

Osteoarthritis and the Complement Cascade

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Clinical Medicine Insights: Arthritis and
Musculoskeletal Disorders
Volume 11: 1–12
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DOI: 10.1177/1179544117751430



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

RECEIVED: September 5, 2017. **ACCEPTED:** December 7, 2017.

TYPE: Review

FUNDING: The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is supported by the B. Braun Foundation (EMID: c6c8a1ca067517d0).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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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 ligament³—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 cartilage¹⁰ were identified as important causal factors. Even though repetitive physiological mechanical loading is an essential regulator for the metabolic activity of the chondrocytes,¹¹ excessive 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 1 β (IL-1 β), tumor necrosis factor α (TNF- α), and matrix metalloproteinase (MMP)-1, MMP-3, and MMP-9.^{12,13} 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.^{2,14,15} Hence, the major contribution of IL-1 β and TNF- α to OA pathogenesis, both representing key pro-inflammatory and catabolic cytokines, has been well



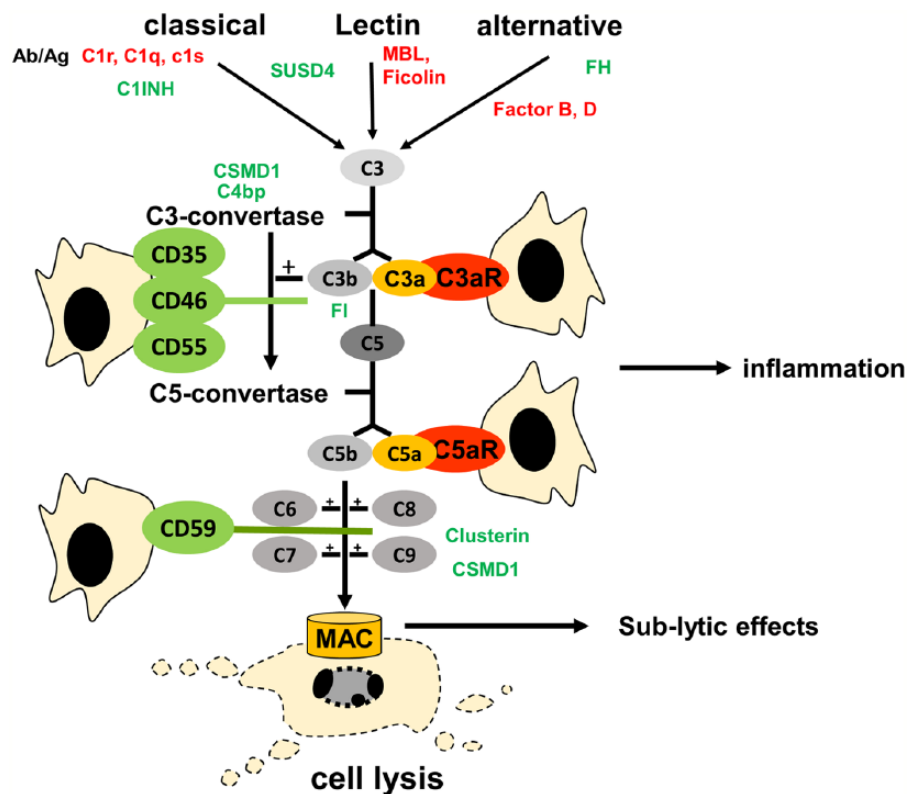


Figure 1. Simplified scheme of complement activation. Ab/Ag, antibody-antigen interaction; C1-INH, C1 inhibitor; C4bp, C4-binding protein; CSMD1, CUB and Sushi multiple domains 1; FH, factor H; FI, factor I; MBL, mannose-binding lectin; SUSD4, Sushi domain-containing protein 4.

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 angiogenic 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

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 ³⁰
	Cell proliferation	Renal mesangial cells	Brandt et al ³¹
		Aortic smooth muscle cells	Niculescu et al ³²
		Schwann cells	Dashiell et al ³³
	Expression of growth factors such as PDGF, bFGF	Endothelial cells	Benzaquen et al ³⁴
	Induces apoptosis	Renal mesangial cells	Nauta et al ³⁵
	Inhibits apoptosis	Oligodendrocytes and Schwann cells	Rus et al ³⁶
C5a	Inhibits apoptosis	Neutrophil granulocytes	Lee et al ³⁷
	Induces apoptosis	Neuronal cell line	Farkas et al ³⁸
	Early increase and later suppression in gene expression of CD46, CD55, CD59	Tenocytes	Own unpublished work
		Chemotaxis	MSCs
	Chemotaxis	Neutrophil granulocytes	Ehrengruber et al ⁴⁰
	Secretion and activation of MMP-9	Neutrophil and eosinophil granulocytes	DiScipio et al ⁴¹
	C3a	Inhibits apoptosis	Mesangial cells
Production of angiogenic factors, such as VEGF, CXCL8/IL-8, and IL-6		MSCs	DiScipio et al ⁴³
Hemotaxis		Eosinophil granulocytes	Daffern et al ⁴⁴

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.

system, such as RA, systemic lupus erythematosus, hyperacute graft rejection after transplantation, and sepsis.^{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).^{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 surface⁵⁶ (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 β and TNF- α .^{57,58} It has been demonstrated that the MAC inhibitory protein CD59 is most likely involved in protection against OA.⁸ Prominent expression of CD59 was seen on the synoviocytes, endothelial cells, and stromal cells in cases of OA.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^{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.⁶² Stuglics et al⁶⁰ 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 filtrate⁶³ as a consequence of systemic inflammation as in RA and OA.

Table 2. Complement regulators.

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.

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.⁶⁴ Corresponding with these observations, the gene expression of these components was shown in articular cartilage.⁶⁴ 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.^{58,64}

The complement components, C3aR and C5aR, and the CRPs, CD46, CD55, and CD59, were expressed not only in articular chondrocytes but also in nonarticular chondrocytes⁵⁸ and in intervertebral disc-derived fibrochondrocytes.⁶⁵ Interestingly, in synovial membrane, an extracellular deposition of CD55 produced by synovial fibroblasts and attached to the intimal collagen fiber network was shown.⁶⁶ The expression of CD35 could not be demonstrated in cultured chondrocytes.⁵⁸ As C4 is involved in the classical pathway, complement factor B (CFB) is represented only in the alternative pathway.⁶⁷ 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.⁶⁷ In cultured chondrocytes, complement expression was regulated by the pro-inflammatory cytokines IL-1 β , TNF- α , and IFN- γ .⁶⁴ The complement component C1s (complement C1s subcomponent) was increased by TNF- α , which is a key factor in the OA.⁶⁸ Both CFB and C3 were amplified by IL-1 β stimulation in cultured chondrocytes and synoviocytes.⁶⁷

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 proteins, C3 and C5, and the anaphylatoxin receptors, C3aR and C5aR, respectively.⁶⁹ 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 macroscopical feature in OA joints.⁸ Overexpression of C5aR exclusively in osteoblasts led to impaired fracture healing in mice, and the C5aR was suggested to increase osteoclast activity called osteoclastogenesis.^{70,71} The CRP CD59a is possibly a sex-dependent regulator of bone growth maintaining bone density and bone stability, as it is observed exclusively in

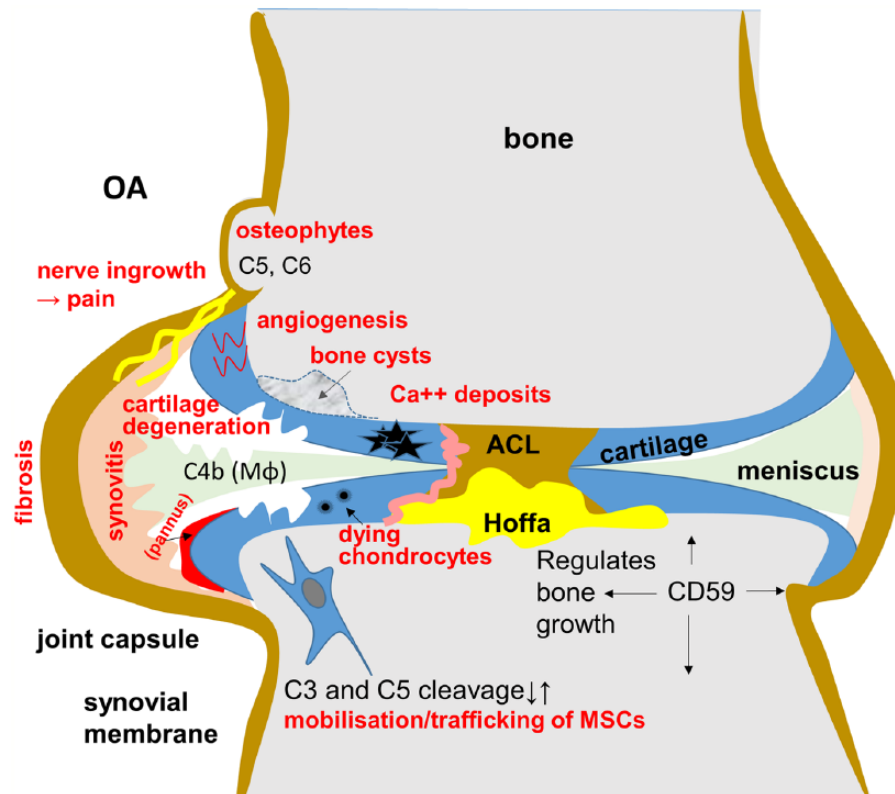


Figure 2. Simplified scheme depicting complement involvement in OA. OA indicates osteoarthritis.

male CD59a knockout mice.⁷² 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).^{15,73,74}

Mesenchymal stromal cells, eg, 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.⁷⁵

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.^{76,77} Complement plays an important role in fatty tissue, adipocytes produce complement proteins,⁷⁸ and complement activation can lead to low-grade inflammation in adipose tissue.²¹ Metabolic disbalances in fat tissue are observed in metabolic diseases, such as diabetes mellitus, which represent a predisposition for OA.⁷⁹ Moreover, C3aR and C5aR are involved in the development of adipocytes' insulin resistance through macrophage infiltration and the activation of adipose tissue.²¹ Taken together, fatty tissue synthesizes and regulates complement components, which present a source for the release of the anaphylatoxins C3a and C5a.⁸⁰

Ligaments and tendons express complement factors.^{81,82} 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-1 β . Furthermore, it impairs the gene expression of the cytoprotective CRPs, CD46 and CD55, in human tenocytes in vitro.⁸¹ 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 CD46, were induced under these conditions.⁸² 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.³

Even in the fibrocartilaginous meniscus tissue, C4d deposits have been detected immunohistochemically in the ECM areas of mucoid degeneration or fibrillation after meniscectomy.⁸³ In some of the samples, an association with the macrophage marker CD68 was found.⁸³ However, the degeneration of the meniscus may not only go along with the onset of gonarthrosis but also might actively contribute to it.⁸⁴

Complement Regulation and Activation in Cartilage and Chondrocytes

Complement activation and OA

Complement activation has been described to be critical for the development of OA.⁸ Wang et al stated that complement activation results in the formation of TCC on chondrocytes, which

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-1 β 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.⁸⁶ 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 MSCs⁹⁶ 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.^{6,98} 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,^{8,98} aggrecan,^{8,99} 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-1 β .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 pathway^{110,111} 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 al⁶⁰ 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 β , IL-6, and TNF- α (r , 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 neopeptides.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 manose-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 properdin¹⁰⁰ suggesting no protective effect of COMP in OA. Whether COMP contributes to OA complement activation as reported for RA remains questionable.¹⁰⁰

Clark et al¹¹⁷ 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 vaso-inhibins, 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 vaso-inhibins 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 vaso-inhibins 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 vaso-inhibins 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 (vaso-inhibins) 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 micro-damage 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

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	Koobkokkrud et al ¹¹⁸
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 al ¹¹⁹
Aggrecan	Specific and major proteoglycan in cartilage ECM	(+) The C-type lectin of the aggrecan G3 domain activates complement	Wang et al ⁸ , Furst et al ⁹⁹
GAGs, eg, CS	Major component of aggrecan	(+) Factor H provides binding sites for GAGs (-) Complement factors, such as CFB, C1s, C3, and C1r, were decreased by CS	Li et al ¹⁴⁰ , Clark et al ¹¹⁷ , Calamia et al ¹⁰⁷
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	Sofat ⁹⁷ , Dragomir et al ¹⁰⁵
COMP	Mediates collagen fibrillogenesis	(+) COMP induces activation and deposition of C3b and C9 via the alternative pathway in RA (-) COMP inhibits the classical and the lectin pathways due to direct interaction with the stalk region of C1q and mannose-binding lectin in RA Both could not be shown in OA by Happonen et al ¹⁰⁰	Blom ²³ , Happonen et al ^{100,141}
Fibronectin and its fragments	Fibronectin regulates cell differentiation, adhesion, and migration	(Effects not shown) binding to the C1q component of complement	Barilla et al ¹⁴² , Casons et al ¹⁴³
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 al ¹¹⁰ , Krumdieck et al ¹¹¹
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 al ⁸ , Sofat ⁹⁷ , Sjoberg et al ⁹⁸
Chondroadherin and osteoadherin (SLRP)	Cell-matrix interaction binds to collagens and $\alpha_2\beta_1$ integrin	(+) Activates complement by binding to C1q, interaction with factor H	Sjoberg et al ¹¹²
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-Bryan ⁶ , Land ¹⁴⁴
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) $\alpha_2\beta_1$ integrin interacted with C1q Direct interaction between TLR and C5a/C5aR signaling, shown in immune cells	Hajishengallis and Lambris ¹⁴⁵ , Holst et al ¹⁴⁶ , Sillat et al ¹⁴⁷ , Zutter and Edelson ¹⁴⁸

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.

with the control group.¹³⁸ A meta-analysis showed that the strengthening exercises combined with other modalities, such as stretching and aerobic exercises, provide benefit in OA.¹³⁹ 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.

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,¹⁰⁷ 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.¹¹⁹ 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 κ B 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 joint¹¹⁷ 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.

Acknowledgements

The authors would like to thank Ms Lynne-Brennan for proofreading.

Author Contributions

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

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