

Review

Impact of the complement cascade on posttraumatic cartilage inflammation and degradation

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Summary. The limited ability of articular cartilage to recover from injury, remains an unsolved clinical challenge in orthopaedic surgery. Persistent injury of the articular surface can lead to the development of posttraumatic osteoarthritis. The local inflammatory response contributes to the pathogenesis of osteoarthritis by inducing chondrocyte apoptosis and the de-regulation of chondrocyte matrix remodelling. The role of the complement system in contributing to secondary inflammation-mediated cartilage degradation represents a newer field of investigation. The purpose of this review article is to summarize the known complement-mediated actions in cartilage homeostasis and injury. This article focuses on the known effects of complement on secondary chondrocyte apoptosis, and the interplay of the complement system with pro-inflammatory cytokines. Pharmacological therapies related to complement inhibition will be discussed as they potentially represent a new avenue for attenuating the effect of the complement system on cartilage repair.

Key words: Chondrocyte, Complement, Cartilage injury, Apoptosis, Cytokines

Introduction

Trauma-induced cartilage injuries are significant injuries that, in certain circumstances, may lead to early posttraumatic arthritis. The self-repair capacity of cartilage is diminished because differentiated chondrocytes do not proliferate. When repair does take place the defect is filled with fibrocartilage which is unable to sustain the joint loads, a situation that is not

apparent with native hyaline cartilage. Hence, secondary osteoarthritis is the longterm consequence of cartilage injury (Martin et al., 2004; Kurz et al., 2005). The pathogenesis of osteoarthritis is dominated by catabolic and inflammatory processes initiated by pro-inflammatory cytokines, such as IL-1 β and TNF- α (Fernandes et al., 2002). The enhanced cytokine release leads to up-regulation of matrix degrading enzymes and inhibition of cartilage-specific matrix production. The ensuing result is cartilage destruction and joint inflammation (Martin et al., 2004; Kurz et al., 2005). TNF- α is a recognized inductive agent of chondrocyte apoptosis (Fischer et al., 2000; Aizawa et al., 2001; Aigner and Kim, 2002; Schuerwegh et al., 2003). Chondrocyte death by direct mechanical injury, apoptosis and necrosis are typical sequelae of traumatic joint injuries (D'Lima et al., 2002; Murray et al., 2004). Following trauma, the surviving chondrocytes have been shown to decrease their biosynthetic activity (Kurz et al., 2005). Recent evidence suggests that the direct contact of articular cartilage with blood induces secondary mechanisms of cartilage damage due to proteoglycan loss and chondrocyte apoptosis (Hooiveld et al., 2003a-e). The destructive cascade induced by exposure to intrarticular hemorrhage does not appear to be related to length of exposure in both *in vivo* and *in vitro* models (Hooiveld et al., 2003e). The molecular basis of blood-induced cartilage damage and chondrocyte apoptosis is not yet fully understood, but has to be considered in the case of traumatic joint injuries which are usually accompanied with haemarthrosis.

Posttraumatic activation of the complement cascade plays an important role in the pathophysiology of injured tissue degradation and repair (Stahel et al., 1998; Gasque, 2004; Guo and Ward, 2005; Fleming and Tsokos, 2006; Hietbrink et al., 2006; Khalil et al., 2006; Machens et al., 2006). The complement system is an innate part of the immune system which consists of proteolytic and regulatory components that act to protect

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the host from microbial infections and tissue damage (Volanakis and Frank, 1998; Mastellos et al., 2003; Carroll, 2004; Rus et al., 2005; Morgan et al., 2005a). Gene expression of complement components, receptors and regulators is found in most tissue and has also been recognized to be present in cartilage (Davies et al., 1994; Bradley et al., 1996; Hyc et al., 2001, 2003; Khan et al., 2001; Onuma et al., 2002). Activation of the complement system has been implicated in the pathogenesis of inflammatory arthritis and cartilage injury (Cooke et al., 1975; Moskowitz and Kresina, 1986; Howell, 1989; Vetto et al., 1990; Jha et al., 2005; Sjoberg et al., 2005; Banda et al., 2006; Mizuno, 2006). Induction of local complement synthesis was observed in hypertrophic cartilage (Andrades et al., 1996). In addition to the complement systems, inflammatory properties, the cascade is known to be involved in regulation of cellular apoptosis and clearance of apoptotic bodies under various pathological conditions (Farkas et al., 1998; Fishelson et al., 2001; Attali et al., 2004; Bohana-Kashtan et al., 2004; Leinhase et al., 2006a, b; Thurman et al., 2006). Interestingly, apoptotic bodies have been shown to activate all known complement activation pathways (Fishelson et al., 2001).

The present review is designed to discuss the importance of the complement system in posttraumatic joint inflammation, cartilage homeostasis and apoptosis based on the recent literature available from experimental and clinical studies in the field. Since several potent protective regulators and inhibitors of the complement system have been developed and characterized in recent years (Bhole and Stahl, 2003; Morgan and Harris, 2003; Holers and Thurman, 2004; Brook et al., 2005; Kulkarni et al., 2005; Purushottam et al., 2005; Thurman et al., 2006), new strategies for pharmacological attenuation of the complement-mediated inflammatory response in injured cartilage will be discussed.

The complement system

The complement system consists of more than 30 proteins, inhibitors, and cell-bound receptors (Volanakis and Frank, 1998; Morgan, 2000). Complement activation through either the alternative, classical, or the lectin pathway (Fig. 1) leads to opsonization of pathogens initiating phagocytosis, increasing vascular permeability, recruitment of phagocytic cells, augmentation of acute phase response, B-cell activation, and finally, cytolysis by membrane pore formation (Morgan, 2000; Onuma et al., 2002; Carroll, 2004).

The classical and lectin pathways are triggered by binding of antibodies to complement component C1q or specific carbohydrates to mannose binding protein (MaBP) (Volanakis and Frank, 1998; Morgan, 2000). The alternative pathway is activated by factor B, properdin, and C3b (Hourcade, 2006; Thurman and Holers, 2006) but also continuously activated through a process termed “tick over”, as a constitutive first line of defense (Thurman and Holers, 2006). Under normal

physiologic conditions, the complement inhibitors and cell surface regulatory proteins control this process, but in response to tissue trauma their natural balance might be disturbed (Stahel et al., 1998; Hietbrink et al., 2006). In this regard, the uncontrolled overwhelming activation of the complement cascade may cause an “innocent bystander” injury by homologous cell lysis (Gasque, 2004; Glovesky et al., 2004; Morgan et al., 2005b).

All three complement activation pathways converge at the central component C3, which is activated by C3 convertases of either pathway (Fig. 1) (Volanakis and Frank, 1998; Morgan, 2000). Cleavage of C3 by C3 convertases leads to formation of the C3b split product, which acts as an opsonin by covalent binding of pathogen surfaces and by release of soluble C3a, a potent inflammatory mediator implicated in cell activation and chemotaxis (Volanakis and Frank, 1998; Morgan, 2000). Anaphylatoxin C3a mediates its biological functions by binding to the C3a receptor (C3aR) expressed on various cell-types (Ames et al., 1996; Nataf et al., 1999).

The C5a anaphylatoxin which is generated via cleavage of C5 by C5 convertases is of central importance in complement-mediated inflammatory responses due to its potent and highly pleiotropic inflammatory properties (Volanakis and Frank, 1998; Nataf et al., 1999; Morgan, 2000; Allegretti et al., 2005). C5a binds to its receptor (C5aR; CD88) which has been shown to be widely expressed on cell-surfaces, including

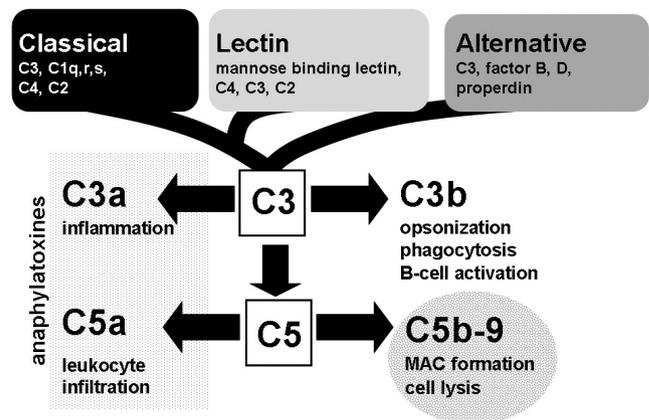


Fig. 1. Simplified scheme of the complement activation pathways and function. Within the complement system, three pathways are capable of initiating the process that result in C3 activation: classical, alternative and lectin. The classical and lectin pathways are triggered by binding of antibodies to complement component C1 or specific sugars to mannose binding protein or mannose binding protein associated serin proteinase. The alternative pathway is activated by factor B and D, and C3b. Complement activation ultimately can induce cell damage either directly or via the membrane attack complex (MAC) or indirectly through C5a mediated recruitment and activation of phagocytic cells. Complement activation furthermore leads to release of the inflammatory anaphylatoxins C3a and C5a.

Complement activation and cartilage injury

human articular chondrocytes (Onuma et al., 2002). Therefore, inhibiting C5a may be a promising approach in complement-mediated disorders (Morgan and Harris, 2003; Allegretti et al., 2005). The larger split product derived from C5 cleavage is C5b which consequently binds to C6, C7, C8, and multiple C9 molecules, thus initiating the terminal complement pathway with formation of the membrane attack complex (MAC; C5b-9). This induces a transmembrane pore disrupting the phospholipid bilayers of cell membranes and leads to lysis of target cells (Morgan, 1999; Huang et al., 2005; Barnum and Szalai, 2006).

Natural soluble complement inhibitors and regulatory proteins

Because of the detrimental effects of activated complement, a tight regulation of the complement cascade is necessary to protect cells from self destruction (Singhrao et al., 2000; Gasque, 2004; Cole et al., 2006). For this reason, complement inhibitors and regulatory proteins (CRP) are present in most tissues. Typical soluble complement inhibitors include C1 inhibitor (C1-INH), C4b binding protein (C4bp), factor H, factor I, and clusterin (Morgan, 2000; Gasque, 2004). CRPs represent a group of cell surface glycoproteins which protect the healthy tissue from complement mediated damage by the soluble complement proteins (Morgan, 2000; Gasque, 2004). Most cells possess these CRPs on their surface membrane which tightly regulate local complement activity. Typical CRPs are the human cell surface glycoproteins CD35 (complement receptor type 1; CR1), CD46 (membrane cofactor protein; MCP), the CD55 (decay accelerating factor; DAF) and CD59 (protectin) (Morgan, 2000; Gasque, 2004). On the chondrocyte surface, several CRPs such as CD46, CD55 and CD59 have been demonstrated (Hyc et al., 2003). Protein and mRNA expression of these proteins was increased by exposure to the pro-inflammatory cytokines IL-1 β and TNF- α in cartilage explants and also on chondrocyte monolayer cultures *in vitro* (Davies et al., 1994; Hyc et al., 2003). However, gene expression of CD35 could not be shown in chondrocytes (Hyc et al., 2003). These CRPs are differentially regulated by various cytokines (Hyc et al., 2003). Additionally, Gonzalez-Rubio and coworkers demonstrated the complement inhibitory Factor I in synovial fluid of arthritic joints. Factor I is a complement inhibitor regulating classical and alternative pathways (Gonzalez-Rubio et al., 1996). Other regulatory proteins such as C1-INH, C4-bp, factor H, S-protein and clusterin have not been localized in synovial joint fluid at this point. Properdin, the only known naturally occurring positive complement regulator has also been shown in cartilage (Andrades et al., 1996). Diminished expression or function of CRPs has been identified as a cause of several human inflammatory diseases (Thurman and Holers, 2006). Modulation of natural CRPs or application of compounds with complement regulatory function may be a therapeutic tool to compensate

enhanced complement activity (Fig. 2). The presence of CRPs on chondrocytes implies an essential participation of the tightly regulated complement system in chondrocyte homeostasis (Davies et al., 1994). CRPs are an important precondition to protect the chondrocytes directly from activated complement which might be derived from various cell sources in the joint.

Sources of complement in the joint and synovia

Complement activation was implicated more than a decade ago in the pathophysiology of inflammatory joint diseases, such as rheumatoid arthritis and osteoarthritis (Vetto et al., 1990; Olesky et al., 1991). As early as 1966, Fell and colleagues reported that when activated complement was added to cartilage *in vitro* it lead to direct sever cartilage degradation (Fell et al., 1966). Recently, a study of Hansson et al., (2004) revealed that an autoimmune disease with prevalent manifestation in cartilage, relapsing polychondritis, may also be mediated by pathways associated with un-regulated complement activation (Hansson et al., 2004). Complement may enter the synovial fluid derived from synovial cells, infiltrating leukocytes and synovial blood supply, or from the haemarthrosis related to the initial trauma. Complement can also be produced by articular cartilage chondrocytes (Gulati et al., 1994; Bradley et al., 1996; Sadallah et al., 1997). It remains unclear to which extent chondrocytes contribute to the joint complement "pool". Moreover, an intensive signalling crosstalk between these sources in activation and regulation of the complement machinery can be supposed and un-regulated leading to articular cartilage destruction (Sakiyama et al., 1997) (Fig. 2). Up to now, knowledge regarding the distribution and function of the complement system in cartilage remains fragmentary.

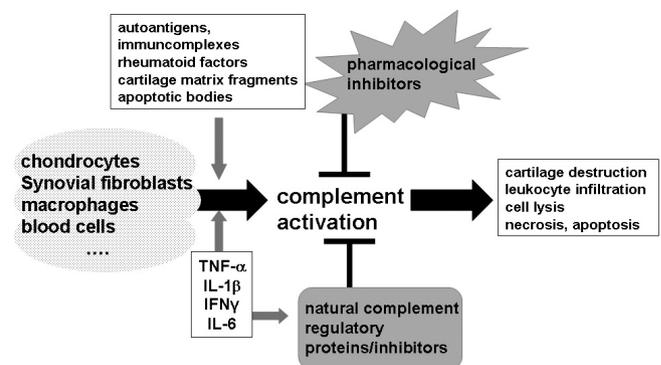


Fig. 2. Regulation and sources of complement activity in the joint and cartilage. Various cell types deriving from inflamed synoviales or cartilage contribute complement factors in response to several stimuli such as immunocomplexes or cartilage matrix fragments. Complement activity is amplified by pro-inflammatory cytokine signalling. Natural inhibitors or pharmacological compounds are able to modulate complement activity. Excessive complement activation in the joint results in cartilage degradation, inflammation and chondrocyte apoptosis.

The synovial membrane represents the immediate immunological environment for the articular cartilage (Berumen-Nafarrate et al., 2002). The synovia contains highly specialized fibroblast like cells producing the synovia which is the prerequisite for regular articular cartilage function and chondrocytes nutrition (Berumen-Nafarrate et al., 2002). Various cytokines and growth factors gain entry via the synovial fluid to the articular cartilage and are essential for articular cartilage balance (Okazaki et al., 2001; Berumen-Nafarrate et al., 2002). Under inflammatory conditions, elevated pro-inflammatory cytokine concentrations severely affect cartilage homeostasis (Fernandes et al., 2002). Most components of the complement system have also been found in synovial fluid and membranes (Gulati et al., 1994). Gulati et al. compared the mRNA expression of components of the classical pathway in normal, osteoarthritic and rheumatoid arthritis synovial membranes (Gulati et al., 1994). The authors concluded that all components of the classical complement pathway were present in all three types of synovial membranes and to a various extent synthesized locally by different cell types, such as macrophages and fibroblasts (Gulati et al., 1994). Factor I, another complement inhibitor, could be demonstrated in elevated concentrations under various inflammatory joint conditions in the synovial fluid (Gonzalez-Rubio et al., 1997) which may be indicative for the onset of compensatory mechanisms involving the complement system. In rheumatoid arthritis, acute arthritis and active osteoarthritis, deposits of C3c, C9 and weak signals of the complement regulatory protein CD59 could be shown in synovial vasculature and intercellular matrix of the synovial lining (Konttinen et al., 1996). Under chronic osteoarthritis conditions these components were no longer detectable suggesting that their upregulation is limited to active inflammatory conditions (Konttinen et al., 1996). The complement regulatory protein CD35 has been demonstrated in the synovial fluid (Sadallah et al., 1997). The presence of anaphylatoxin C5a could be demonstrated in the synovial fluid of rheumatoid arthritis patients (Hogasen et al., 1995). Obviously, activation of complement components in rheumatoid arthritis and acute arthritides seems to be associated with a decreased protection of synovial cells against cellular effects and lysis mediated by the complement membrane attack complex (Konttinen et al., 1996).

Complement expression by chondrocytes

As indicated above, complement has been implicated in cartilage destruction in rheumatoid arthritis and osteoarthritis. Most authors hypothesized that the components involved may be derived from noncartilaginous sources (Cooke et al., 1975; Vetto et al., 1990). Complement has, however, been demonstrated to be produced from cartilage tissue (Andrades et al., 1996; Bradley et al., 1996). Expression of a broad range of complement proteins and mRNA was noted in chondrocytes (Hyc et al., 2003). Most complement

components of the classical and alternative pathways could be detected immunohistochemically and at the gene expression level in chondrocytes under various conditions (Sakiyama et al., 1991; Andrades et al., 1996; Bradley et al., 1996; Nakagawa et al., 1997a,b).

In contrast, C1r, C1-INH, C4bp and factor I were not detected in cartilage tissue (Bradley et al., 1996; Nakagawa et al., 1997). The role of these complement proteins in cartilage has not been defined. By the use of *in vitro* cultures, the complement profile of chondrocytes might depend on their differentiation state (Bradley et al., 1996; Nakagawa et al., 1997b). Bradley et al. (1996) studied the distribution of complement components in chondrocyte cultures *in vitro* and concluded that cultured chondrocytes had a complement profile that is most similar to that of cultured fibroblasts and less similar to that of articular chondrocytes *in vivo* (Bradley et al., 1996). Nakagawa and coworkers found a novel function for C1s in rheumatoid cartilage: degradation of the cartilage matrix (Nakagawa et al., 1999). Moreover, it has been shown recently that extracellular cartilage matrix components are able to activate the complement system (Barilla and Carsons, 2000; Sjoberg et al., 2005). Fibromodulin activated both the alternative and classical complement pathways by directly binding to C1q and also fibronectin fragments bind to C1q (Barilla and Carsons, 2000; Sjoberg et al., 2005). Lectin motifs have also been demonstrated in proteoglycans of the cartilage matrix suggesting that all three complement pathways can be activated in cartilage (Day et al., 1994). Matrix fragments released in response to cartilage trauma or during osteoarthritis, are well known to accelerate cartilage and synovial inflammation via induction of pro-inflammatory cytokines and may induce complement activation (Yasuda, 2006). The complement activation cascade may therefore start in the cartilage and be amplified by invading complement proteins from various other cell sources of the joint.

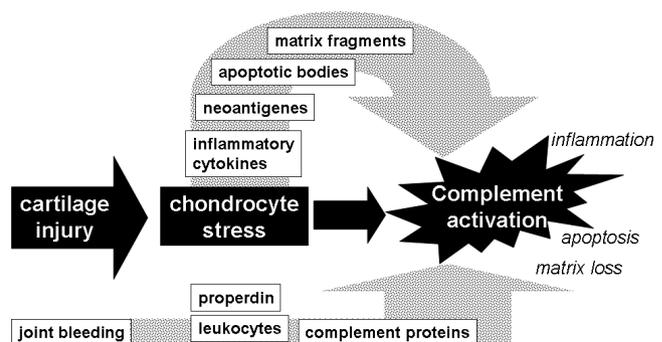


Fig. 3. Hypothetic response of articular chondrocytes to cartilage injury which leads to activation of the complement system. Stressed chondrocytes produce elevated levels of pro-inflammatory cytokines, express neoantigens and undergo apoptosis. Additionally, cartilage extracellular matrix fragments are released from the cartilage defect. Altogether, enhanced complement activity may lead to joint inflammation, chondrocyte apoptosis and cartilage matrix loss.

Enchondral ossification – role of complement

Since most complement components are broadly expressed in cartilage (Sakiyama et al., 1991; Andrades et al., 1996; Bradley et al., 1996; Nakagawa et al., 1997a,b), the question arises whether this implies a physiological function in cartilage. Interestingly, the complement system is evidently involved in physiological processes in cartilage such as enchondral ossification, which is the basis for long bone development (Andrades et al., 1996; Sakiyama et al., 1997). During bone development, a chondral precursor of the future bone is transformed to bone tissue (Andrades et al., 1996). In this process, the cartilage undergoes various differentiation stages from resting to proliferating and subsequently hypertrophic cartilage (Andrades et al., 1996). Finally, at distinct areas, the primary and secondary ossification centers, the cartilage matrix mineralizes, the hypertrophic chondrocytes die and the cartilage matrix will be degraded and subsequently substituted by mineralized bone matrix (Andrades et al., 1996). C1s, which is an integral part of the first component of complement C1, initiating the classical pathway, is induced in the secondary ossification center (Sakiyama et al., 1997). The extent of C1s synthesis correlated well with hypertrophic alterations of chondrocytes (Sakiyama et al., 1997). In contrast, normal articular cartilage did not stain for C1s (Sakiyama et al., 1997). C1s synthesis increased with chondrocyte differentiation induced by ascorbic acid (Nakagawa et al., 1997b). In contrast, transforming growth factor- β and basic fibroblast growth factor which inhibit chondrocyte hypertrophic differentiation, suppressed C1s production (Nakagawa et al., 1997b). An interaction of C1s with insulin-like growth factor binding protein could also be demonstrated in the synovial fluid (Clemmons et al., 2002). Sakiyama et al. (1998) could show that inhibition of basic fibroblast growth factor activity by complement C1s may be mediated by covalent binding of C1s with basic fibroblast growth factor (Sakiyama et al., 1998). Taken together, these studies suggest an intimate interaction between the complement system and key anabolic growth factors in cartilage indicating a possible involvement in cartilage differentiation and the continuous cartilage remodelling process. The complement proteins C3 and factor B were equally expressed in the resting and proliferation zone (Hourcade, 2006). Properdin, which is a positive regulator of complement activation (Hourcade, 2006) was evident in the resting and hypertrophy zone during enchondral ossification, but not in the proliferation zone (Hourcade, 2006). Complement proteins C5 and C9, which are “key” components of the terminal complement pathway, were found only in the hypertrophic zone indicating that they might mediate or accompany chondrocyte death in the hypertrophic area required for ossification and bone formation (Andrades et al., 1996). The alternative complement pathway may therefore play a substantial

role in transformation and terminal differentiation of chondrocytes (Andrades et al., 1996). In osteoarthritis, a progressive ossification process can be observed in the form of osteophyte development (Bonnet and Walsh, 2004). One may thus hypothesize that the complement system might also take part in these processes.

Complement activation in cartilage injury and osteoarthritis

Based on the aforementioned data from the literature, an involvement of complement activation in secondary cartilage injury, albeit not proven, must be assumed (Hietbrink et al., 2006). Complement activation has been shown to occur as a typical sequelae of tissue trauma (Stahel et al., 1998; Bellander et al., 2001; Hietbrink et al., 2006). The initial traumatic insult may induce deficiencies of regulatory proteins on cells which become necrotic or on injured cells at the defect area (Thurman and Holers, 2006). Alternatively, infiltrating cells such as neutrophils bring in C3 and properdin that increase complement levels specifically at the trauma site by providing additional substrates for the alternative pathway (Thurman and Holers, 2006). Since the alternative complement pathway is continuously activated through a spontaneous process called “tick over” trauma-induced disturbances in the balance of inhibitory and regulatory factors on the cells lead directly to activation of this complement pathway (Thurman and Holers, 2006). Therefore, the alternative pathway seems to be a candidate for trauma-induced complement activation in cartilage (Thurman and Holers, 2006). Additionally, the alternative and classical pathway can be activated by released cartilage matrix fragments (Sjoberg et al., 2005). Other cartilage matrix proteoglycans contain lectin motifs or complement binding protein like domains which may be accessible during cartilage injury for complement activation via the lectin pathway (Chen et al., 2002; Day et al., 2004). During cartilage trauma associated with joint bleeding, properdin, a blood derived positive complement regulator can enhance complement activation (Hourcade, 2006; Thurman and Holers, 2006). The stress-activated chondrocytes in the defect area may produce increased levels of pro-inflammatory cytokines leading to enhanced complement activity and to development of posttraumatic arthritis (Nakagawa et al., 1999; Onuma et al., 2002). Both the alternative and the classical complement pathways are involved in the effector phase of arthritis (Hietala et al., 2004) and have been implicated in the early phases of pathogenesis of osteoarthritis (Cooke et al., 1975; Moskowitz and Kresina, 1986; Howell, 1989; Vetto et al., 1990). Corvetta and colleagues determined more than a decade ago that the terminal complement complex (MAC) is present in synovial tissue samples of human osteoarthritic joints, but in none of the controls (Corvetta et al., 1992). C3 and C9 neopeptides and also the complement regulatory protein CD59 were localized in

synovial tissue of osteoarthritic knees (Kemp et al., 1992). Compared to rheumatoid arthritis, the complement activity in osteoarthritis seems to be limited to the active, inflammatory phases (Partsch et al., 1991; Kontinen et al., 1996). Active arthritis is characterized by enhanced pro-inflammatory cytokine activity, the same changes are recognized following trauma to cartilage since stressed chondrocytes respond by up-regulating their pro-inflammatory cytokine production (Lotz, 2001).

Interrelation of complement and cytokines in joint inflammation

The pro-inflammatory cytokines IL-1 β and TNF- α play a pivotal role in inflammatory joint diseases, such as rheumatoid arthritis and active osteoarthritis leading to the catabolic damage of cartilage (Feldmann et al., 1996; Blanco, 1999). Cytokines have been shown to regulate complement synthesis since complement synthesis in cultured chondrocytes was modulated by the cytokines IL-1 β , TNF- α and IFN γ (Bradley et al., 1996). C5aR was up-regulated in rheumatoid cartilage and in normal cartilage by IL-1 β , but not in osteoarthritic cartilage (Onuma et al., 2002). Gonzalez-Rubio and colleagues found a correlation between the expression levels of the complement inhibitor Factor I and the pro-inflammatory cytokine IL-6 (Gonzalez-Rubio et al., 1997). Chondrocytes stimulated with cytokines such as IL-1 β , TNF- α and IL-4 demonstrated up-regulation of complement regulatory proteins, probably as a compensatory mechanism of enhanced complement activation (Davies et al., 1994; Hyc et al., 2001, 2003). IL-1 β and TNF- α stimulated CD46, CD55 and CD59 whereas IL-4 stimulated CD46 only. C1s production by chondrocytes was increased by the pro-inflammatory cytokine TNF- α *in vitro* leading to cartilage matrix degradation (Nakagawa et al., 1999). Taken together, cytokines differentially modulate complement expression in cartilage indicating that complement pathways may crosstalk with pro-inflammatory cytokine signalling. On the other hand, recombinant Vaccinia virus complement control protein (rVCP), an effective complement inhibitor, was able to lead to down regulation of pro-inflammatory cytokines IL-12, TNF- α , IL-6 (Purushottam et al., 2005). Accordingly, complement receptor type 1-related protein (Crry-Ig) and anti-C5 antibodies diminished pro-inflammatory cytokines in a collagen induced arthritis model (Banda et al., 2002) suggesting that the activated complement system seems to represent a positive amplification loop in pro-inflammatory cytokine signalling circuits.

Complement and chondrocyte cell death

In the past, complement-mediated cell lysis has been presented as a classical example of necrotic cell death. Later, pro- and anti-apoptotic effects of complement have been recognized in various cell types (Fishelson et

al., 2001; Bohana-Kashtan et al., 2004). Complement plays a substantial role in the recognition and clearance of the cellular remnants of apoptosis (Attali et al., 2004; Liu et al., 2004; Cole et al., 2006). Apoptotic cells activate all three pathways (classical, alternative, lectin) of the complement cascade (Fishelson et al., 2001). C5a has been shown to activate typical executioners of apoptosis the effector and initiator caspases caspase-3, -8, -9, and to provoke cytochrome C release from mitochondria (Guo et al., 2000) and therefore, clearly exerts a pro-apoptotic capacity which involves intrinsic (mitochondrial) and extrinsic (death receptor mediated) apoptotic pathways. Accordingly, complement-mediated lysis was prevented by caspase-6, -8, -9 or -10 inhibitors in early apoptotic cells (Attali et al., 2004). Factor B has pro-apoptotic effects (Uwai et al., 2000) and inhibition of factor B has been shown to protect from apoptosis (Thurman et al., 2006).

Particularly the alternative complement pathway might play a significant role in complement-mediated apoptosis since posttraumatic neural cell apoptosis was ameliorated in animals lacking the alternative pathway after traumatic brain injury (Leinhase et al., 2006b).

The pro-apoptotic capacity of the complement system might depend on other cell type-specific factors. Binding of C5a to its receptor C5aR initiated apoptosis in neuroblastoma cells (Farkas et al., 1998). In contrast, C5a is able to protect neurons from cell death by activation of the MAPKinase cascade (via ERK1/2) (Mukherjee et al., 2001). Additionally, sublytic doses of the MAC have been shown to induce cell stimulatory effects (Fishelson et al., 2001). The impact of complement activation on chondrocyte apoptosis has not been investigated yet. It is well known that enhanced apoptotic cell death of chondrocytes is a typical feature in the pathogenesis of osteoarthritis (Fischer et al., 2000; Aizawa et al., 2001; Aigner and Kim, 2002; Schuerwegh et al., 2003). The removal of apoptotic cells in cartilage still remains enigmatic. Up to now, the involvement of the complement system in the clearance of apoptotic cell bodies in cartilage has not been recognized. Increase in complement proteins in the hypertrophic cartilage during enchondral ossification may indicate a possible role of these components in apoptosis and cartilage matrix degradation (Andrades et al., 1996). The removal of cell residues is important since apoptotic cell bodies lead to expression of neoepitopes and predisposes one to the development of autoimmune disease (Fishelson et al., 2001). The ability to block selectively pro-apoptotic pathways by selective modulation of complement activation pathways may be a promising therapeutic tool.

Current pharmacological approaches for complement inhibition

The complement system is tightly regulated to avoid self destruction of the tissue (Bohana-Kashtan et al., 2004). In recent years, an increasing amount of

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Table 1. Summary of currently investigated complement inhibitory or regulatory compounds which might have a therapeutic relevance in complement-mediated disorders.

COMPOUND	TARGET	INHIBITED	REFERENCES
Crry Ig	C3	CP, AP, LP	Banda et al., 2002, Leinhase et al., 2006a
Recombinant Vaccinia virus complement control protein (rVCP)	C3b	CP, AP	Purushottam et al., 2005
Anti-factor B neutralizing monoclonal antibody	factor B	AP	Thurman et al., 2006
Anti-C5 antibodies	C5	CP, AP, LP	Banda et al., 2002, Brook et al., 2005
Soluble CR1 (sCR1)	C3-, C5- convertase	AP, CP	Brook et al., 2005
Compstatin	C3	?	Brook et al., 2005
Curcumin	?	CP, AP	Kulkarni et al., 2005
Rosmarinic acid	C5 convertase	CP, AP	Kulkarni et al., 2005
Quercetin	?	AP, CP	Kulkarni et al., 2005

compounds inhibiting cross-points of the complement cascade has been implicated in therapeutical approaches in complement-mediated diseases (Brook et al., 2005; Kulkarni et al., 2005; Purushottam et al., 2005; Leinhase et al., 2006a-b; Fleming and Tsokos, 2006). Since complement activation seems to be involved in arthritis progression, modulating complement activity may be an approach in arthritis therapy (Low and Moore, 2005). Recently, the rVCP has been found to ameliorate collagen-induced arthritis in a mouse model by limiting cartilage degradation (Purushattam et al., 2005).

With regard to treatment of other well known complement-mediated diseases, anti-complement prodrugs with a complement regulatory capacity have been designed (Harris et al., 2003). These prodrugs are cleaved at the site of inflammation releasing the fully active complement regulator. Several complement inhibitors are currently being tested for the use in various disorders (Burruva et al., 2005). These drugs have not yet been approved by regulatory agencies but seem to represent a future therapeutic concept (Kulkarni et al., 2005).

Compounds with complement inhibitory capacities have been studied in the last few years such as inhibitory antibodies directed against C5 (Brook et al., 2005), blocking antibodies directed against factor B (Thurman et al., 2006), the murine complement inhibitor Crry-Ig, a well known mouse specific complement inhibitor which represents a functional homologue of the human complement regulatory proteins CD55 and CD46 and soluble complement receptor 1 (Banda et al., 2002; Fleming and Tsokos, 2006), rVCP (Purushottam et al., 2005), curcumin, a major component of the culinary spice turmeric derived from *Curcuma longa* and rosmarinic acid (Kulkarni et al., 2005) as summarized in Table 1. The main question is whether chondrocyte apoptosis can be modulated by selective inhibition of specific complement pathways. Apart from curcumin the effects of these compounds have not been studied in cartilage. Curcumin is an effective inhibitor of several other catabolic pathways in chondrocytes and obviously

exhibits anti-apoptotic effects (Schulze-Tanzil et al., 2004; Shakibaei et al., 2005), its complement inhibitory effect in cartilage is not yet known. These compounds modulate various complement pathways. Inhibition of downstream complement components is more promising since the essential early functions of complement such as opsonization microbials and the natural immune clearance will not be affected by these agents (Brook et al., 2005).

Concluding remarks

Essential components of the complement system are synthesized by chondrocytes, but the role of the complement system in traumatic condition of cartilage, and the relationship to the development of posttraumatic osteoarthritis is poorly understood. The regulation of complement components by pro-inflammatory cytokines suggests that the complement cascade may be involved in several catabolic pathways and might contribute to arthritis pathogenesis. Studies investigating the role of complement in chondrocyte apoptosis or cartilage injury are lacking. Further work needs to be done to define the role of the complement system in cartilage homeostasis and to decide whether modulating complement activity using inhibitors of distinct complement pathways may be a useful approach in cartilage repair and the prevention or treatment of osteoarthritis.

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