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Interactions of the complement system with molecules of extracellular matrix; relevance for joint diseases.

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Key words: Complement, Rheumatoid arthritis, Osteoarthritis, Extracellular matrix
Abbreviations: ACPA, anti-citrullinated peptide antibody; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AIA, antigen-induced arthritis; C4BP, C4b-binding protein; CAIA, collagen antibody-induced arthritis; CIA, collagen-induced arthritis; COMP, cartilage oligomeric matrix protein; CR, complement receptor; CS, chondroitin sulfate; ECM, extracellular matrix; GAG, glycosaminoglycan; IαI, inter-α-trypsin inhibitor; IL, interleukin; MAC, membrane attaack complex; MASP, mannose-binding lectin associated serine protease; MBL, mannose-binding lectin; MMP, matrix metalloproteinase; OA, osteoarthritis; PRELP, proline arginine-rich end leucine-rich repeat protein, RA, rheumatoid arthritis; SLRP, small leucine-rich repeat protein; TLR, toll-like receptor; TNF, tumor necrosis factor

Abstract

Rheumatoid arthritis (RA) is a highly disabling disease affecting all structures of the joint. Understanding the pathology behind the development of RA is essential for developing targeted therapeutic strategies as well as for developing novel markers to predict disease onset. Several molecules normally hidden within the cartilage tissue are exposed to complement components in the synovial fluid upon cartilage breakdown. Some of these have been shown to activate complement and toll-like receptors, which may enhance an already existing inflammatory response, thereby worsening the course of disease. Other cartilage-resident molecules have in contrast shown to possess complement-inhibitory properties. Knowledge about mechanisms behind pathological complement activation in the joints will hopefully lead to methods which allow us to distinguish patients with pathological complement activation from those where other inflammatory pathways are predominant. This will help to elucidate which patients will benefit from complement inhibitory therapies, which are thought to aid a specific subset of patients or patients at a certain stage of disease. Future challenges are to target the complement inhibition specifically to the joints to minimize systemic complement blockade.

1. Introduction

The early view of the complement system as solemnly a defense against invading pathogens has been challenged by the evidence of the importance of complement in multiple processes such as clearance of apoptotic cells and immune complexes, regulation of adaptive immune responses and regulation of cellular effects such as cell proliferation and migration. Therefore it is not surprising that misdirected complement activation and the lack of correct complement regulation may lead to severe inflammatory conditions. An example of this is the inflammatory joint disease RA, in which pathological complement activation occurs in the affected joints propagating a disease process leading to progressive tissue destruction.

RA is a common joint disease affecting an estimated 0.5-1% of the population worldwide with prevalence increasing with age. RA is a systemic autoimmune disease which is more common in women than in men, suggesting that hormonal factors may play a role for disease development (Islander et al., 2010). Even though any joint can be affected, the small peripheral joints of the hands and feet tend to be more susceptible. The disease course of RA may be further complicated by an increased risk of cardiovascular disease (Holmqvist et al., 2010) and other comorbidities. RA is characterized by a persistent inflammation of the synovial membrane lining the joint, with associated infiltration of macrophages, granulocytes, T-cells and B-cells. In RA, macrophage-like synovial cells produce an excess of pro-inflammatory cytokines and proteases whereas fibroblast-like synoviocytes change their morphology by becoming more invasive, allowing them to invade the cartilage tissue thereby promoting tissue destruction. Also the underlying bone is affected in RA as activation of osteoclasts leads to bone erosion also contributing to joint deformation (Scott et al., 2010).

2. Complement activation in arthritis

There is substantial evidence of complement involvement in RA from studies of both patients and animal models (Okroj et al., 2007). Results from rodent arthritis models emphasized the role of the alternative pathway for disease development whereas the classical pathway seemed less important. In a collagen-induced arthritis (CIA) model, C5 (Wang et al., 2000) or factor B deficiency protected mice from the disease whereas C4 deficiency did not (Banda et al., 2006). Likewise, C1q or mannose-binding lectin (MBL) deficiency did not protect mice from arthritis in a collagen antibody-induced arthritis (CAIA) model (Banda et al., 2007). In contrast, MASP1/3$^{-/-}$ mice have a less severe disease outcome in CAIA, which most likely relates to absence of alternative pathway activity in these mice due to the absence of activated factor D (Banda et al., 2010). Recently, deletion of properdin in the myeloid lineage by conditional gene targeting was shown to reduce disease severity in a K/BxN model of arthritis (Kimura et al., 2010). Cumulatively, the evidence supports the role of the alternative pathway in the development of RA in mice. This is perhaps somewhat surprising considering that CAIA model is dependent on effector mechanisms elicited by antibodies, one of which should be the classical complement pathway. It appears that this pathway may be less significant relatively to the alternative pathway in mice as compared to man. Mice lacking the C5aR or C3aR also have milder disease in arthritis models showing that at least a part of the pathology is related to anaphylatoxin production and the interaction between anaphylatoxins and their cellular receptors (Banda et al., 2011). Furthermore, deletion of complement receptor 2 (CR2) results in a diminished arthritis severity in the CIA model with lower antibody production towards collagen II and citrullinated peptides, showing the importance of complement mediated adaptive immune responses (Kuhn et al., 2008).

In humans, complement activation products, such as C3d (Swaak et al., 1987), have been found in the joints of patients with RA and complement deposition can be detected in the synovium by immunohistological staining (Konttinen et al., 1996). Circulating complexes of C1q and C4 were found in the plasma of patients with RA at a much higher concentration during active disease than during remission indicating ongoing complement activation through the classical pathway (Wouters et al., 2006). Also elevated levels of C3a (Moxley and Ruddy, 1985) and C5a have been demonstrated in the synovial fluid of RA patients (Jose et al., 1990), as well as soluble C5b-9 complexes (Brodeur et al., 1991; Morgan et al., 1988). This shows that complement activation occurs locally within the joint with the potential to stimulate further inflammatory responses.

A recent study of both mice and man has shown that complement is also activated in the joints during osteoarthritis (OA), a joint disease originally characterized as a non-inflammatory arthritis. Elevated levels of C3a and sC5b-9 were found in the synovial fluid in patients with early and late stage disease and the complement membrane attack complex (MAC) was found deposited on the cartilage tissue (Wang et al., 2011). The authors further showed that mice lacking C5 or C6 were protected against induced OA, and inversely, mice deficient in CD59a, a potent inhibitor of MAC, showed a more severe disease phenotype (Wang, et al., 2011). Other studies have shown that synovial inflammation can be observed in OA patients already at early stages of disease with thickening of the synovial lining, increased vascularity, inflammatory cell infiltration and increased production of pro-inflammatory cytokines such as IL-1α and -1β or TNF (Smith et al., 1997). Using histological staining, T-helper and -suppressor lymphocytes, B-lymphocytes and macrophages were demonstrated in the synovial membrane of osteoarthritis patients (Revell et al., 1988). Further, RT-PCR was used to compare the synovial cytokine profiles in addition to cellular infiltration in RA and OA patients. Even though cellular infiltration was more pronounced in RA synovium, the cytokine profiles were similar in both diseases (Wagner et al., 1997). Moreover, moderately elevated CRP levels can be found in patients with early OA (Saxne et al., 2003; Spector et al., 1997). Therefore it appears that inflammation plays a significant role in the pathogenesis of various type of arthritis and complement is one of the key players regulating this inflammatory response.

3. Why is complement activated in arthritis?

One of the main triggers of classical pathway complement activation are deposited or circulating immune complexes, which in the context of RA includes autoantibodies such as rheumatoid factor or anti-collagen II, in complex with their autoantigens. Anti-citrullinated peptide antibodies (ACPA) have shown the potential to directly activate both the classical and surprisingly also the alternative pathways of complement whereas the lectin pathway is unaffected by ACPA (Trouw et al., 2009). Interestingly, it was shown that the glycosylation pattern of the Fc-region of IgG molecules is altered in RA and an increase of these altered IgG glycoforms lacking the outermost galactose were found at elevated levels in the synovial fluid in RA. These IgG glycoforms have shown an increased affinity for MBL and may trigger lectin pathway activation in the joint (Malhotra et al., 1995).

Apoptotic and necrotic cells have in many studies been proven to induce C1q, MBL and ficolin binding and further complement activation through the classical or lectin pathway (Korb and Ahearn, 1997; Ogden et al., 2001). Also the alternative pathway has been shown to be activated on the surface of apoptotic cells (Matsui et al., 1994), an effect that might be attributable to the ability of certain apoptotic cells to bind properdin (Kemper et al., 2008; Xu et al., 2008). Since apoptotic (Webb et al., 2002) and necrotic cells bind complement inhibitors C4b-binding protein (C4BP) and factor H as well, complement activation downstream of C3-convertase formation is inhibited and therefore the release of

C3a and C5a is limited (Leffler et al., 2010; Trouw et al., 2005; Trouw et al., 2007). Moderate deposition of early opsonins, C1q and C3b/iC3b, is most likely beneficial due to promotion of phagocytosis (Ogden, et al., 2001) but problems arise when complement activation is over stimulated, the load of dying cells increases or their uptake is disturbed. In RA, apoptosis of synovial macrophages, T-cells and fibroblast-like synoviocytes has been observed with the level of apoptosis varying with the disease course (Firestein et al., 1995; Nishioka et al., 1998), potentially contributing to pathological complement activation.

In addition, more recent studies indicate that various extracellular matrix (ECM) components released by proteolytic cleavage during cartilage damage in joint diseases represent another putative trigger of complement.

3.1 Small leucine-rich repeat proteins (SLRPs)

Several structural proteins of the joint tissues have recently been implicated in complement regulation in RA. These include the family of small leucine-rich repeat proteins (SLRPs) that are abundant in the cartilage tissue. Cartilage components are under physiological conditions exposed to complement in the synovial fluid to a very limited extent, mainly following release in tissue regeneration. However, production of proteases in the joint tissue with ensuing tissue damage increases the level of released cartilage components and their display of surface epitopes is altered (Heinegård, 2009).

SLRPs are small proteoglycans found in a variety of extracellular matrices where they stabilize tissue structure by cross-linking different components. All SLRPs contain a characteristic region with 10-12 leucine-rich repeats flanked by cystein loops in the N- and C-termini. The N- and C-terminal extensions provide each SLRP unique features with diversity in both amino acid sequences and glycosaminoglycan content (McEwan et al., 2006). Many of the SLRPs participate in the regulation of collagen fiber synthesis by regulating the rate of assembly and termination of fibrillation to maintain proper thickness of the intact collagen fibers (Douglas et al., 2006; Hedbom and Heinegård, 1993).

Most SLRPs are enriched within the cartilage although a wide tissue distribution can be found for many SLRP-members. Fibromodulin and Proline arginine-rich end leucine-rich repeat protein (PRELP) are found in cartilage as well as in tendon, sclera and aorta and less abundantly in several other tissues (Heinegård et al., 1986). PRELP is furthermore quite uniquely expressed in basement membranes of skin, Bowman's capsule and testis. Osteoadherin is only expressed in bone (Sommarin et al., 1998) whereas chondroadherin is found in different types of cartilage, in bone and to a low extent in tendon (Larsson et al., 1991). Decorin and biglycan are the most abundantly expressed SLRPs, found among other tissues in cartilage, bone, skin, tendon, smooth muscle and cornea (Bianco et al., 1990; Day et al., 1986).

Two SLRPs, osteoadherin and fibromodulin are potent activators of complement interacting with the globular heads of C1q, whereas chondroadherin binds and activates C1q to a lower extent (Sjöberg et al., 2005; Sjöberg et al., 2009a). Since these proteins also capture C4BP and factor H, complement activation is limited to the early steps of the classical pathway (Happonen et al., 2009; Sjöberg, et al., 2005; Sjöberg, et al., 2009a). Whether SLRP-induced complement activation promotes inflammation or non-inflammatory clearance might depend on both the level of released SLRP as well as on their presentation of available surface structures. Decorin (Krumdieck et al., 1992) and biglycan are inhibitory as they bind the stalk region of C1q. Both decorin and biglycan also bind MBL, but only biglycan has the ability to inhibit the lectin pathway (Groeneveld et al., 2005). PRELP does not activate complement, but can directly inhibit MAC formation as well as the alternative C3-convertase in addition to binding C4BP (Happonen, et al., 2009;

Happonen et al., 2012a). Importantly, the presence of fibromodulin (Wang, et al., 2011) and PRELP (Happonen, et al., 2012a) has been demonstrated in arthritic synovial fluid, which is essential for the possibility of interactions with complement.

3.2 Cartilage oligomeric matrix protein (COMP)

COMP is a structural molecule in the cartilage where it serves both as a catalyst of collagen I and II fibrillogenesis in the young (Halasz et al., 2007; Rosenberg et al., 1998) as well as a tissue stabilizer through interactions with different collagens and matrilins in the adult (Mann et al., 2004). Structurally COMP is a pentamer consisting of five identical subunits that are linked together in their N-termini through formation of a coiled-coil structure stabilized by disulphide bridges (Oldberg et al., 1992). When cartilage explants are stimulated with IL-1β, COMP is released from the tissue through a process putatively mediated by matrix metalloproteinase (MMP)-1, -3, -9, 13 (Ganu et al., 1998) and ADAMTS-4 (Dickinson et al., 2003), -7 and -12 (Liu et al., 2006a; Liu et al., 2006b) and possibly other, yet unrecognized, proteases. COMP-release can also be seen *in vivo* in patients with different types of joint diseases, where COMP can be detected both in the synovial fluid and in the serum during active disease (Saxne and Heinegård, 1992). Interestingly, patients with advanced joint destruction have low levels of COMP in their circulation due to the low amount of intact cartilage left in affected joints (Månsson et al., 1995; Neidhart et al., 1997). Different types of COMP-degradation fragments can be found in the synovial fluid of healthy individuals, those with OA and those with RA. In RA it appears that COMP is more readily cleaved with an increased presence of cleavage fragments between 50-70 kDa in size. In healthy controls and OA patients, the most abundant fragments are between 80 and 100 kDa (Neidhart, et al., 1997). Intriguingly, injection of rat COMP subcutaneously into rats or mice induces the development of chronic arthritis (Carlsen et al., 1998; Carlsen et al., 2008). It is furthermore possible to induce arthritis in mice using anti-COMP antibodies in a serum transfer model (Carlsen, et al., 2008). This shows that, at least in rodents, COMP is arthritogenic. Another study showed the presence of COMP-reactive antibodies in the sera of RA patients, but not in healthy controls or patients with osteoarthritis (Souto-Carneiro et al., 2001). The authors were furthermore able to isolate B-cells recognizing COMP-sequences from the synovial membrane of RA patients showing that COMP can induce an immune response also in humans.

COMP has a dual effect on complement as it can both bind to C1q and MBL thereby inhibiting classical and lectin pathway activities as well as activate the alternative pathway by binding C3 and properdin (Happonen et al., 2010). An interaction between CR2 in complex with CD19 and the B-cell receptor through C3dg/C3d-opsonized antigen markedly reduces the threshold for B-cell activation (Dempsey et al., 1996). Thereby complement deposition on COMP might be one of the factors driving the antibody-response towards the molecule. Interestingly, the RA-specific antibodies against COMP are directed against the C-terminal domain (Souto-Carneiro, et al., 2001), which is the same domain that stimulates complement activation (Happonen, et al., 2010). As an indication of COMP-induced complement activation occurring *in vivo,* complexes between COMP and C3b can be found in the circulation of patients with RA and other rheumatic diseases. The level of these complexes decreased in RA patients upon treatment with TNF inhibitors, indicating that they fluctuate with disease activity. However, this decrease followed different kinetics than the general improvement as measured by a decrease in CRP or the disease activity score (DAS) showing that COMP-C3b is not merely another measure of inflammation, but rather a measure of a more complex process of tissue remodeling (Happonen et al., 2012b).

3.3 Other ECM molecules

The NC4 domain of cartilage-specific collagen IX, which can be liberated from collagen IX by the action of MMP-13 (Danfelter et al., 2007), inhibits the formation of the MAC and increases the co-factor activity of C4BP and factor H (Kalchishkova et al., 2011). This may serve to down-regulate complement activity locally in the joint. The cartilage specific collagen II has in contrast been shown to trigger alternative pathway complement activation in guinea-pig serum (Hanauske-Abel et al., 1982). Recent results show in addition, that aggrecan can activate complement (Wang, et al., 2011) and that the complement-activating domain appears to lie in the protein core as opposed to the glycosaminoglycan chains (C. Melin-Fürst, personal communication). Two other ECM proteins, laminin and fibronectin, which are abundant in basement membranes but also expressed in cartilage, have also been shown to bind C1q but these interactions involve the collagen-like stalk of C1q and do not mediate complement activation (Bing et al., 1982; Bohnsack et al., 1985). Fibronectin may in addition participate in driving cartilage breakdown as it interacts with TLR4 to stimulate aggrecanase activity in articular cartilage (Sofat et al., 2012).

The cartilage tissue is rich in heavily glycosylated proteins with complex glycosaminoglycan (GAG) structures. GAGs have in several studies been shown to have an impact on complement although this specificity is strictly related to the GAG composition and more importantly to the amount and location of sulfation of the sugar residues. Properdin was shown to bind apoptotic T-cells via heparan sulfate (HS) and chondroitin sulfate (CS) attached to cell surface proteoglycans, and this interaction was, as expected, dependent on the sulfation pattern of the GAG chains (Kemper, et al., 2008). The GAG-chains of another proteoglycan secreted by malignant plasma cells, serglycin, which is rich in highly sulfated CS, was shown in contrast to bind C1q and MBL and thereby inhibit complement (Skliris et al., 2011). Since charge patterns formed by variation in the sugar residues of the GAG backbone and their sulfation are extremely versatile, the interaction between different GAGs and their ligands becomes very specific and therefore not all highly sulfated GAGs affect complement activity. No specific GAG-structures within the cartilage has to date been shown to regulate complement, but it would not be surprising if such interactions were found due to the vast amount of various and distinct proteoglycans found in the tissue.

Inter-α-trypsin inhibitor (IαI)

IαI is a serum protease inhibitor consisting of two heavy chains connected to protease inhibitor bikunin (Fries and Blom, 2000) via a CS chain (Blom et al., 1999; Zhuo et al., 2004). It has been demonstrated that IαI can inhibit both murine (Garantziotis et al., 2007) and human complement (Okroj et al., 2012) and this activity is mediated by the heavy chains of the protein. Furthermore, the heavy chains of IαI can be transferred from the CS to hyaluronan chains by TNF-stimulated gene-6 protein (TSG-6), a protein expressed during inflammation (Rugg et al., 2005). Heavy chain-hyaluronan complexes can be found both in synovial fluid and serum of patients with RA and the serum levels of heavy chain-hyaluronan were found to be higher in RA patients than in healthy controls (Kida et al., 1999; Yingsung et al., 2003). Furthermore, a correlation between IαI/heavy chain-containing complexes and hemolytic activity of human synovial fluid from RA patients was found. Importantly, intramolecular changes in the heavy chains induced by TSG-6-binding do not alter the complement inhibitory properties of IαI (Okroj, et al., 2012). Therefore heavy chains of IαI may limit complement activation and consumption within the synovium.

4. The dual role of ECM proteins

In addition to their structural role in the matrix, several ECM components have signaling functions in their released and soluble form. In the case of hyaluronan, smaller fragments generated by degradation processes stimulate inflammatory responses in cells whereas the intact molecule appears not to (reviewed in (Jiang et al., 2011). In a lung injury model in mice, the inflammatory response initiated by hyaluronan fragments was dependent on both TLR2 and TLR4. Fragments of hyaluronan isolated from patients with acute lung injury were further able to stimulate TLR2 and TLR4 *in vitro* (Jiang et al., 2005). Therefore degradation of hyaluronan, a process also known to occur during arthritis, may act as a danger signal for the immune system by triggering signaling via, among others, TLRs. It is known that native hyaluronan does not inhibit complement as opposed to e.g. heparin (Chang et al., 1985; Kazatchkine et al., 1981), but its complement activating properties have been not been studied.

TLR2 and TLR4 may be stimulated by the SLRP-member biglycan, thereby upregulating the synthesis of TNF and macrophage inflammatory protein (MIP)-2 (Schaefer et al., 2005). Likewise, decorin may activate macrophage TLR2 and TLR4 to induce TNF and IL-12p70 production (Merline et al., 2011). Intriguingly, in contrast to these pro-inflammatory features, decorin and biglycan are the two SLRPs that were shown to inhibit complement through either the classical or both the classical and the lectin pathways by binding C1q and MBL (Groeneveld, et al., 2005; Krumdieck, et al., 1992). It is possible that these functions act in parallel as a means to control the inflammatory response or that one pathway is predominant to the other depending on the microenvironment, stage of disease and on the type of biglycan and decorin fragments released from the tissue. This could be compared to the ability of the complement-activating SLRPs to also bind complement inhibitors to reduce terminal pathway activation and thereby minimize the overall complement activity. Since little is known about how the different SLRPs are proteolytically modified during disease, it is still unclear whether also smaller fragments of the proteins released in disease share these immunomodulatory properties.

5. Therapeutics in RA

The treatment of RA has advanced dramatically in the past 20-30 years due to novel therapeutics such as methotrexate, TNF inhibitors, IL-inhibitors and CD20-depletion. However, a clinical challenge lies in treating a heterogeneous patient population such as RA patients, where different underlying disease mechanisms may prevail and the dominating pro-inflammatory pathways may vary even within a given patient during the disease course. Therefore it is not surprising that patients respond very differently to the available therapeutics. Despite the presence of several different biological agents targeting a variety of cytokines, there is still a subset of patients who fail to achieve remission either due to non-responsiveness or acquired drug resistance (Colmegna et al., 2012). Furthermore, a disadvantage of the currently available agents is their systemic immunosuppressive effect, which blocks host-protective inflammatory responses towards pathogens in addition to disease-mediated inflammation. As a result, patients treated with anti-cytokine therapies are more susceptible to severe infections. Therefore it is important to develop further anti-inflammatory strategies that will down regulate not only cytokines but also other key players of the immune response, such as complement, and importantly, aim at targeting inhibitors to the actual site of the pathological immune response.

5.1 Complement inhibition in RA

Due to the evidently important role of complement in RA, several therapeutic studies interfering with complement activation have been carried out in animal models of RA. Blocking the cleavage of C5 with monoclonal antibodies targeting C5 both prevented development of CIA in mice as well as ameliorated an already established disease (Wang et al., 1995). This has been confirmed by other studies where C5-targeting antibodies reduced disease activity in an antigen-induced arthritis (AIA) model in rats (Fischetti et al., 2007). Further, vaccination of mice with a recombinant vaccine that induces C5a-neutralizing antibodies protected the mice from disease in CAIA and CIA models (Nandakumar et al., 2010). Inhibition of complement at the level of C3-convertase by injecting mice with C4BP also ameliorated disease activity in both models via inhibition of both classical and alternative complement pathways (Blom et al., 2009). In a recent study on rats and mice, a fusion protein between a C5-blocking antibody and a peptide targeting specifically inflamed synovium (MT07) was used to prevent and treat AIA and CAIA models of arthritis (Macor et al., 2012). MT07 could both prevent the onset of arthritis as well as cure an established disease showing that blocking C5 activity can prevent arthritis in these rodent models. Taken together, these data show that complement activation is crucial for the initiation and progression of arthritis in rodent disease models and inhibiting complement activation may prove to be therapeutically beneficial in the treatment of arthritis.

In humans, a small-scale study has been carried out where patients were orally administered a cyclic peptide (PMX53), which binds the C5aR with high affinity without having an agonistic effect, thereby blocking the anaphylactic effect of C5a released upon complement activation. This peptide did not ameliorate synovial inflammation in the patient cohort, suggesting its efficacy only in animal models (Vergunst et al., 2007). However, novel C5-inhibitors currently under trial will hopefully prove to be applicable also for human use (Woodruff et al., 2011). Importantly, since the discovery of the pathological role of complement in OA (Wang, et al., 2011) it will be intriguing to see whether complement inhibition therapy would benefit these patients as well.

5.2 Blocking joint-specific complement activation

One approach to increase the specificity of immunosuppression to the actual site of inflammation is to inhibit the action of tissue-specific inflammatory stimulators. In the context of complement involvement in RA, this could mean inhibiting cartilage-specific molecules from triggering complement activation. One way of doing this would be to use small inhibitory peptides or $F(ab')_2$-fragments interfering with the interaction between the complement recognition molecules, e.g. C1q, MBL, ficolins or properdin, and members of the SLRP-family or COMP. Such peptides should not induce immunosuppressive effects systematically as their targets mainly are present in the joint. In this approach it might be desirable to target and block the liberated cartilage constituent, as this would leave the complement recognition molecules free to interact with other ligands. Furthermore, the amount of liberated cartilage components should be lower than the amount of circulating complement, which would significantly lower the amount of inhibitor needed for effective inhibition. In order for such approaches to be feasible it is vital to expand the knowledge of how these cartilage components are liberated and if/how they are proteolytically processed when released from the tissue. In addition, the interaction between complement initiators and their cartilage ligands needs to be studied in detail to allow identification of key residues mediating their interaction. However, it might be necessary to inhibit several of these interactions simultaneously in order to achieve sufficient complement down-regulation due to the vast amount of complement stimulating molecules present in the joint. In addition, it is not clear yet whether SLRP-mediated complement activation only drives

pathological inflammation or if it also serves as a means to increase the clearance of these released protein fragments since complement activation downstream of C3-convertases is limited. Most likely normal tissue turnover releasing minor amounts of SLRPs results in low-grade complement recognition and thereby promotes clearance, whereas disease-induced tissue degradation may stimulate inflammation by overwhelming the complement system (Sjöberg et al., 2009b). A major issue that still needs to be clarified is whether the protein fragments liberated from the cartilage possess the same properties with respect to interactions with complement as the intact proteins.

Another attempt to target complement inhibition to the joint was carried out by fusing factor H with CR2, which results in concentration of administered factor H to sites with deposited C3b. Mice injected with factor H-CR2 showed a milder disease phenotype in a CAIA-model with less tissue damage, inflammation and complement deposition (Banda et al., 2009). As mentioned above, successful prevention and treatment of arthritis was also obtained in rodents using the C5-blocking fusion protein MT07, which targets a C5-blocking antibody to inflamed synovium with the help of an attached peptide. MT07 was shown to preferentially home to inflamed joints and left circulating C5 functional (Macor, et al., 2012). Using such fusion proteins would both reduce the amount of inhibitor needed for effective inhibition as it acts to concentrate the protein to the site of ongoing complement activation as well as in optimal cases leave circulating complement active. These proteins are therefore attractive candidates for further therapeutic development.

Due to the finding of several extracellular matrix proteins inhibiting complement (Groeneveld, et al., 2005; Happonen, et al., 2010; Happonen, et al., 2012a; Kalchishkova, et al., 2011) it might be possible to design new recombinant complement inhibitors based on the complement regulatory mechanisms of these endogenous ligands. Several studies in mice have indicated that inhibition of MAC-formation has a protective effect against RA. $CD59^{-/-}$ mice show a more pronounced disease phenotype than their wild type counterparts in the AIA model (Williams et al., 2004). Furthermore, rats injected with a membrane-targeted rat CD59 derivative (sCD59-APT542) had a milder disease course in the AIA model than rats injected with PBS alone (Fraser et al., 2003). Since PRELP and the NC4-domain of collagen IX inhibit MAC formation by blocking C9 polymerization (Happonen, et al., 2012a; Kalchishkova, et al., 2011), they are interesting candidate molecules to utilize for further development of a synthetic inhibitor. Biglycan, decorin and COMP block the early classical and lectin pathways and effective parts of these molecules might be used to block specific pathways of complement. However, with this strategy the problem of systemic complement inhibitory effects remains.

6. Novel diagnostic approaches

In order for erosive damage to be prevented it is critical that patients are diagnosed at an early stage of disease and interventions are applied immediately at this stage. In addition, it would be crucial to know which patient will benefit from which treatment, since customized therapy will both prevent disease progression and be cost-effective. This on the other hand requires that the diagnostic methods used in the clinic are able to distinguish disease-specific markers before the actual clinical disease onset as well as markers telling us about the underlying pathology in each individual patient. As the pathogenesis of RA is very complex it might be necessary to target several inflammatory pathways at the same time to block the cycle of tissue damage.

Extensive research is carried out with the aim to identify disease-specific cleavage fragments of cartilage components to be used as biomarkers. Protease- and disease-specific cleavage fragments of among others aggrecan, collagen II, collagen IX and SLRPs have been described (Danfelter, et al., 2007; Flannery, 2006; Fosang et al., 2003; Heathfield et

al., 2004), which tell us about the proteolytic activity in the joint. These findings pave the way in developing novel neo-epitope specific antibodies to be used in diagnosing and monitoring the state of the cartilage tissue. Whether such neo-epitope formation occurs already before disease onset seems likely but remains to be elucidated. Since protease expression and activity is under the regulation of pro-inflammatory cytokines, knowledge about which proteases are active during different stages of disease might guide in selecting proper cytokine-blocking therapy.

To single out the patients who will benefit from complement inhibition therapy, novel diagnostics need to be developed to distinguish in which patients complement is actually driving the inflammation. One such marker could be to monitor formation of complexes between cartilage proteins and complement activation products, such as COMP and C3b. Serum COMP-C3b levels were shown to be elevated in several rheumatic diseases compared to healthy controls and are therefore on their own not applicable for diagnosis of a particular disease (Happonen, et al., 2012b). However, presence of such complexes might indicate which patients have pathologic complement activation and therefore would benefit from complement inhibition therapy. Furthermore, COMP-C3b was shown to decrease in RA patients upon TNF inhibition, which indicates that these complexes might be useful in monitoring responsiveness to treatment. Worth mentioning is also that the decrease in COMP-C3b upon TNF inhibition was very variable between patients with some responding very dramatically and others remaining at their baseline (Happonen, et al., 2012b) . Since this did not parallel changes in CRP or DAS, it seems that COMP-C3b measures a different component of the disease process. This knowledge might shed light on molecular alterations occurring in the cartilage upon therapy not available by monitoring conventional inflammatory parameters. It would be of high interest to see if similar complexes can be found between fibromodulin or osteoadherin, two strong complement activators of the SLRP-family, and C3b or C4b. Using a combination of such markers might provide additional information not available by monitoring only individual markers. These assays could be further increased in sensitivity by using neo-epitope-specific antibodies against the cartilage proteins as well as complement activation products.

7. Conclusion

Even though both the diagnostics and treatment of arthritis have progressed in leaps during the recent decades, there is still a need for further development in order to be able to customize therapies for individual needs of each patient. For this to be possible it is crucial that the pathological mechanisms underlying both RA and OA are elucidated and that novel diagnostic approaches are explored to distinguish between different pathological changes regulating disease progression. The fact that cartilage resident proteins are able to regulate complement is highly intriguing and opens up possibilities for developing both inhibitors of cartilage-induced joint inflammation as well as novel biomarkers of disease. The role of these molecules in the induction of arthritis still needs to be elucidated, but their immune-regulating properties are becoming clear. Since we are incessantly exposed to a certain degree of cartilage proteins due to tissue regeneration, some sort of tolerance must be mounted towards these possibly pathogenic ligands. It is possible that this tolerance is broken in joint disease upon an increased burden or by a changed microenvironment or alternatively by the exposure of epitopes normally hidden within the released proteins. Complement deposition on the newly liberated molecules may then stimulate auto-antibody production and trigger further responses by the adaptive immune system. Therefore preventing such pathological complement activity is of outmost importance in order to prevent autoimmunity.

References

Banda, N. K., Thurman, J. M., Kraus, D., Wood, A., Carroll, M. C., Arend, W. P. and Holers, V. M., 2006. Alternative complement pathway activation is essential for inflammation and joint destruction in the passive transfer model of collagen-induced arthritis. J Immunol 177, 1904-1912.

Banda, N. K., Takahashi, K., Wood, A. K., Holers, V. M. and Arend, W. P., 2007. Pathogenic complement activation in collagen antibody-induced arthritis in mice requires amplification by the alternative pathway. J Immunol 179, 4101-4109.

Banda, N. K., Levitt, B., Glogowska, M. J., Thurman, J. M., Takahashi, K., Stahl, G. L., Tomlinson, S., Arend, W. P. and Holers, V. M., 2009. Targeted inhibition of the complement alternative pathway with complement receptor 2 and factor H attenuates collagen antibody-induced arthritis in mice. Journal of immunology 183, 5928-5937.

Banda, N. K., Takahashi, M., Levitt, B., Glogowska, M., Nicholas, J., Takahashi, K., Stahl, G. L., Fujita, T., Arend, W. P. and Holers, V. M., 2010. Essential role of complement mannose-binding lectin-associated serine proteases-1/3 in the murine collagen antibody-induced model of inflammatory arthritis. J Immunol 185, 5598-5606.

Banda, N. K., Hyatt, S., Glogowska, M., Takahashi, K., Merkel, T. J., Stahl, G. L., Lu, B., Gerard, C., Wetsel, R. A., Arend, W. P. and Holers, V. M., 2011. Independent roles for complement C3a and C5a receptors and the membrane attack complex in the pathogenesis of collagen antibody-induced arthritis in mice: Potential therapeutic implications. Molecular Immunlogy 48, 1670.

Bianco, P., Fisher, L. W., Young, M. F., Termine, J. D. and Robey, P. G., 1990. Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. J Histochem Cytochem 38, 1549-1563.

Bing, D. H., Almeda, S., Isliker, H., Lahav, J. and Hynes, R. O., 1982. Fibronectin binds to the C1q component of complement. Proc Natl Acad Sci U S A 79, 4198-4201.

Blom, A. M., Mörgelin, M., Oyen, M., Jarvet, J. and Fries, E., 1999. Structural characterization of inter-alpha-inhibitor. Evidence for an extended shape. J Biol Chem 274, 298-304.

Blom, A. M., Nandakumar, K. S. and Holmdahl, R., 2009. C4b-binding protein (C4BP) inhibits development of experimental arthritis in mice. Ann Rheum Dis 68, 136-142.

Bohnsack, J. F., Tenner, A. J., Laurie, G. W., Kleinman, H. K., Martin, G. R. and Brown, E. J., 1985. The C1q subunit of the first component of complement binds to laminin: a mechanism for the deposition and retention of immune complexes in basement membrane. Proc Natl Acad Sci U S A 82, 3824-3828.

Brodeur, J. P., Ruddy, S., Schwartz, L. B. and Moxley, G., 1991. Synovial fluid levels of complement SC5b-9 and fragment Bb are elevated in patients with rheumatoid arthritis. Arthritis Rheum 34, 1531-1537.

Carlsen, S., Hansson, A. S., Olsson, H., Heinegård, D. and Holmdahl, R., 1998. Cartilage oligomeric matrix protein (COMP)-induced arthritis in rats. Clin Exp Immunol 114, 477-484.

Carlsen, S., Nandakumar, K. S., Bäcklund, J., Holmberg, J., Hultqvist, M., Vestberg, M. and Holmdahl, R., 2008. Cartilage oligomeric matrix protein induction of chronic arthritis in mice. Arthritis Rheum 58, 2000-2011.

Chang, N. S., Boackle, R. J. and Armand, G., 1985. Hyaluronic acid-complement interactions--I. Reversible heat-induced anticomplementary activity. Mol Immunol 22, 391-397.

Colmegna, I., Ohata, B. R. and Menard, H. A., 2012. Current understanding of rheumatoid arthritis therapy. Clinical pharmacology and therapeutics 91, 607-620.10.1038/clpt.2011.325

Danfelter, M., Önnerfjord, P. and Heinegård, D., 2007. Fragmentation of proteins in cartilage treated with interleukin-1: specific cleavage of type IX collagen by matrix metalloproteinase 13 releases the NC4 domain. J Biol Chem 282, 36933-36941.

Day, A. A., Ramis, C. I., Fisher, L. W., Gehron-Robey, P., Termine, J. D. and Young, M. F., 1986. Characterization of bone PG II cDNA and its relationship to PG II mRNA from other connective tissues. Nucleic Acids Res 14, 9861-9876.

Dempsey, P. W., Allison, M. E., Akkaraju, S., Goodnow, C. C. and Fearon, D. T., 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 271, 348-350.

Dickinson, S. C., Vankemmelbeke, M. N., Buttle, D. J., Rosenberg, K., Heinegård, D. and Hollander, A. P., 2003. Cleavage of cartilage oligomeric matrix protein (thrombospondin-5) by matrix metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motifs. Matrix Biol 22, 267-278.

Douglas, T., Heinemann, S., Bierbaum, S., Scharnweber, D. and Worch, H., 2006. Fibrillogenesis of collagen types I, II, and III with small leucine-rich proteoglycans decorin and biglycan. Biomacromolecules 7, 2388-2393.

Firestein, G. S., Yeo, M. and Zvaifler, N. J., 1995. Apoptosis in rheumatoid arthritis synovium. J Clin Invest 96, 1631-1638.

Fischetti, F., Durigutto, P., Macor, P., Marzari, R., Carretta, R. and Tedesco, F., 2007. Selective therapeutic control of C5a and the terminal complement complex by anti-C5 single-chain Fv in an experimental model of antigen-induced arthritis in rats. Arthritis Rheum 56, 1187-1197.

Flannery, C. R., 2006. Usurped SLRPs: novel arthritis biomarkers exposed by catabolism of small leucine-rich proteoglycans? Arthritis Res Ther 8, 106-107.

Fosang, A. J., Stanton, H., Little, C. B. and Atley, L. M., 2003. Neoepitopes as biomarkers of cartilage catabolism. Inflammation research 52, 277-282.

Fraser, D. A., Harris, C. L., Williams, A. S., Mizuno, M., Gallagher, S., Smith, R. A. and Morgan, B. P., 2003. Generation of a recombinant, membrane-targeted form of the complement regulator CD59: activity in vitro and in vivo. J Biol Chem 278, 48921-48927.

Fries, E. and Blom, A. M., 2000. Bikunin--not just a plasma proteinase inhibitor. The international journal of biochemistry & cell biology 32, 125-137.

Ganu, V., Goldberg, R., Peppard, J., Rediske, J., Melton, R., Hu, S. I., Wang, W., Duvander, C. and Heinegård, D., 1998. Inhibition of interleukin-1alpha-induced cartilage oligomeric matrix protein degradation in bovine articular cartilage by matrix metalloproteinase inhibitors: potential role for matrix metalloproteinases in the generation of cartilage oligomeric matrix protein fragments in arthritic synovial fluid. Arthritis Rheum 41, 2143-2151.

Garantziotis, S., Hollingsworth, J. W., Ghanayem, R. B., Timberlake, S., Zhuo, L., Kimata, K. and Schwartz, D. A., 2007. Inter-alpha-trypsin inhibitor attenuates complement activation and complement-induced lung injury. Journal of immunology 179, 4187-4192.

Groeneveld, T. W., Oroszlan, M., Owens, R. T., Faber-Krol, M. C., Bakker, A. C., Arlaud, G. J., McQuillan, D. J., Kishore, U., Daha, M. R. and Roos, A., 2005. Interactions of the extracellular matrix proteoglycans decorin and biglycan with C1q and collectins. J Immunol 175, 4715-4723.

Halasz, K., Kassner, A., Mörgelin, M. and Heinegård, D., 2007. COMP acts as a catalyst in collagen fibrillogenesis. J Biol Chem 282, 31166-31173.

Hanauske-Abel, H. M., Pontz, B. F. and Schorlemmer, H. U., 1982. Cartilage specific collagen activates macrophages and the alternative pathway of complement: evidence for an immunopathogenic concept of rheumatoid arthritis. Ann Rheum Dis 41, 168-176.

Happonen, K. E., Sjöberg, A. P., Mörgelin, M., Heinegård, D. and Blom, A. M., 2009. Complement inhibitor C4b-binding protein interacts directly with small glycoproteins of the extracellular matrix. Journal of immunology 182, 1518-1525.

Happonen, K. E., Saxne, T., Aspberg, A., Mörgelin, M., Heinegård, D. and Blom, A. M., 2010. Regulation of complement by cartilage oligomeric matrix protein allows for a novel molecular diagnostic principle in rheumatoid arthritis. Arthritis Rheum 62, 3574-3583.

Happonen, K. E., Fürst, C. M., Saxne, T., Heinegård, D. and Blom, A. M., 2012a. PRELP Protein Inhibits the Formation of the Complement Membrane Attack Complex. J Biol Chem 287, 8092-8100.10.1074/jbc.M111.291476

Happonen, K. E., Saxne, T., Geborek, P., Andersson, M., Bengtsson, A. A., Hesselstrand, R., Heinegård, D. and Blom, A. M., 2012b. Serum COMP-C3b complexes in rheumatic diseases and relation to anti-TNF-alpha treatment. Arthritis Res Ther 14, R15.

Heathfield, T. F., Onnerfjord, P., Dahlberg, L. and Heinegard, D., 2004. Cleavage of fibromodulin in cartilage explants involves removal of the N-terminal tyrosine sulfate-rich

region by proteolysis at a site that is sensitive to matrix metalloproteinase-13. J Biol Chem 279, 6286-6295.

Hedbom, E. and Heinegård, D., 1993. Binding of fibromodulin and decorin to separate sites on fibrillar collagens. J Biol Chem 268, 27307-27312.

Heinegård, D., Larsson, T., Sommarin, Y., Franzen, A., Paulsson, M. and Hedbom, E., 1986. Two novel matrix proteins isolated from articular cartilage show wide distributions among connective tissues. J Biol Chem 261, 13866-13872.

Heinegård, D., 2009. Proteoglycans and more--from molecules to biology. Int J Exp Pathol 90, 575-586.

Holmqvist, M. E., Wedren, S., Jacobsson, L. T., Klareskog, L., Nyberg, F., Rantapaa-Dahlqvist, S., Alfredsson, L. and Askling, J., 2010. Rapid increase in myocardial infarction risk following diagnosis of rheumatoid arthritis amongst patients diagnosed between 1995 and 2006. J Intern Med 268, 578-585.

Islander, U., Jochems, C., Lagerquist, M. K., Forsblad-d'Elia, H. and Carlsten, H., 2010. Estrogens in rheumatoid arthritis; the immune system and bone. Mol Cell Endocrinol 335, 14-29.

Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G. D., Mascarenhas, M. M., Garg, H. G., Quinn, D. A., Homer, R. J., Goldstein, D. R., Bucala, R., Lee, P. J., Medzhitov, R. and Noble, P. W., 2005. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med 11, 1173-1179.

Jiang, D., Liang, J. and Noble, P. W., 2011. Hyaluronan as an immune regulator in human diseases. Physiol Rev 91, 221-264.

Jose, P. J., Moss, I. K., Maini, R. N. and Williams, T. J., 1990. Measurement of the chemotactic complement fragment C5a in rheumatoid synovial fluids by radioimmunoassay: role of C5a in the acute inflammatory phase. Ann Rheum Dis 49, 747-752.

Kalchishkova, N., Melin Fürst, C., Heinegård, D. and Blom, A. M., 2011. The NC4 domain of the cartilage-specific collagen IX inhibits complement directly due to attenuation of membrane attack formation and indirectly through binding and enhancing activity of complement inhibitors C4B-binding protein and factor H. J Biol Chem 286, 27915-27926.

Kazatchkine, M. D., Fearon, D. T., Metcalfe, D. D., Rosenberg, R. D. and Austen, K. F., 1981. Structural determinants of the capacity of heparin to inhibit the formation of the human amplification C3 convertase. J Clin Invest 67, 223-228.

Kemper, C., Mitchell, L. M., Zhang, L. and Hourcade, D. E., 2008. The complement protein properdin binds apoptotic T cells and promotes complement activation and phagocytosis. Proc Natl Acad Sci U S A 105, 9023-9028.

Kida, D., Yoneda, M., Miyaura, S., Ishimaru, T., Yoshida, Y., Ito, T., Ishiguro, N., Iwata, H. and Kimata, K., 1999. The SHAP-HA complex in sera from patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 26, 1230-1238.

Kimura, Y., Zhou, L., Miwa, T. and Song, W. C., 2010. Genetic and therapeutic targeting of properdin in mice prevents complement-mediated tissue injury. J Clin Invest 120, 3545-3554.

Konttinen, Y. T., Ceponis, A., Meri, S., Vuorikoski, A., Kortekangas, P., Sorsa, T., Sukura, A. and Santavirta, S., 1996. Complement in acute and chronic arthritides: assessment of C3c, C9, and protectin (CD59) in synovial membrane. Ann Rheum Dis 55, 888-894.

Korb, L. C. and Ahearn, J. M., 1997. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J Immunol 158, 4525-4528.

Krumdieck, R., Hook, M., Rosenberg, L. C. and Volanakis, J. E., 1992. The proteoglycan decorin binds C1q and inhibits the activity of the C1 complex. J Immunol 149, 3695-3701.

Kuhn, K. A., Cozine, C. L., Tomooka, B., Robinson, W. H. and Holers, V. M., 2008. Complement receptor CR2/CR1 deficiency protects mice from collagen-induced arthritis and associates with reduced autoantibodies to type II collagen and citrullinated antigens. Mol Immunol 45, 2808-2819.

Larsson, T., Sommarin, Y., Paulsson, M., Antonsson, P., Hedbom, E., Wendel, M. and Heinegård, D., 1991. Cartilage matrix proteins. A basic 36-kDa protein with a restricted distribution to cartilage and bone. J Biol Chem 266, 20428-20433.

Leffler, J., Herbert, A. P., Norstrom, E., Schmidt, C. Q., Barlow, P. N., Blom, A. M. and Martin, M., 2010. Annexin-II, DNA, and histones serve as factor H ligands on the surface of apoptotic cells. J Biol Chem 285, 3766-3776.10.1074/jbc.M109.045427

Liu, C. J., Kong, W., Ilalov, K., Yu, S., Xu, K., Prazak, L., Fajardo, M., Sehgal, B. and Di Cesare, P. E., 2006a. ADAMTS-7: a metalloproteinase that directly binds to and degrades cartilage oligomeric matrix protein. Faseb J 20, 988-990.

Liu, C. J., Kong, W., Xu, K., Luan, Y., Ilalov, K., Sehgal, B., Yu, S., Howell, R. D. and Di Cesare, P. E., 2006b. ADAMTS-12 associates with and degrades cartilage oligomeric matrix protein. J Biol Chem 281, 15800-15808.

Macor, P., Durigutto, P., De Maso, L., Garrovo, C., Biffi, S., Cortini, A., Fischetti, F., Sblattero, D., Pitzalis, C., Marzari, R. and Tedesco, F., 2012. Treatment of arthritis models by targeting synovial endothelium with a neutralizing recombinant antibody to C5. Arthritis Rheum 10.1002/art.34430

Malhotra, R., Wormald, M. R., Rudd, P. M., Fischer, P. B., Dwek, R. A. and Sim, R. B., 1995. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1, 237-243.

Mann, H. H., Ozbek, S., Engel, J., Paulsson, M. and Wagener, R., 2004. Interactions between the cartilage oligomeric matrix protein and matrilins. Implications for matrix assembly and the pathogenesis of chondrodysplasias. J Biol Chem 279, 25294-25298.

Månsson, B., Carey, D., Alini, M., Ionescu, M., Rosenberg, L. C., Poole, A. R., Heinegård, D. and Saxne, T., 1995. Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J Clin Invest 95, 1071-1077.

Matsui, H., Tsuji, S., Nishimura, H. and Nagasawa, S., 1994. Activation of the alternative pathway of complement by apoptotic Jurkat cells. FEBS Lett 351, 419-422.

McEwan, P. A., Scott, P. G., Bishop, P. N. and Bella, J., 2006. Structural correlations in the family of small leucine-rich repeat proteins and proteoglycans. J Struct Biol 155, 294-305.

Merline, R., Moreth, K., Beckmann, J., Nastase, M. V., Zeng-Brouwers, J., Tralhao, J. G., Lemarchand, P., Pfeilschifter, J., Schaefer, R. M., Iozzo, R. V. and Schaefer, L., 2011. Signaling by the matrix proteoglycan decorin controls inflammation and cancer through PDCD4 and MicroRNA-21. Science signaling 4, ra75.

Morgan, B. P., Daniels, R. H. and Williams, B. D., 1988. Measurement of terminal complement complexes in rheumatoid arthritis. Clin Exp Immunol 73, 473-478.

Moxley, G. and Ruddy, S., 1985. Elevated C3 anaphylatoxin levels in synovial fluids from patients with rheumatoid arthritis. Arthritis Rheum 28, 1089-1095.

Nandakumar, K. S., Jansson, A., Xu, B., Rydell, N., Ahooghalandari, P., Hellman, L., Blom, A. M. and Holmdahl, R., 2010. A recombinant vaccine effectively induces c5a-specific neutralizing antibodies and prevents arthritis. PLoS One 5, e13511.

Neidhart, M., Hauser, N., Paulsson, M., DiCesare, P. E., Michel, B. A. and Hauselmann, H. J., 1997. Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. Br J Rheumatol 36, 1151-1160.

Nishioka, K., Hasunuma, T., Kato, T., Sumida, T. and Kobata, T., 1998. Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of synovial tissue. Arthritis Rheum 41, 1-9.

Ogden, C. A., deCathelineau, A., Hoffmann, P. R., Bratton, D., Ghebrehiwet, B., Fadok, V. A. and Henson, P. M., 2001. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 194, 781-795.

Okroj, M., Heinegård, D., Holmdahl, R. and Blom, A. M., 2007. Rheumatoid arthritis and the complement system. Ann Med 39, 517-530.

Okroj, M., Holmquist, E., Sjölander, J., Corrales, L., Saxne, T., Wisniewski, H. G. and Blom, A. M., 2012. Heavy chains of inter alpha inhibitor (IalphaI) inhibit the human complement system at early stages of the cascade. J Biol Chem 10.1074/jbc.M111.324913

Oldberg, A., Antonsson, P., Lindblom, K. and Heinegård, D., 1992. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. J Biol Chem 267, 22346-22350.

Revell, P. A., Mayston, V., Lalor, P. and Mapp, P., 1988. The synovial membrane in osteoarthritis: a histological study including the characterisation of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis 47, 300-307.

Rosenberg, K., Olsson, H., Mörgelin, M. and Heinegård, D., 1998. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. J Biol Chem 273, 20397-20403.

Rugg, M. S., Willis, A. C., Mukhopadhyay, D., Hascall, V. C., Fries, E., Fulop, C., Milner, C. M. and Day, A. J., 2005. Characterization of complexes formed between TSG-6 and inter-alpha-inhibitor that act as intermediates in the covalent transfer of heavy chains onto hyaluronan. J Biol Chem 280, 25674-25686.

Saxne, T. and Heinegård, D., 1992. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. Br J Rheumatol 31, 583-591.

Saxne, T., Lindell, M., Månsson, B., Petersson, I. F. and Heinegård, D., 2003. Inflammation is a feature of the disease process in early knee joint osteoarthritis. Rheumatology (Oxford) 42, 903-904.

Schaefer, L., Babelova, A., Kiss, E., Hausser, H. J., Baliova, M., Krzyzankova, M., Marsche, G., Young, M. F., Mihalik, D., Gotte, M., Malle, E., Schaefer, R. M. and Grone, H. J., 2005. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest 115, 2223-2233.

Scott, D. L., Wolfe, F. and Huizinga, T. W., 2010. Rheumatoid arthritis. Lancet 376, 1094-1108.

Sjöberg, A., Önnerfjord, P., Mörgelin, M., Heinegård, D. and Blom, A. M., 2005. The extracellular matrix and inflammation: fibromodulin activates the classical pathway of complement by directly binding C1q. J Biol Chem 280, 32301-32308.

Sjöberg, A. P., Manderson, G. A., Mörgelin, M., Day, A. J., Heinegård, D. and Blom, A. M., 2009a. Short leucine-rich glycoproteins of the extracellular matrix display diverse patterns of complement interaction and activation. Mol Immunol 46, 830-839.

Sjöberg, A. P., Trouw, L. A. and Blom, A. M., 2009b. Complement activation and inhibition: a delicate balance. Trends Immunol 30, 83-90.

Skliris, A., Happonen, K. E., Terpos, E., Labropoulou, V., Borset, M., Heinegård, D., Blom, A. M. and Theocharis, A. D., 2011. Serglycin inhibits the classical and lectin pathways of complement via its glycosaminoglycan chains: Implications for multiple myeloma. Eur J Immunol 41, 437-449.

Smith, M. D., Triantafillou, S., Parker, A., Youssef, P. P. and Coleman, M., 1997. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 24, 365-371.

Sofat, N., Robertson, S. D. and Wait, R., 2012. Fibronectin III 13-14 domains induce joint damage via Toll-like receptor 4 activation and synergize with interleukin-1 and tumour necrosis factor. J Innate Immun 4, 69-79.10.1159/000329632

Sommarin, Y., Wendel, M., Shen, Z., Hellman, U. and Heinegård, D., 1998. Osteoadherin, a cell-binding keratan sulfate proteoglycan in bone, belongs to the family of leucine-rich repeat proteins of the extracellular matrix. J Biol Chem 273, 16723-16729.

Souto-Carneiro, M. M., Burkhardt, H., Muller, E. C., Hermann, R., Otto, A., Kraetsch, H. G., Sack, U., Konig, A., Heinegard, D., Muller-Hermelink, H. K. and Krenn, V., 2001. Human monoclonal rheumatoid synovial B lymphocyte hybridoma with a new disease-related specificity for cartilage oligomeric matrix protein. Journal of immunology 166, 4202-4208.

Spector, T. D., Hart, D. J., Nandra, D., Doyle, D. V., Mackillop, N., Gallimore, J. R. and Pepys, M. B., 1997. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 40, 723-727.

Swaak, A. J., Van Rooyen, A., Planten, O., Han, H., Hattink, O. and Hack, E., 1987. An analysis of the levels of complement components in the synovial fluid in rheumatic diseases. Clin Rheumatol 6, 350-357.

Trouw, L. A., Nilsson, S. C., Goncalves, I., Landberg, G. and Blom, A. M., 2005. C4b-binding protein binds to necrotic cells and DNA, limiting DNA release and inhibiting complement activation. J Exp Med 201, 1937-1948.

Trouw, L. A., Bengtsson, A. A., Gelderman, K. A., Dahlbäck, B., Sturfelt, G. and Blom, A. M., 2007. C4b-binding protein and factor H compensate for the loss of membrane-bound complement inhibitors to protect apoptotic cells against excessive complement attack. J Biol Chem 282, 28540-28548.

Trouw, L. A., Haisma, E. M., Levarht, E. W., van der Woude, D., Ioan-Facsinay, A., Daha, M. R., Huizinga, T. W. and Toes, R. E., 2009. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum 60, 1923-1931.

Vergunst, C. E., Gerlag, D. M., Dinant, H., Schulz, L., Vinkenoog, M., Smeets, T. J., Sanders, M. E., Reedquist, K. A. and Tak, P. P., 2007. Blocking the receptor for C5a in patients with rheumatoid arthritis does not reduce synovial inflammation. Rheumatology (Oxford) 46, 1773-1778.

Wagner, S., Fritz, P., Einsele, H., Sell, S. and Saal, J. G., 1997. Evaluation of synovial cytokine patterns in rheumatoid arthritis and osteoarthritis by quantitative reverse transcription polymerase chain reaction. Rheumatol Int 16, 191-196.

Wang, Q., Rozelle, A. L., Lepus, C. M., Scanzello, C. R., Song, J. J., Larsen, D. M., Crish, J. F., Bebek, G., Ritter, S. Y., Lindstrom, T. M., Hwang, I., Wong, H. H., Punzi, L., Encarnacion, A., Shamloo, M., Goodman, S. B., Wyss-Coray, T., Goldring, S. R., Banda, N. K., Thurman, J. M., Gobezie, R., Crow, M. K., Holers, V. M., Lee, D. M. and Robinson, W. H., 2011. Identification of a central role for complement in osteoarthritis. Nat Med 17, 1674-1679.

Wang, Y., Rollins, S. A., Madri, J. A. and Matis, L. A., 1995. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. Proc Natl Acad Sci U S A 92, 8955-8959.

Wang, Y., Kristan, J., Hao, L., Lenkoski, C. S., Shen, Y. and Matis, L. A., 2000. A role for complement in antibody-mediated inflammation: C5-deficient DBA/1 mice are resistant to collagen-induced arthritis. J Immunol 164, 4340-4347.

Webb, J. H., Blom, A. M. and Dahlback, B., 2002. Vitamin K-dependent protein S localizing complement regulator C4b-binding protein to the surface of apoptotic cells. Journal of immunology 169, 2580-2586.

Williams, A. S., Mizuno, M., Richards, P. J., Holt, D. S. and Morgan, B. P., 2004. Deletion of the gene encoding CD59a in mice increases disease severity in a murine model of rheumatoid arthritis. Arthritis Rheum 50, 3035-3044.

Woodruff, T. M., Nandakumar, K. S. and Tedesco, F., 2011. Inhibiting the C5-C5a receptor axis. Mol Immunol 48, 1631-1642.

Wouters, D., Voskuyl, A. E., Molenaar, E. T., Dijkmans, B. A. and Hack, C. E., 2006. Evaluation of classical complement pathway activation in rheumatoid arthritis: measurement of C1q-C4 complexes as novel activation products. Arthritis Rheum 54, 1143-1150.

Xu, W., Berger, S. P., Trouw, L. A., de Boer, H. C., Schlagwein, N., Mutsaers, C., Daha, M. R. and van Kooten, C., 2008. Properdin binds to late apoptotic and necrotic cells independently of C3b and regulates alternative pathway complement activation. J Immunol 180, 7613-7621.

Yingsung, W., Zhuo, L., Morgelin, M., Yoneda, M., Kida, D., Watanabe, H., Ishiguro, N., Iwata, H. and Kimata, K., 2003. Molecular heterogeneity of the SHAP-hyaluronan complex. Isolation and characterization of the complex in synovial fluid from patients with rheumatoid arthritis. J Biol Chem 278, 32710-32718.

Zhuo, L., Hascall, V. C. and Kimata, K., 2004. Inter-alpha-trypsin inhibitor, a covalent protein-glycosaminoglycan-protein complex. J Biol Chem 279, 38079-38082.

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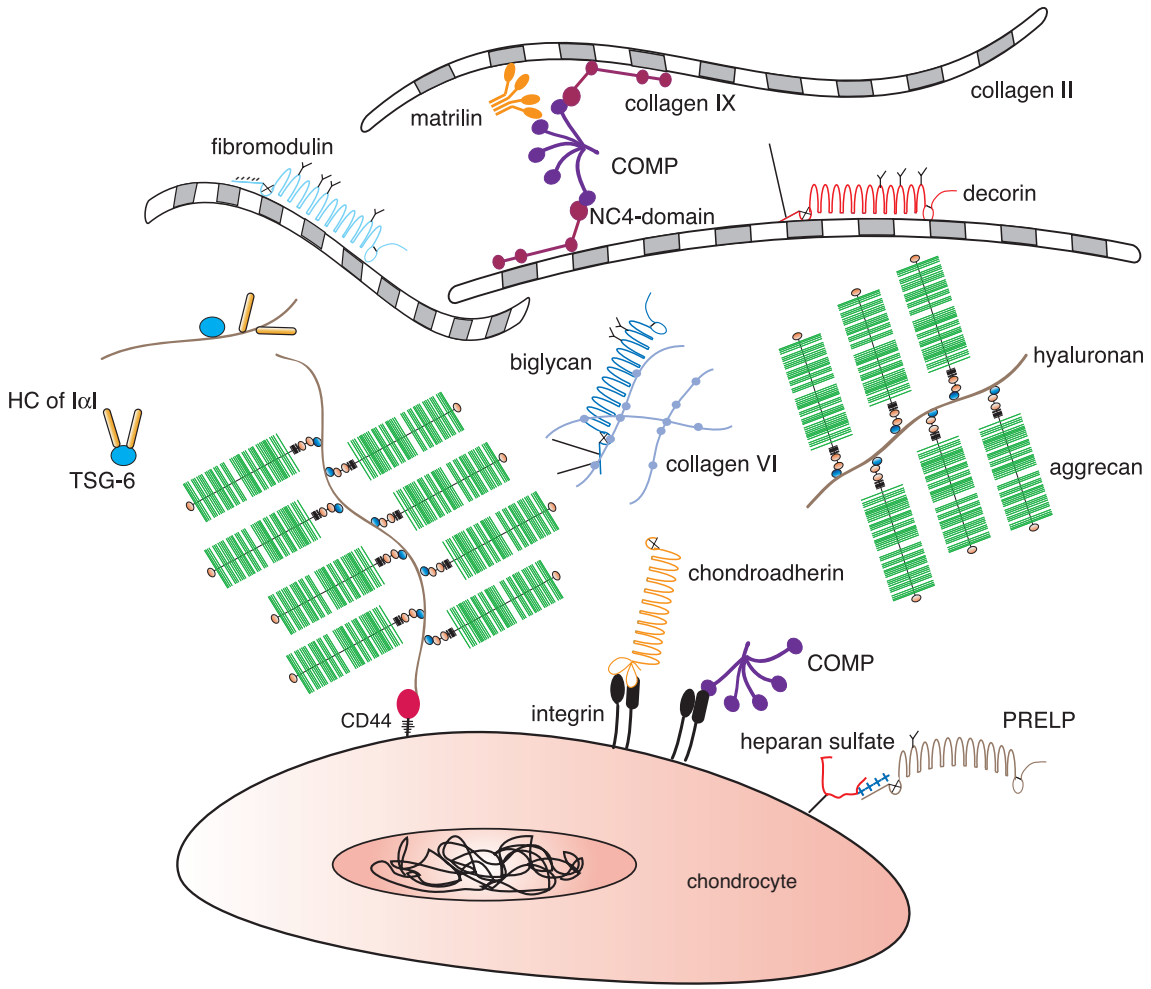

Figure 1. *Articular cartilage.* The articular cartilage found in joints has a low number of cells but an extensive extracellular matrix. This matrix is composed of collagen networks and various linker proteins regulating the assembly and organization of the tissue. Several of these structural proteins also have modulatory effects on the cartilage resident chondrocytes by interacting with cell-surface receptors. COMP, cartilage oligomeric matrix protein; HC, heavy chains; IαI, inter-α-trypsin inhibitor; PRELP, proline arginine-rich end leucine-rich repeat protein; TSG-6, tumor necrosis factor-stimulated gene-6 protein.

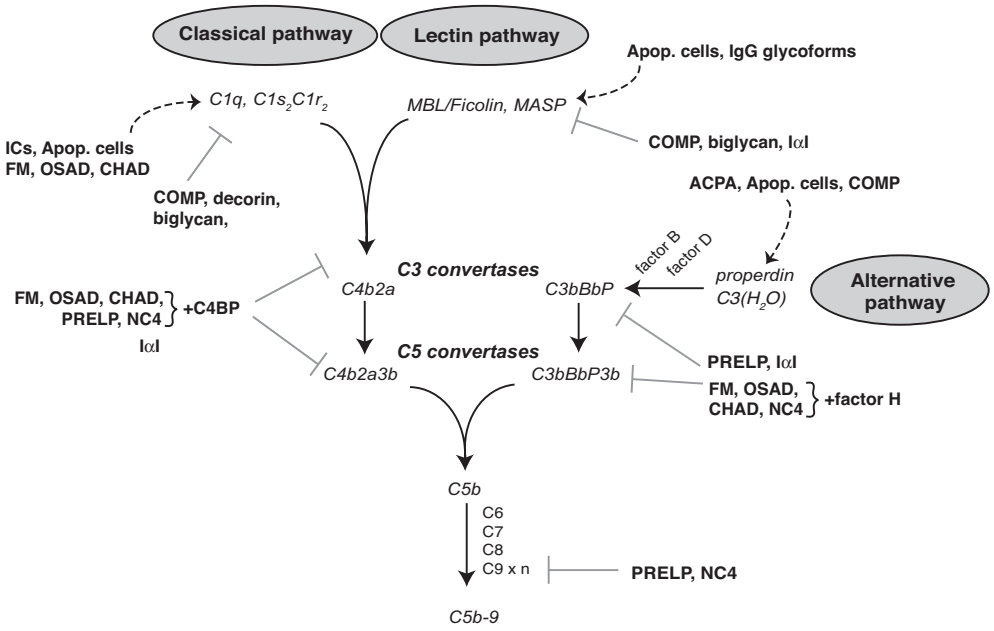

Figure 2. *Complement regulators in arthritis.* The classical pathway can be activated by immune complexes, apoptotic and necrotic cells, fibromodulin, osteoadherin or chondroadherin, whereas COMP, biglycan and decorin inhibit the early classical pathway by binding C1q. The lectin pathway can be initiated by apoptotic cells and possibly glycoforms of IgG. COMP, biglycan and IαI can in contrast inhibit lectin pathway activation. The alternative pathway may be triggered by apoptotic cells, anti-citrullinated peptide antibodies (ACPA) or COMP. Several ECM proteins can bind C4BP and factor H and therefore locally downregulate the classical and alternative C3 convertases (C4b2a and C3bBbP, respectively). IαI can on its own inhibit both the classical and alternative C3-convertase, whereas PRELP only inhibits the alternative convertase. The assembly of the membrane attack complex is inhibited by both PRELP and the NC4-domain of collagen IX. ACPA, anti-citrullinated peptide antibody; Apop. Cells, apoptotic cells; CHAD, chondroadherin; FM, fibromodulin; ICs, immune complexes; OSAD, osteoadherin.