest*. 1986;78:494–501.