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Complement in Disease
-
Extracellular Proteins as Complement Regulators

Kaisa Happonen

Doctoral Thesis

LUND UNIVERSITY
Faculty of Medicine

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Abstract

Complement activation occurs during inflammatory joint diseases such as rheumatoid arthritis (RA) and is thought to contribute to the chronic inflammation observed within the joints. Previous studies have shown that certain cartilage components of the small leucine-rich repeat protein (SLRP)-family regulate complement activity, thereby possibly contributing to disease progression. The aim of this thesis is to find new interactions between cartilage components and complement, which could provide useful information for the development of novel therapeutic and diagnostic approaches.
We found that complement-activating SLRPs also bind the complement inhibitor C4b-binding protein (C4BP) leading to a downregulation of the terminal complement pathway. This may be a mechanism to minimize release of anaphylatoxins and direct the complement response towards clearance of released cartilage constituents.

We further found that one member of the SLRP-family, PRELP, inhibits complement directly by binding C9 and inhibiting the formation of the lytic membrane attack complex. PRELP was also found to inhibit the assembly of the alternative pathway C3-convertase and thereby affects two stages of the complement cascade.

We found that cartilage oligomeric matrix protein (COMP), which has been shown to be released into the circulation during erosive joint diseases, has a dual role in complement activation; COMP inhibits the classical and lectin pathways whereas it activates the alternative pathway. COMP-induced complement activation can be seen *in vivo* by the presence of circulating COMP-C3b complexes. Such complexes are present in several rheumatic diseases but absent in healthy controls. COMP-C3b reflects disease activity in RA and levels are decreased upon TNF- inhibition, which might provide an opportunity to use COMP-C3b as a marker of disease progression in RA. COMP is to our knowledge the first cartilage component whose complement activating properties have been demonstrated *in vivo*.

We further showed that serglycin, a proteoglycan secreted by multiple myeloma cells, inhibits the classical and lectin pathways of complement and that cell surface expression of serglycin leads to protection of these cells from complement attack. This might interfere with immunotherapies that aim at directing complement responses towards multiple myeloma cells.

Taken together, several novel interactions between endogenous ligands and complement have been found, which might regulate the progression of different disease processes.

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It is wiser to find out than to suppose

- Mark Twain

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List of papers

This doctoral thesis is based on the following papers, which will be referred to in the text with their respective roman numerals:

I. ***Happonen K.E.****, Saxne T., Aspberg A., Mörgelin M., Heinegård D., Blom A.M.* Regulation of complement by COMP allows for a novel molecular diagnostic principle in rheumatoid arthritis. *Arthritis Rheum. 2010 Dec;62(12):3574-83*

II. ***Happonen K.E.****, Saxne T., Geborek P., Andersson M., Bengtsson, A.A., Hesselstrand R., Heinegård D, Blom A.M.* Serum COMP-C3b complexes in rheumatic diseases and relation to anti-TNF-alpha treatment. *Submitted*

III. ***Happonen K.E.****, Sjöberg A. P., Mörgelin M., Heinegård D., and Blom A. M.* Complement inhibitor C4b-binding protein interacts directly with small glycoproteins of the extracellular matrix. *J.Immunol 2009 Feb 1;182(3):1518-1525*

IV. ***Happonen K.E.****, Melin-Fürst C., Heinegård D, Blom A.M.* Proline arginine-rich end leucine-rich repeat protein (PRELP) inhibits the formation of the complement membrane attack complex. *Submitted*

V. *Skliris A,* ***Happonen K.E.****, Terpos E., Labropoulou V., Børset M., Heinegård D., Blom A.M., Theocharis A.D.* Serglycin inhibits the classical and lectin pathways of complement via its glycosaminoglycan chains: Implications for multiple myeloma. *Eur J Immunol. 2011 Feb;41(2):437-49*

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Abbreviations

ACPA	anti-citrullinated peptide antibody
ADAMTS	a disintegrin and metalloproteinase with thrombospondin motifs
AIA	antigen-induced arthritis
AMD	age-related macular degeneration
AS	ankylosing spondylitis
ATG	anti-thymocyte globulin
CAIA	collagen-antibody induced arthritis
CFHL	complement factor H-like protein
CFHR	complement factor H-related protein
CIA	collagen induced arthritis
COMP	cartilage oligomeric matrix protein
CR	complement receptor
CRP	C-reactive protein
CS	chondroitin sulfate
C4BP	C4b-binding protein
DAF	decay accelerating factor
DAS	disease activity score
GAG	glycosaminoglycan
aHUS	atypical hemolytic uremic syndrome
IL	interleukin
MAC	membrane attack complex
MASP	MBL-associated serine protease
MBL	mannan-binding lectin
MCP	membrane cofactor protein
MM	multiple myeloma
MMP	matrix metalloproteinase
MPGN	membranoproliferative glomerulonephritis
OA	osteoarthritis
PAD	peptidylarginine deiminase
PNH	paroxymal nocturnal hemoglobinuria
PRELP	proline arginine-rich end leucine rich repeat protein
PsA	psoriatic arthritis
RA	rheumatoid arthritis
ReA	reactive arthritis
SLE	systemic lupus erythematosus
SLRP	small leucine-rich repeat protein
SSc	systemic sclerosis
TIMP	tissue inhibitor of metalloproteinase
TNF-α	tumor necrosis factor-α
VAS	visual analogue scale

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease affecting the joints. Already remains of Native Americans from 4500 BC found in Tennessee show signs of a disease, which is thought to be RA. Nevertheless, the first documentation describing RA symptoms did not arise until 123 AD in India where a disease characterized by swollen, painful joints striking hands and feet was described. This disease obtained its current name by Sir Alfred Baring Garrod in 1859, although its first proper medical description is acknowledged the French surgeon Augustin Jacob Landré-Beauvais in the early 1800's. In the 17th century, RA was treated with Peruvian bark, which is known to contain the anti-inflammatory component quinine. Later on also Willow bark was used, which contains salicylate, an anti-inflammatory compound that is the active ingredient of modern aspirin. Since then, major developments in the treatment of RA have taken place. However, the understanding of how and why RA actually develops is far from satisfactory even today. It is known that RA is a multicomponent disease involving many inflammatory pathways, among them the complement system, but the trigger behind this inflammatory cascade is still unknown. In this thesis I discuss roles of complement in RA and possible mechanisms behind pathological complement activation at the molecular level within the joint. Also the role of complement in cancer is touched upon, as one of the papers involved in this thesis concerns a complement inhibitory molecule secreted by certain cancer cells.

The complement system

The complement system was first described in the late 19th century as a heat-labile factor in serum with bactericidal effects (*1-3*). Later on, the importance of complement as an adjuvant for the adaptive immune response was recognized (*4*). The perception of the role of complement has, however, developed even further since then. Today complement is recognized as an important immunological sensor and effector mechanism, that in interplay with the adaptive immunity as well as other parts of the innate immune system defends the host against infection and regulates the inflammatory response in various conditions. Several novel regulatory roles have been designated complement in the recent years and formerly unknown proteins involved in both complement activation and regulation have been discovered. Pathways of cross-talk between complement and other parts of immunity are being unraveled and bring a new complexity to immune regulation, both in health and in disease.

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

Complement activation

Complement can be activated through three distinct pathways, depending on the molecular trigger. Immune complexes or various endogenous proteins, such as C-reactive protein (CRP) (*5*), initiate the classical pathway whereas the lectin pathway recognizes mainly polysaccharides present on pathogenic surfaces. The alternative pathway serves both as an amplification loop for the other two pathways and as a complement activation pathway of its own. All three pathways merge at the level of the C3-convertases, after which they proceed through a common terminal pathway. Complement activation proceeds through a proteolytic cascade with activated enzymes cleaving downstream complement proteins circulating as zymogens. This allows the presence of high amounts of circulating complement to ensure a rapid and efficient response upon stimuli.

The classical pathway

The main recognition unit of the classical pathway is the multimeric C1-complex consisting of one molecule of C1q in association with a Ca^{2+}-dependent $C1s_2C1r_2$-tetramer (for review, see (*6*)). C1q is structurally a heterohexamer where each monomer is composed of an A-, B- and C-chain, which are transcribed from three separate genes (*7*). These three chains polymerize to form a tripple-helical structure, which further assemble in the N-termini to form the intact C1q molecule with a collagen-like bundle in one end and six globular heads protruding in the other end. C1q recognizes its activating ligands mainly through the charge-patterns formed in the interface of the A-, B-, and C-chains in the head region. In addition to most subtypes of IgG and IgM (*8, 9*), activators of C1q include CRP (*10*), certain extracellular proteins of the small-leucine-rich repeat family (*11, 12*) and apoptotic cells (*13*), to name a few.

The $C1s_2C1r_2$-tetramer binds the C1q molecule at the stalk region where the six monomers diverge to form the protruding globular heads. Upon ligand recognition by C1, a conformational change in the C1q subunit activates the C1r molecule, which thereby activates the C1s subunit (*14*). C1s contains the main enzymatic activity of the complex, further cleaving molecules of C4 and C2 (*15*). The C1-complex is dynamic in character and constantly dissociates and re-associates in circulation. This disassembly is further enhanced by the C1-inhibitor (C1inh), which binds both C1r and C1s resulting in their release from the C1-complex (*16*).

Upon activation of C1s, C4 is cleaved into C4a, a small peptide that is released into circulation, and C4b, which binds to the complement activating surface via a highly reactive thioester (*17*). C1s further cleaves C2 to form the smaller C2b fragment, which is released, and the larger C2a fragment, which associates with C4b to form the classical pathway C3-convertase, C4b2a. This convertase is an enzymatic complex, which

can cleave several molecules of C3, thereby both promoting anaphylaxis and terminal complement pathway activation.

The lectin pathway

The molecules participating in lectin pathway activation resemble in many ways those of the classical pathway. The ligand recognition units, mannose-binding lectin (MBL) or ficolins in association with mannose-binding lectin-associated serine proteases (MASPs) form a complex similar to that of the C1-complex. MBL, which is a C-type lectin, contains multiple carbohydrate recognizing domains and can therefore bind a broad variety of polysaccharides on pathogenic surfaces. MBL is an oligomer composed of subunits containing three polypeptide chains that polymerize to form a collagen like stalk region and a carbohydrate recognizing head-region. In functional MBL, 2-6 of these subunits assemble in their N-termini to form a molecule with a collagenous stalk region in one end and the ligand recognition units protruding in the other end (for review, see (*18*)). Similarly to C1q, MBL can also recognize altered endogenous ligands, such as apoptotic and necrotic cells (*19*), which leads to enhanced phagocytosis and non-inflammatory clearance of dying cells.

Three different types of ficolins can replace MBL in the MBL-MASP complex, ficolin-1 (M-ficolin), ficolin-3 (H-ficolin) and ficolin-2 (L-ficolin). Ficolins-3 and -2 are soluble and produced mainly by the liver similarly to MBL whereas ficolin-1 is membrane-bound and produced by monocytes, the lungs and the spleen (*20*). Ficolins contain an N-terminal domain where oligomer assembly occurs, a collagen-like region and a carbohydrate-recognizing fibrinogen-like domain in the C-terminus. Structurally the ficolin subunits are composed of trimers, which further form oligomeric structures of four or more subunits.

Three types of MASPs can associate with MBL or ficolins, MASP-1, MASP-2 or MASP-3. Both MASP-1 and MASP-2 are able to cleave C2, whereas only MASP-2 can cleave C4 (*21*). Since MASP-3 lacks C2/C4-cleaving activity, it might be considered a negative regulator of the MBL-MASP-complex (*22*). However, the physiological role of MASP-3 is still not completely characterized and it might have other, yet undiscovered, substrates. Two non-functional truncated variants of the MASPs, small MBL-associated protein (sMAP) (*23*) or MAp44 (*24*) can furthermore compete with MASP-2 for MBL/ficolin-binding and by blocking the assembly of functional MBL-MASP complexes with proteolytic activity they inhibit the lectin pathway. The lectin pathway C3 convertase is formed when C4 and C2 are cleaved by MASPs and C4b and C2a combine to form a functional enzymatic complex as for the classical pathway.

The alternative pathway

The alternative pathway is both a direct pathway of complement activation as well as an amplification loop for the classical and lectin pathways. The central component of this pathway is C3, which contains a highly reactive internal thioester bond that lies hidden within the molecule (*25*). This thioester undergoes spontaneous hydrolysis at a low rate in circulation rendering a molecule of C3(H_2O), which structurally resembles C3b and is able to participate in the formation of the alternative pathway C3-convertase (*26*). C3(H_2O) or C3b formed through the activity of classical and lectin pathway associates with factor B (fB) which in the presence of factor D is cleaved into Bb. This results in the formation of the alternative pathway C3-convertase built of either C3(H_2O)Bb or C3bBb.

Already in 1954, Pillemer and colleagues demonstrated a role for properdin in complement activation (*27*). Today it is known that properdin can act both as a platform for alternative pathway C3-convertase assembly (*28, 29*) as well as a stabilizer of the convertase itself (*30*). Properdin has been shown to bind apoptotic and necrotic cells and thereby target complement activation to these altered structures for rapid removal (*31, 32*). Moreover, Spizer and colleagues showed that properdin binds zymosan thereby enhancing the ability of zymosan to induce alternative pathway activation (*29*). However, the target specificity of properdin seems to largely depend on the state of properdin polymerization. Properdin occurs naturally as dimers, trimers and tetramers but might undergo polymerization upon long term storage or repeated freezing/thawing (*33*). The interaction between necrotic cells or zymosan with naturally occurring oligomers of properdin was confirmed whereas rabbit erythrocytes, that in a previous study were shown to bind properdin, do not bind any of the naturally occurring properdin variants (*34*).

The alternative pathway can also be activated by factors normally considered belonging to the classical and lectin pathways. Matsushita and Okada showed that sheep erythrocytes opsonized with C4b were able to activate the alternative pathway in the absence of C2 or in the presence of EGTA, which would inhibit the activity of the Ca^{2+}-dependent C1-complex (*35*). Furthermore, May and Frank demonstrated that sheep erythrocytes opsonized with sufficient amounts of antibody were lysed in serum depleted of C2 or C4, but not C6, showing that the alternative pathway was activated by C1-immunoglobulin complexes on the surface of the cells (*36*). Furthermore MASP-1 may directly cleave C3 (*21*) and activates factor D, which is secreted in an inactive form (*37*), thereby contributing to alternative pathway activation.

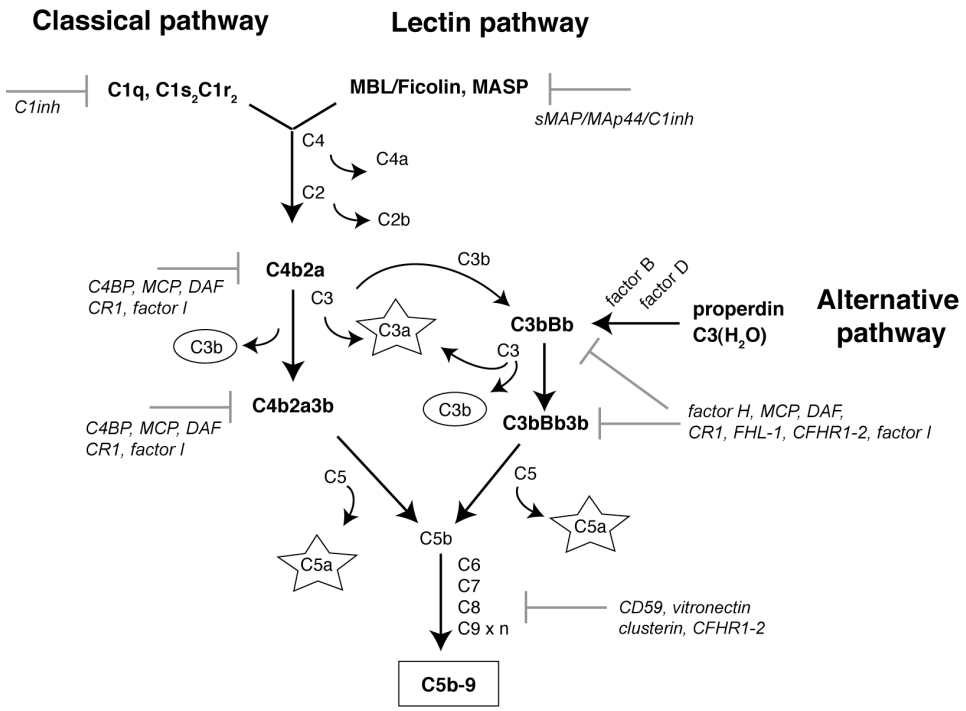

Figure 1. *The complement system*. Complement can be activated through three distinct pathways, the classical, the lectin and the alternative pathway. This leads to the formation of C3-convertases, C4b2a or C3bBb, with the ability to cleave C3 resulting in release of the anaphylatoxin C3a and the opsonin C3b. Association of C3b with the C3-convertases changes their substrate specificity to C5. C5-cleavage results in the release of the C5a-peptide with anaphylactic properties and the generation of C5b, the first component of the MAC. Association of C6, C7, C8 and several molecules of C9 to C5b leads to the formation of a membrane-disrupting pore in the target cell. Complement inhibitors target mainly the C3/C5-convertases or the formation of the MAC.

The terminal pathway

The C3-convertases formed through any of the three pathways cleave molecules of C3 into C3a, a potent anaphylatoxin, and C3b, the main opsonin of the complement system. C3b can further bind to the C3-convertase to form C3bBbC3b or C4bC2aC3b, which changes the substrate specificity of the enzymatic complex to C5. These C5-convertases cleave C5 to release the small anaphylatoxin C5a and the first component of the membrane attack complex (MAC), C5b. C5b associates with C6 and further C7 to form a C5b-7 intermediate with a hydrophobic region exposed within C7 that promotes membrane

attachment (*38*). Binding of C8 to C5b-7 further stabilizes membrane attachment and creates a binding site for C9. Upon binding to C5b-8, C9 undergoes a conformational change which allows for self-polymerization and formation of a lytic pore in the cell membrane (*39*) (Fig. 1).

C5-cleavage with release of active C5a has also been shown to occur in the absence of active C3-convertases. Huber-Lang and colleagues showed that C5a is produced in $C3^{-/-}$ mice by thrombin, demonstrating a connection between coagulation and the complement system (*40*).

Effector mechanisms of complement

Opsonization

The activity of the C3-convertases leads to surface deposition of C3b on the complement-activating target. Deposited C3b is further processed by factor I and its cofactors to iC3b, C3dg and C3d, which are ligands for complement receptor 2 (CR2), a molecule expressed mainly on the surface of B cells and follicular dendritic cells. 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 (*4*). Therefore complement deposition onto a surface greatly enhances its antigenic properties and acts to enhance the immunological response towards the target.

The iC3b cleavage fragment readily interacts with CR3 and 4, which are expressed on macrophages, neutrophils and natural killer cells. This interaction leads to an enhanced phagocytosis of the complement-opsonized target, and in the presence of additional stimulatory molecules on pathogenic surfaces, to cytotoxicity. In the absence of other danger signals, however, this phagocytosis does not provoke pro-inflammatory signaling (*41*), which forms the basis for complement mediated clearance of self-ligands. Also CR1 can mediate phagocytosis of complement-opsonized particles even though it seems not to be an equally important phagocytic receptor as CR3 (*42*). In the circulation, CR1 is mainly expressed on erythrocytes. Binding of C3b-opzonized immune complexes to CR1 on erythrocytes leads to the transport of these complexes to tissue resident macrophages in the liver and spleen where they are taken up in a Fc-receptor -dependent manner. This promotes safe clearance of immune complexes from the circulation (*41*). Also C1q, MBL and ficolins can act as opsonins promoting phagocytosis (*43*), which is especially important in the removal of apoptotic cells.

Anaphylaxis

Another important effector function of complement is the production of the anaphylatoxins C3a and C5a with both chemoattractant and immune-modulatory functions. These cleavage products exert their functions by binding to their respective G-protein coupled receptors, C3aR and C5aR, which are expressed on most types of myeloid cells, but also in several tissues. Signaling through these receptors leads to various responses, depending on the affected cell type. As mediators of inflammation, C3a and C5a regulate smooth muscle cell contraction, vasodilation and the permeability of small blood vessels (*44*). Mast cells and basophils secrete histamine, dendritic cells upregulate the expression of costimulatory molecules and therefore become more potent antigen presenters (*45, 46*), and T-cells increase their proliferation and longevity (*47, 48*) in response to C3a or C5a. Complement receptor regulated antigen presentation by dendritic cells also influence T-cell differentiation (*48*). During an infection, signaling through both TLR2 and C5aR on dendritic cells induce naive CD4+ Th cells to undergo Th1 differentiation, whereas in the absence of C5aR signaling the Th cells are driven towards Th17 and Foxp3+ Treg differentiation (reviewed in (*49*)). Therefore danger recognition by multiple inflammatory pathways co-operate to trigger a pro-inflammatory response by the adaptive immune system. C5a, C3a or their processed variants C3a-*des arg* and C5a- *des arg* also bind the C5a-like receptor 2, C5L2. C5L2 is a non-signaling scavenger receptor, which is postulated to negatively regulate anaphylatoxin mediated inflammation by immobilizing excess C5a and C3a in the circulation. C3a/C3a- *des arg* stimulation of C5L2 has, however, been shown to stimulate triglyceride synthesis and increase glucose uptake in adipocytes and skin fibroblasts (*50*).

MAC-formation

As a defense mechanism against pathogens, the MAC is mainly effective in killing Gram negative bacteria. This is verified by the susceptibility for especially *Neisserial* infections in persons with deficiencies in MAC-components (*51*). It is likely that several MAC need to deposit onto the bacterial surface in order to trigger direct cell lysis. However, a small number of MAC-formed pores can also act as a passage for lysozyme to enter the periplasmic space and digest peptidoglycan, thereby disrupting the cell wall structure (*52*).

In humans, nucleated cells also require multiple MAC to penetrate the cell membrane for the cells to undergo lysis, whereas erythrocytes are lysed more easily. As a protection mechanism, sublytic MAC on the cell membrane can be removed either by endocytosis or exocytosis in a very rapid process to prevent the disruption of membrane integrity (*53*). Host cells are further protected from lysis by the MAC-inhibitor CD59, which binds the cell surface through a glycosylphosphatidylinositol (GPI)-anchor. Defects in GPI-anchoring of proteins lead to the absence of CD59 on erythrocyte membranes and

promotes the development of paroxymal nocturnal hemoglobinuria (PNH), a disease where erythrocytes are lysed due to inadequate terminal pathway protection (*54*).

MAC can also mediate non-lethal signals, including cell cycle activation (*55*), proliferation and migration (*56*) to the cell when present at sublytic concentration. This shows that complement activation has several regulatory effects on self-cells, which brings a diversity to the cellular response of complement attack.

Complement regulation

Since complement activation can have deleterious effects when occurring at the wrong time or on the wrong surface, autologous surfaces are protected by both soluble and membrane-bound complement inhibitors. The main targets of these inhibitors are the C3-convertases or MAC assembly (Fig. 2). Most complement inhibitors are composed of complement control protein (CCP)-domains, which assemble into a similar fold despite their relatively high sequence diversity.

C4b-binding protein (C4BP)

C4BP is the main soluble inhibitor of the classical and lectin pathways. C4BP is a glycoprotein of approximately 570 kDa, which by binding C4b can both inhibit the formation and accelerate the decay of the C3- and C5-convertases (*57, 58*). Furthermore, C4BP can act as a cofactor for the serine protease factor I in the inactivation of deposited C4b (*59*) and to a lesser amount C3b (*60*). C4BP exists in several isoforms in the circulation, of which the most common one contains seven identical α-chains and one unique β-chain. The β-chain binds with high affinity to the anticoagulant vitamin-K dependent protein S (PS) (*61*). During acute-phase response, a type of C4BP lacking the β-chain is secreted, which prevents depletion of PS from the circulation (*62*). The α-chains are composed of 8 CCP-domains whereas the β-chain contains only three CCP-domains. The cofactor activity and decay accelerating activities all reside in the four outermost CCP-domains of the α-chains (*60, 63*).

In addition to being hi-jacked by several pathogens as a defense against host immunity (for review, see (*64*)), C4BP has been shown to bind apoptotic and necrotic cells (*65, 66*), CRP (*67*) as well as several proteins of the extracellular matrix (*68*). This leads to the recruitment of C4BP to vulnerable surfaces in need of extra protection from complement and down regulation of the lytic terminal complement pathway with the associated release of C5a.

Factor H

Factor H is the main soluble inhibitor of the alternative pathway comprising 20 CCP-domains in a head-to-tail arrangement. By binding to C3b, factor H affects both the C3- and C5-convertases of the alternative pathway (*69*) and also acts as a co-factor for factor I in the inactivation of C3b (*70*). The main complement regulatory site on factor H resides within the 4 most N-terminal CCP-domains whereas the C-terminus acts as the target recognition site by interacting with deposited C3b and cell surface glycosaminoglycans (GAGs) (*69*).

Factor H belongs to a protein family also comprising complement factor H-like protein 1 (FHL-1) as well as five other factor H-related proteins, CFHR1-5. FHL-1 is an alternative splice variant of factor H and contains the complement regulatory N-terminal region in addition to a short extension at the C-terminus and therefore displays complement inhibitory activity (*71*). CFHR1 blocks C5-convertase activity and interferes with MAC surface-deposition and assembly (*72*) whereas CFHR2 is suspected to inhibit the alternative C3-convertase and MAC-formation (*73*). CFHR3-4 lack complement regulatory activity on their own but they have been shown to enhance the cofactor activity of factor H (*74*). In addition, CFHR5 might have a weak cofactor and decay accelerating activity but the evidence of this is inconclusive (*75, 76*). CFHR5 has further been suggested to influence the interaction between iC3b and it's ligands (*76*).

Other convertase inhibitors

In addition to the soluble complement inhibitors C4BP, factor H and factor I, several membrane bound complement inhibitors are expressed on most cells throughout the body. Decay accelerating factor (DAF) containing four CCP domains and a GPI-tail, which mediates cell attachment, inhibits the assembly and promotes the decay of classical and alternative pathway C3- and C5-convertases by binding deposited C3b and C4b (*77*). DAF lacks, however, co-factor activity for factor I. Membrane cofactor protein (MCP) on the other hand, lacks decay accelerating activity but is a potent cofactor for factor I in the cleavage of both C3b and C4b (*78*). Complement receptor 1 (CR1), containing 30 CCP domains, a transmembrane domain and a cytoplasmic domain carries both decay accelerating activity as well as co-factor activity in addition to having an important function in immune complex processing and clearance (*79*). Importantly, CR1 is the only co-factor that promotes the cleavage of C3b to C3dg by factor I, which results in a change of receptor specificity to CR2 (*80, 81*) (Fig. 2)

Complement inhibition at the level of MAC

Some complement inhibitors are directed against the assembly of the MAC thereby preventing target cell lysis. CD59 is a membrane bound complement inhibitor, which inhibits C9 from incorporating into the forming MAC by binding both C8 and C9 (*82, 83*). Clusterin, also called apolipoprotein J, binds C7, C8 and C9 and therefore inhibits proper assembly and membrane attachment of MAC (*84*). A similar complement inhibitory activity has been designated vitronectin, which also binds MAC already at C5b-7 stage and inhibits membrane insertion of the forming MAC (*85*). Vitronectin also directly binds C9 thereby inhibiting its polymerization (*86*).

Other host complement regulators

The nephritic factor is the only positive regulator of the alternative pathway C3-convertase in addition to properdin. It is an autoantibody with specific affinity for the alternative pathway C3/C5-convertases, which by binding to the convertase prevents its dissociation and thereby leads to enhanced complement activity and eventually complement consumption in the circulation (*87*). The nephritic factor has been found mainly in the circulation of patients with membranoproliferative glomerulonephritis (MPGN) where it is thought to contribute to disease progression. However, not all nephritic factors bind the same epitope of the convertases so their efficiency in convertase stabilization might vary (*88*).

C1inh belongs to the serpin family of protease inhibitors and may act on C1r, C1s, MASP-1 or MASP-2. C1inh dissociates C1r and C1s from an activated C1-complex, mainly through an interaction with C1r (*89*). It has also been demonstrated that C1inh can bind to the C1-complex and thereby inhibit its activation (*90, 91*). C1inh further binds both MASP-1 and MASP-2 and inactivates these proteases (*21*). In addition to inhibiting the complement system, C1inh also regulates the plasma kallikrein-kinin system by inhibiting kallikrein, factor XIIa and factor XIa thereby forming a link between the complement and the contact system (for review on the functions of C1inh, see (*92*)).

Carboxypeptidase N is an enzyme mainly involved in bradykinin metabolism, but also cleaves and partially inhibits the anaphylatoxins formed upon complement activation. It cleaves a C-terminal arginine from C3a and C5a leading to the formation of C3a-*des arg* and C5a-*des arg*, which results in their inability to stimulate histamin release from basophils and mast cells. Due to the inability of C3-*des arg* to interact with the C3aR, the modified molecule has lost most of its biological function. C5a-*des arg* does, however, still have chemoattractant activity (*93*). A similar anaphylatoxin-inactivating activity has been demonstrated for carboxypeptidase R (*94*).

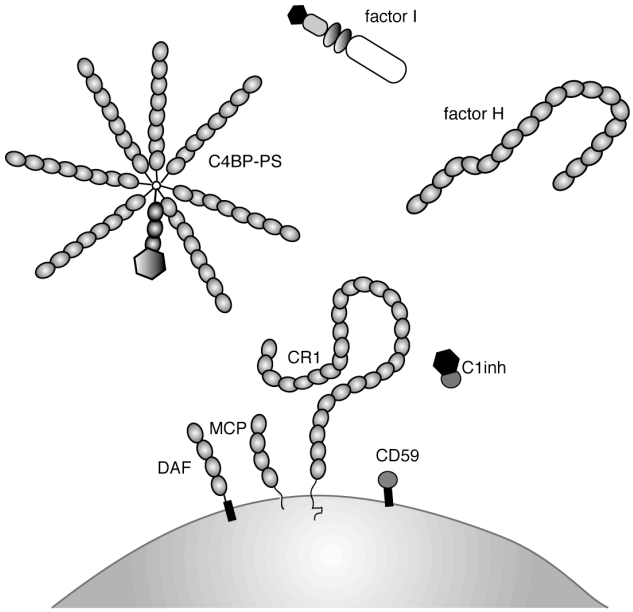

Figure 2. *Complement inhibitors*. Several soluble or membrane-bound complement inhibitors protect host tissues against misdirected complement activation. Most of these contain CCP-domains as their active subunits.

Complement mediated diseases

Due to the wide range of inflammatory responses that complement can mediate, it is not surprising that complement is involved in several diseases. Inadequate complement activation may lead to an increased susceptibility to infections and autoimmune diseases whereas misdirected complement activation lead to diseases such as atypical hemolytic uremic syndrome (aHUS) or age-related macular degeneration (AMD) where host tissues are not efficiently protected against complement attack.

Deficiencies in early classical pathway components, especially C1q, lead to the development of systemic lupus erythematosus (SLE), which has been suggested to relate to an impaired clearance of apoptotic cells (for a review of complement in SLE, see (*95*)). Deficiencies in lectin pathway components mainly lead to an increased susceptibility to infections, especially during early childhood when the adaptive immunity has not matured (*96*). Deficiencies in MAC-components are associated with an increased risk of meningococcal infections (*51*), similarly to properdin-deficiency (*97*). C3 deficiency is extremely rare and usually leads to recurrent pyogenic infections or MPGN (*98*).

Mutations in complement inhibitors also promote disease development. Abnormalities in factor H lead to the development of MPGN or aHUS, where pathological complement deposition occurs in the kidneys (*99*). aHUS has in addition been linked to

mutations in factor I, MCP, factor B, C3 and autoantibodies against factor H (*100*). Polymorphisms in factor H also predispose to AMD, which is the major cause of blindness in the elderly (*101*). Deficiency of C1inh leads to hereditary angioedema, which is not related to the complement regulatory role of C1inh, but to insufficient regulation of the bradykinin-forming pathway (*102*). In addition, the absence of CD59 on erythrocytes due to an impaired GPI-anchoring of the protein leads to PNH as mentioned above (*54*). Mutations in C4BP have in one study been shown to associate with aHUS (*103*), but interestingly no C4BP deficiency has been reported in literature showing that such deficiency is most likely lethal.

Misdirected or excessive complement activation in the absence of specific complement mutations may also lead to pathology. In ischemia reperfusion, blood flow is interrupted to a tissue and when restored, complement recognizes the hypoxic vessel endothelium as an altered-self structure thereby promoting tissue damage even further (*104*). In RA complement recognizes several ligands within the joint and promotes the development of chronic inflammation (*105*). Complement deposition has been shown to occur in atherosclerotic lesions and is thought to contribute to the development and growth of the lesions. However, it is also possible that classical and lectin pathway-mediated clearance of apoptotic cells and debris helps to control the progression of lesion growth, and thereby the pathological role has been designated mainly the later stages of the complement cascade, especially C5b-9 (*106, 107*). In allergic asthma complement has a dual role. Production of C3a and C5a seems to drive allergic inflammation during the effector phase, whereas C5aR signaling might be protective during the allergen sensitization phase by orchestrating Th2-responses (*108*). Complement activation has also been connected to Alzheimer's disease, although whether this actually promotes inflammation is not known. C1q can bind directly to amyloid deposits in the brain as well as to a variety of apoptotic brain cells thereby activating the classical pathway. Due to additional binding of C4BP, MAC-formation and anaphylatoxin release is limited. Therefore C1q might enhance the clearance of apoptotic cells and misfolded proteins and as such have an anti-inflammatory role, but under conditions where the immune system is overwhelmed by excessive exposure of complement-activating ligands, the balance might be tipped over to a pro-inflammatory response (reviewed in (*109*). Finally, complement has also been associated with early stages of prion disease. Opsonization of PrP^{Sc} with complement might facilitate its transport to lymphoid tissues by dendritic cells enhancing its invasion of host tissue (*110*).

Taken together, this shows that a strictly controlled complement activity is needed to achieve potent anti-microbial effects while leaving host tissues protected against complement-mediated damage.

Inflammatory joint diseases

Inflammatory joint diseases, such as RA, have a major impact on both the patients' well being and also a large economic impact in society. In most cases, chronic inflammation within the joint leads to irreversible tissue destruction impairing the mobility of the joint in addition to causing pain and discomfort. Several rheumatic conditions are associated with joint inflammation; RA, SLE, psoriatic arthritis (PsA) and ankylosing spondylitis (AS) to name a few. The underlying reason for the joint inflammation is usually an autoimmune reaction towards epitopes exposed in the affected tissues, but the actual trigger for this autoimmune reaction is still unknown. Osteoarthritis (OA) is the most common type of degenerative arthritis and even though OA is not an autoimmune disease, it has been associated with a local inflammatory response in the affected joints. The following chapters will focus on RA and OA since they are specifically important for this thesis.

Rheumatoid arthritis

RA is the most common type of arthritis triggered by the immune system affecting an estimated 0.5-1% of the population worldwide with prevalence increasing with age. RA is more common in women than in men, which suggests that hormonal factors may play a role for disease development (*111*). Even though any joint can be affected, the small peripheral joints of the hands and feet tend to be more susceptible to display symptoms. The disease course of RA may be further complicated by an increased risk for cardiovascular disease (*112*) and other comorbidities.

Molecular alterations within the joint

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. This altered invasive phenotype of the synovial membrane with infiltration of inflammatory cells is often referred to as a "pannus" (Fig. 3). Also the underlying bone is affected in RA as activation of osteoclasts leads to bone erosion also contributing to joint deformation (for review, see (*113*)).

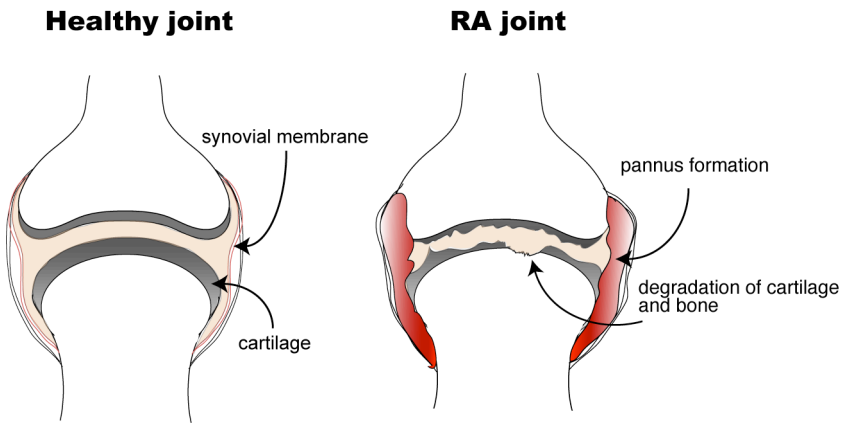

Figure 3. *The joint*. In a healthy joint the bone surface is covered with a layer of cartilage, which provides a smooth surface for bone movement. I RA, the cartilage and the underlying bone are degraded due to secretion of proteases by both infiltrated inflammatory cells in the pannus-tissue as well as by chondrocytes in the cartilage.

The articular cartilage tissue, which is the main type of cartilage found in the joints, consists of a low number of chondrocytes surrounded by an extensive extracellular matrix (Fig. 4). The most abundant proteins in the matrix are different types of collagens associated with linker molecules forming a network of high tensile strength. Aggrecan is a highly negatively charged proteoglycan abundantly distributed in the matrix, which aids in retaining water within the cartilage to provide shock-absorbing properties to the tissue. Disruption this molecular network is a hallmark of RA as well as OA.

One way to study cartilage degradation in disease is to subject cartilage explants to pro-inflammatory cytokines mimicking the situation during disease, with the objective to induce protease secretion by cells present within the tissue. Studies like this have revealed a highly coordinated event of protein degradation and release, aggrecan being the first molecule to be fragmented. Aggrecan release is followed by degradation of fibromodulin, cartilage oligomeric matrix protein (COMP) and collagen IX, where after the fibrillar collagen network is cleaved (*114*). Therefore it seems that smaller structural proteins are being released before the extensive collagen network can be degraded. Understanding this process of tissue breakdown is of importance both for monitoring cartilage destruction as well as well as finding means to inhibit it.

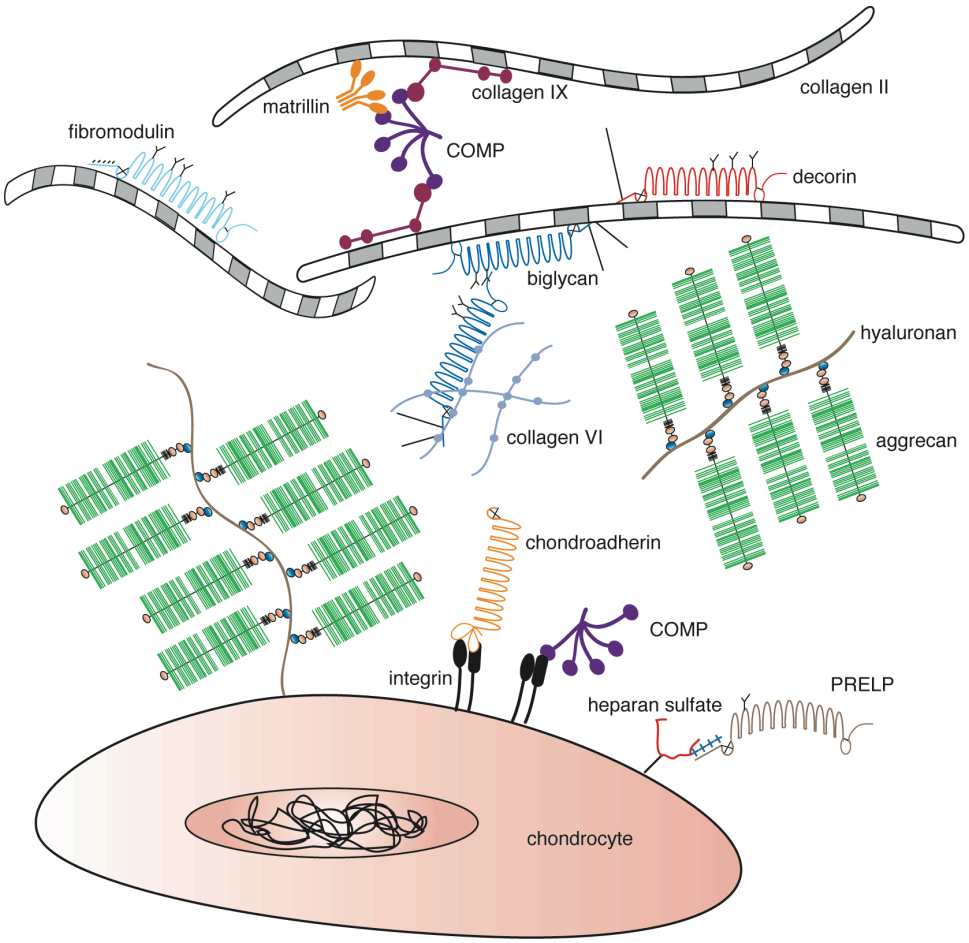

Figure 4. *Articular cartilage.* The articular cartilage found in joints has a low number of cells but an extensive extracellular matrix. This matrix is composed of collagen networks and various linker proteins regulating the assembly and organization of the tissue. Several of these structural proteins also have modulatory effects on the cartilage resident chondrocytes. Aggrecan bound to hyaluronan acts as a water attractant due to its highly negative charge and thereby contributes to the shock-absorbing properties of the tissue.

An important mediator of cartilage destruction in RA and OA is the family of matrix metalloproteinases, MMPs (*115*). Under normal conditions MMP activity is regulated by TIMPs, tissue inhibitors of metalloproteinases. However, in arthritis MMP expression is up-regulated and MMPs are found at a high molar excess compared to TIMPs (*116*) thereby promoting excess cartilage degradation. The different MMPs can together cleave most components of cartilage (*117*). MMP-13 is especially important in

arthritis, since it cleaves not only collagen II (*118*), but also collagen IX (*119*), aggrecan (*120*), fibromodulin (*121*) and COMP (*122*). MMP secretion by synovial cells or chondrocytes is stimulated by cytokines such as interleukin (IL)-1β or tumor necrosis factor (TNF)-α (*123*), which also act to suppress cartilage anabolic activity (*124, 125*). Recent studies show that C3a-mediated signaling is essential for IL-1β production by isolated human macrophages, which indicates that complement activation may have a direct impact on the process of cartilage degradation (*126*).

Due to the detrimental effects of excessive MMP activity, attempts to selectively inhibit MMPs in RA have been carried out. The most promising results have been obtained in a mouse model of collagen induced arthritis (CIA), where inhibition of c-Fos/activator protein-1, a transcription factor regulating MMP synthesis, both prevented disease onset as well as resolved symptoms when the inhibitor was administered during active disease (*127*).

Another important family of cartilage degrading enzymes is the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. ADAMTS-4 and -5 are the main aggrecan-cleaving proteases in human cartilage (*128*) whereas ADAMTS-4 also cleaves COMP (*129*). Both enzymes cleave also biglycan and decorin (*130*). COMP can furthermore be cleaved by ADAMTS-7 and -12 (*131, 132*). ADAMTS-4, -7 and -12 expression is induced in cartilage explants by IL-1 and TNF-α whereas ADAMTS-5 seems to be constitutively expressed (*133*). Inhibition of ADAMTS-4 and -5 expression has proven to be beneficial in CIA in mice (*134*) whereas deletion of the genes was showed to prevent the progression of OA (*135*). No clinical trials have been reported with specific ADAMTS inhibitors. Glucosamine and hyaluronan have been used in the treatment of osteoarthritis, albeit with controversial clinical effect. *In vitro* studies have shown that glucosamine may inhibit ADAMTS activity (*136*) as well as reduce IL-1β stimulated expression of ADAMTS-4 and -5 by chondrocytes (*137*). Hyaluronan has similarly been shown to inhibit IL-1β induced ADAMTS-4 expression in osteoarthritic chondrocytes (*138*). However, hyaluronan has been shown to have several other effects on different cell types present in the joint, which has been found to largely depend on the molecular size of the molecule (*139*). Therefore any possible effect that hyaluronan might have on ADAMTS secretion or activity will be in context of other cellular changes induced by the same molecule.

Another interesting observation is that the C1-complex subcomponent C1s can cleave collagens type I and II (*140*) and activate MMP-9 (*141*), a collagen-cleaving protease. Whether C1s actually has this activity also *in vivo* is still uncertain.

Risk factors for RA

Twin studies have shown that approximately 65% of the risk factors contributing to RA can be attributed to genetics (*142, 143*) with a major contribution by different *HLA-DR* alleles within the major histocompatibility (MHC) complex (*144*). It is however still unclear if these allelic variations on their own predispose to RA or whether the effect is mediated by an increased predisposition to anti-citrullinated peptide antibodies (ACPA), which themselves are a risk factor for RA (*142, 145*). In Caucasian populations, another important genetic predisposition comes from a single nucleotide polymorphism in the protein tyrosine phosphatase N22 (*PTPN22)* gene (*146*). Polymorphisms in peptidylarginine deiminase (*PADI4)*, which might give a weak predisposition to RA in Caucasians (*147, 148*), seem to strongly contribute to disease susceptibility in Asian populations (*149*). *HLA-DR* and *PTPN22* are both directly involved in T-cell reactivity underlining the importance of T-cell immunity for the development of RA. Mutations in *PADI4* on the other hand are thought to lead to an increase in protein citrullination, which may contribute to the development of autoantigens in the tissues. Interestingly, both the *HLA* region (*150*) and the *PTPN22* gene have in addition been associated with other autoimmune disorders, such as type 1 diabetes mellitus and SLE (*146*).

Even though these genetic factors have been shown to predispose to RA, it is likely that a combined effect of both environmental and genetic factors is needed for triggering disease. An important risk factor for RA is smoking (*151, 152*), but how smoking actually affects the immune system leading to joint inflammation is still unknown. There have been suggestions that smoking increases citrullination of peptides within the lung leading to an increased risk for the production of ACPA (*153, 154*), which would be the first step of the pathogenic events in RA. According to a recent report the effect of smoking is an increase in nonspecific citrullination of proteins as opposed to citrullination of specific antigens (*155*). Other environmental factors that have been implicated in disease initiation are viral or bacterial infections, although this has been questioned due to lack of conclusive evidence (*156*). Obesity, previous blood transfusions (*157*) and stress (*158*) are also risk factors linked to RA, whereas there have been some indications that vitamin D (*159*) might have a protective effect. Several other studies concerning diet have been carried out, and although a high consumption of fruit, vegetables, olive oil and fatty fish has been suggested to have a protective effect, existing evidence are still inconclusive (*160*).

Autoantibodies in RA

Autoantibodies are a common finding in RA. The most diagnostically valuable autoantibodies so far to be characterized are the rheumatoid factor (RF) and the ACPA. RA patients may also have a variety of antibodies against cartilage components, such as collagen II, which are thought to be locally produced. A subset of RA patients also display with antibodies against RA33, the heterogeneous nuclear ribonucleoprotein A2, or glucose-6-phosphate isomerase (*161*). In mice models of arthritis the disease can be triggered by injecting mice with monoclonal antibodies against collagen II. This shows that such antibodies have pathologic effects and may reflect the scenario observed in humans. The following chapters will focus on RF and ACPA due to their high abundance in RA patients and their importance for disease diagnostics.

Rheumatoid factor

The RF is one of the most used molecular serological markers in the diagnostics of RA. RF is an IgM, IgG or IgA subtype antibody against the Fc-region of IgG. Albeit its wide use in RA diagnostics, there are still some issues to overcome concerning the specificity and sensitivity of RF as a diagnostic marker; a systematic review on 151 published studies concluded that IgM RF has a specificity of 79% whereas the sensitivity is around 70% (*162*). However, there seem to be differences between the type of RF found in healthy individuals compared to RA patients. Healthy individuals most often have low affinity IgM subtype RF whereas RA RF often show evidence of affinity maturation and isotype switching to IgG RF (*163*).

Due to the presence of RF also in unaffected individuals, the contribution of RF for the development of RA has been questioned. The possible pathological effect mediated by RF most likely lies in their potential to form immune complexes within the joints leading to complement activation (*164, 165*) and stimulation of cytokine secretion via the interaction of immune complexes with Fc-γ receptors (FcγR) on immune cells. One hypothesis is that specifically IgG subclass RF mediates TNF-α secretion by synovial macrophages by interacting with FcγRIIIa. Expression of FcγRIIIa is high in synovial macrophages (*166*) and interacts mainly with small, preferably dimeric complexes. Circulating IgG RF is present at relatively low concentration and readily forms these dimers (*167*) whereas polymeric IgM subclass RF, which can also be found in healthy individuals, would not trigger FcγRIIIa-mediated TNF-α secretion. A high local production of IgG RF in RA synovium leading to increased concentration of IgG RF could, however, lead to formation of larger complement-fixing immune complexes with the potential to also stimulate B-cell survival and complement activation (for review, see (*168*)). However, evidence for a pro-arthritogenic effect of RF still remains insufficient.

ACPA

Citrullinated peptides are formed by a post-translational modification where arginine is converted to citrulline by enzymes of the peptidyl arginine deiminase (PAD)-family (*169*). Citrullination has been shown to render proteins more arthritogenic and immunogenic and thereby citrullination might be of importance in the development of RA (*170*). Importantly, citrullinated antigens, such as collagen type II, α-enolase or vimentin, can be found in the synovium of patients with RA likewise to antibodies reacting with such antigens (reviewed in (*171*)). ACPA are more specific for RA than RF, less than 5% of the healthy population are positive for ACPA whereas ACPA can be found in approximately 60% of patients with RA (*172*). From a diagnostic perspective, it is further interesting that ACPA can be found in the circulation years before onset of disease (*173*). The presence of ACPA in RA has been associated with a more severe disease course as well as a better responsiveness to methotrexate treatment. A recent study also showed that higher baseline levels of IgG or IgM ACPA are related to a better clinical response to TNF-α inhibition, although the association is not strong enough to predict treatment outcome at the level of individual patients (*174*). Moreover, the genetic association of *HLA-DR* to RA is evidently limited to ACPA-positive RA. Therefore a clinical distinction between ACPA positive and negative RA seems to exist. Dividing RA patients into two subsets based on ACPA-reactivity might be of high value when both studying disease pathology and evaluating treatment outcome (reviewed in (*175*)).

The pathological effects of ACPA are mediated by complement activation (*176*) and stimulation of immune cells. Immune complexes containing ACPA have been shown to induce secretion of TNF-α by macrophages via an interaction with FcγRII on the surface of the macrophages (*177*). IgE-ACPA, which also can be found in the circulation of RA-patients, has the ability to activate basophils when bound to a citrullinated antigen (*178*).

As mentioned above, smoking confers an increased risk for mainly ACPA-positive RA. The connection between smoking and RA has been suggested to result from an increase in citrullination of proteins in the lungs leading to a humoral immune response towards the newly citrullinated peptides. When an inflammatory response later on, by an unknown trigger, arises in the joint, PAD becomes activated and locally deiminates joint molecules. These citrullinated joint molecules might then cross-react with circulating ACPA to form immune complexes, which further stimulate the joint inflammation, eventually leading to chronic RA (discussed in (*175*)). However, this hypothesis still needs further evidence to clarify the relationship between the low amount ACPA-positive individuals in the normal population compared to the amount of smokers worldwide. In any case, a genetic predisposition would be needed in addition to a life style with smoking to increase the susceptibility for disease.

Infections with *Porphyromonas gingivalis*, a pathogen causing periodontitis, have also been proposed to be an environmental trigger for RA. This may be explained by the ability of *P. gingivalis* to produce a bacterial PAD enzyme (*179*), which can deiminate both bacterial antigens and human fibrinogen and α-enolase (*180*) leading to autoantigen formation. However, evidence for a connection between *P. gingivalis* infections and RA are still inconclusive.

Complement activation in RA

There is substantial evidence of complement involvement in RA from studies of both animal models as well as patient studies. Results from rodent arthritis models have emphasized the role of the alternative pathway for disease development whereas the classical pathway has seemed less important. In a CIA-model C5 (*181*) or factor B deficiency protected mice from disease whereas C4 deficiency did not (*182*). Likewise, C1q or MBL deficiency did not protect mice from arthritis in a collagen antibody-induced arthritis (CAIA)-model (*183*). 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 (*184*). Recently Kimura and colleagues showed that deleting properdin in the myeloid lineage by conditional gene targeting reduced disease severity in a K/BxN model of arthritis (*185*). Cumulatively, this evidence supports the role of the alternative pathway in the development of RA. Mice lacing 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 respective cellular receptors (*186*). Furthermore, deletion of CR2 resulted 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 (*187*).

Due to the evidently important role of complement in rheumatoid arthritis, several approaches to therapeutically interfere with pathological complement activation have been carried out. In 1995, Wang and colleagues showed that 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 (*188*). This has been confirmed by other studies where C5-targeting antibodies reduced disease activity in an antigen-induced arthritis model in rats (*189*). Further, vaccination of mice with a recombinant vaccine that induces C5a-neutralizing antibodies showed to protect the mice from disease in a CAIA model (*190*). Inhibition of complement at the level of C3-convertase by injecting mice with C4BP also ameliorated disease activity in a CAIA-model via inhibition of both classical and alternative pathway complement activation (*191*). 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 might prove to be therapeutically beneficial in the treatment of arthritis.

In humans, complement activation products, such as C3d (*192*), have been found in the joints of patients with RA and complement deposition can be detected in the synovium by immunohistological staining (*193*). 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 flare indicating ongoing complement activation through the classical pathway (*194*). Also elevated levels of C3a (*195*) and C5a have been demonstrated in the synovial fluid of RA patients (*196*), similarly to soluble C5b-9 complexes (*197, 198*). This shows that complement activation occurs locally within the joint with the potential to stimulate further inflammatory responses. Interestingly, a recent study shows that genetic variations in C1q leading to elevated serum C1q-levels confers an elevated risk for developing RA (*199*). This might be related to a potential increase in classical pathway mediated complement activation on auto-antigens in the synovium.

As discussed above, C5-inhibition therapy in rodent models of arthritis have proven to be very successful. In humans, only one small-scale study has been published where patients were orally administered a cyclic peptide (PMX53), which binds the C5aR with high affinity without having an agonist 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 (*200*). However, novel C5-inhibitors currently under trials might prove to be applicable also for human use (*201*).

What activates complement in RA?

One of the main triggers of classical pathway complement activation are deposited or circulating immune complexes, which in the context of RA would compose of autoantibodies such as RF or anti-collagen II in complex with their autoantigens. 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 (*176*). Interestingly, it was shown that the glycosylation pattern of the Fc-region of IgG molecules is altered in RA and an increase of these IgG glycoforms lacking the outermost galactose are found at elevated levels in the synovial fluid in RA. These IgG glycoforms have shown an increased affinity for MBL and might therefore trigger lectin pathway activation in the joint (*202*).

Apoptotic and necrotic cells have in many studies been proven to induce C1q and MBL binding and further complement activation through the classical or lectin pathway (*13, 203*). Also the alternative pathway has been shown to be activated on the surface of apoptotic cells (*204*), an effect that might be attributable to the ability of certain apoptotic cells to bind properdin (*31, 32*). Since apoptotic and necrotic cells bind 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 (*65, 66*). Moderate deposition of early opsonins, C1q and C3b, is most likely beneficial due to promotion of phagocytosis (*203*) 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 (*205, 206*) and might therefore contribute to pathological complement activation.

Several structural proteins of the joint have also been implicated in complement regulation in RA. These include the family of small leucine-rich repeat proteins (SLRPs) that are abundantly found in the cartilage tissue. Cartilage components are normally exposed to complement in the synovial fluid to a very limited extent, mainly due to tissue regeneration, which releases low amounts of tissue constituents. However, during RA when tissue damage is pronounced and matrix proteases are secreted by infiltrated macrophages in the synovium, the level of released cartilage components is increased and their display of surface epitopes is altered (*114*). Two SLRPs, osteoadherin and fibromodulin have strong complement activating properties due to their interaction with the globular heads of C1q whereas a third SLRP, chondroadherin binds and activates C1q to a lower extent (*11, 12*). Since these proteins also can capture C4BP and factor H, complement activation is limited to the early steps of the classical pathway ((*11, 12*) and Paper III). Whether the 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 epitopes. Two other members of the SLRP-family, decorin (*207*) and biglycan, have the opposing effect as they bind the stalk region of C1q whilst inhibiting further complement activation. Both decorin and biglycan also bind MBL, but only biglycan has the ability to inhibit the lectin pathway (*208*). PRELP, another member of the SLRP-family, does not activate complement, but can both directly inhibit MAC-formation and the alternative C3-convertase in addition to binding C4BP (Paper III and IV). Cartilage oligomeric matrix protein (COMP) has a dual effect on complement as it can both bind to C1q and MBL thereby inhibiting classical and lectin pathway activity as well as activate the alternative pathway by binding C3 and properdin (Paper I). Furthermore, the NC4 domain of collagen IX, which is released in early RA, inhibits the formation of the MAC and increases the co-factor activity of C4BP and factor H (*209*). The cartilage specific collagen II has in contrast been shown to trigger alternative pathway complement activation in guinea-pig serum (*210*). Recent results show in addition, that the protein core of aggrecan can activate complement (C. Melin-Fürst, personal communication).

The cartilage tissue is rich in heavily glycosylated proteins with complex 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 (*31*). 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 (paper V). 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 proteoglycans found in the tissue.

Since tissue destruction, autoantibody production and cell death together form a very complex process within the joints, the overall impact on complement will be dependent on an interplay between all of these components and will most likely fluctuate during disease progression.

Biological therapeutics in RA

Therapeutics of RA have for a long time included disease modifying anti-rheumatic drugs such as methotrexate, sulfasalazine and leflunomide. However, a new era of RA treatment started upon development of the first biological agents, the TNF-α inhibitors. TNF-α is an important inflammatory mediator in RA due to its role as a pro-inflammatory cytokine and therefore its inhibition was hypothesized to interrupt the self-feeding cycle of inflammation. Feldmann and Maini were pioneers in studying the role of TNF-α in RA. Their hypothesis of a pathogenic role of this cytokine was corroborated in clinical trials showing that monoclonal antibodies blocking the biological function of TNF-α ameliorate disease activity in RA patients who do not respond to conventional therapeutics (*211, 212*). Several monoclonal antibodies and a soluble TNF-α receptor have now been designed for the blockade of TNF-α, and their efficacy in disease amelioration seems to be augmented by simultaneous treatment with methotrexate (*213, 214*). In addition to preventing pro-inflammatory signaling, one of the outcomes of TNF-α inhibition is most likely a reduced MMP and ADAMTS- 4 expression, as seen *in vitro* (*215*), which would lead to a decrease in cartilage degradation.

If and how TNF-α inhibition affects the complement system is still not clear. TNF-α has been shown to induce factor B and C3 expression in hepatocytes whereas the expression of C2 and C4 remained unaltered (*216*). Di Muzio and colleagues showed that TNF-α inhibition in RA patients significantly reduce serum C3- and C4-levels paralleling changes in RF (*217*). In another study, TNF-α inhibition was showed to decrease the amount of complement activation products C3b/c and C4b/c in plasma already within two weeks of treatment start (*218*). The same study showed also a decrease in plasma CRP levels as well as in circulating complexes between CRP and C3d or C4d and therefore concluded that TNF-α inhibition might reduce of CRP-stimulated complement activation. It seems that the possible complement regulatory effects of TNF-α inhibition may be secondary to its ability to downregulate inflammation and the presence

of complement stimulating agents within the joints and might also relate to an altered complement protein synthesis.

Since only 60-70% of RA patients respond to TNF-α inhibition, other strategies for immune-modulation need to be developed. Due to the likely role of complement in the disease progression of RA and to the numerous positive results obtained from animal studies, complement inhibition therapy might provide to be a valuable intervention. As mentioned above, the only reported clinical trial carried out to date concerning complement inhibition in RA is with the C5aR inhibitor PMX53, which despite its therapeutic efficacy in rodent models of arthritis (*219*) did not ameliorate symptoms in RA patients (*200*). However, C5aR still remains an interesting target for complement inhibition since blockade of C5a-C5aR signaling would reduce the anaphylactic effect of complement activation whilst the remainder of the cascade is left intact. Ideally, one would like to inhibit only pathological complement activation while leaving the majority of the complement cascade itself functional. Another way to accomplish this could be to selectively inhibit the interaction between a complement initiator and its ligand. This might also act as a localizing complement therapy if the expression of the ligand itself is limited to certain affected tissues. One example would be the inhibition of the SLRP-C1q interaction, which might target only local pathological complement activation within the joint. However, due to the presence of multiple complement stimulating ligands in the synovium, it might be required to simultaneously block several of these interactions in order to break the inflammatory cycle in RA.

One of the main challenges in the treatment of RA is to predict which patient will respond to which therapy in order to achieve early effective treatment start and avoid progression of disease. Therefore understanding the pathology behind the disease is of outmost importance and developing diagnostics that reveal the underlying mechanism of inflammation would be valuable. As an example it would be of high interest to be able to determine which patients truly have pathological complement activation within the joint to selectively treat these patients with future complement inhibitors, which unfortunately will be extremely expensive.

Osteoarthritis

Osteoarthritis (OA) is the most common type of arthritis. It is an erosive joint disease that progresses slowly and affects all structures of the joint. Disease progression is associated with loss of cartilage matrix and destruction of the underlying bone, development of cartilage or bone outgrowths, modest inflammatory infiltrates in the synovium, muscle weakness and ligament laxity (*220*).

The prevalence of OA increases with age while also gender-specific differences occur. Below the age of 50, OA is more common in men than women whereas at higher age osteoarthritis of knee, hand and foot is more common in women (*221*). This has in some cases been hypothesized to be influenced by an estrogen deficiency after menopause (*222, 223*), although evidence for this is still controversial (*224*). Knee injury (*225*) or destabilization of the joint due to ligament injury (*226*) have also been associated with an increased risk of OA. This is most likely related to the altered mechanical load on the cartilage. Obesity is another factor that often is associated with OA. How obesity predisposes to OA is not simply a factor of enhanced mechanical load since obesity also increases the risk of finger OA (*227*). Therefore adipocytokines and metabolic factors might contribute to disease (*228*). Mutations in several structural cartilage molecules, such as collagen type II or IX, COMP or matrilins lead to a high incidence of OA (*229*) emphasizing the importance of a stabile cartilage structure for prevention of disease outbreak.

In normal cartilage, chondrocytes do not proliferate but focus on orchestrating a balanced tissue degradation and synthesis. In contrast, chondrocytes from OA articular cartilage show signs of proliferation (*230*) but also the rate of chondrocyte apoptosis is increased (*231*). In OA the balance between cartilage synthesis and degradation is disturbed, with degradation being more pronounced. The actual cartilage degradation process most likely follows that described for RA, although an alteration of protease activity may influence the type of protein fragments released from the cartilage. Also the molecular composition and organization of the cartilage is altered due to attempts of tissue rebuilding. For instance, COMP is in healthy cartilage most abundant in the interterritorial matrix, furthest away from the chondrocytes. Upon cartilage degradation, COMP is lost from the interterritorial matrix whereas it increases in abundance close to the chondrocytes (*229*). This is thought to reflect an increased synthesis of COMP by the chondrocytes as a means of damage control, as one of the main functions of COMP is to catalyze collagen II fibrillogenesis (*232*). However, this repair process is highly inefficient when COMP is present in too high amounts compared to collagen II, which is the case in OA (*114*).

OA – an inflammatory disease?

Even though OA has traditionally been considered a non-inflammatory joint disease, evidence is accumulating for the opposite. Synovial inflammation has been observed in 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-α (*233*). Using histological staining, Revell and colleagues showed the presence of T-helper and -suppressor lymphocytes, B-lymphocytes and macrophages in the synovial membrane of OA patients (*234*). Using RT-PCR, Wagner and colleagues compared the synovial cytokine profiles in addition to cellular infiltration in RA and OA patients. They found that even though cellular infiltration was more pronounced in RA synovium, the cytokine profiles were similar in both diseases (*235*). Moreover, moderately elevated CRP levels can be found in patients with early OA (*236, 237*). Therefore it seems likely that the inflammation observed in OA contributes to the disease and might be enhanced by the release of components from the cartilage with the potential to stimulate the immune system. Treatment of patients with inhibitors of TNF-α or IL-1 have in some cases proved beneficial for disease amelioration (reviewed in (*123*)) showing that inhibiting inflammation might benefit at least a subset of OA patients.

The involvement of complement in OA is scarcely studied. C3c and C9 deposition in the synovium of patients with OA has been described, and this was limited to the active phase of the disease (*193*). Another study showed C3 deposition in articular collagenous tissue in a subset of patients with OA, although this staining was relatively weak (*238*). Further, in Paper II we show that COMP released from cartilage in OA is able to activate complement. Therefore a low level of complement activation may take place within the joint in OA even though it is not as pronounced as in RA. This further confirms that an inflammatory component exists in OA.

Complement in spondyloarthropaties

It is known that inflammation is a predominant factor in rheumatic diseases, whereas the role of complement in the different conditions is still not fully characterized. Spondyloarthropaties compose a group of related diseases with different phenotypic characteristics and includes psoriatic arthritis (PsA), ankylosing spondylitis (AS), reactive arthritis (ReA), certain subtypes of juvenile idiopathic arthritis and arthritis related to inflammatory bowel disease.

PsA is an inflammatory joint disease associated with psoriasis of the skin. The disease may involve peripheral and/or axial joints and is commonly associated with enthesitis and dactylitis (*239*). Complement activation seems to occur to a limited extent in PsA, as elevated levels of plasma iC3b, C4d and Bb was found in a small study of patients with psoriasis and PsA (*240*). Another study showed relatively low levels of the complement activation product C3c in synovial fluid of PsA patients whereas the total

synovial fluid C3 levels were higher than in RA or OA (*241*). Other studies have reported decreased erythrocyte expression of CD59 (*242*) or CR1 (*243*), which in the case of CD59 was hypothesized to possibly reflect a decreased tissue expression of CD59 in the synovium as well and thereby render synovial cells more vulnerable to complement mediated damage. It is therefore likely that complement activation occurs during PsA although the contribution of complement to disease may not be as prominent as in RA.

AS presents with mainly axial disease involving the sacroiliac joints, whereas peripheral join involvement is less common (*244*). Studies of complement in AS are scarce. Two studies have shown an increase in C3 and C4 activation products in the plasma of AS patients (*245, 246*) whereas a study by Gabay and colleagues found no such increase in their patient cohort (*247*). Polymeric IgA-subtype antibodies reacting with C1q have been found in a subset of patients with AS but the influence of these antibodies on disease pathology has not been elucidated (*248*).

Reactive arthritis (ReA), also known as Reiter's syndrome, is a type of arthritis occurring after an infection, most often gastrointestinal or venereal. The disease is usually monoarticular and can most commonly involve the joints of the knees and ankles (*249*). C3b and C9 deposition has been observed in the synovium of patients with ReA (*193*) indicating local complement activation within the joints.

In paper II we have studied the presence of complexes between COMP and C3b in serum as an indication of COMP-driven complement activation in patients with spondyloarthropaties. We found that patients with ReA had markedly elevated levels of COMP-C3b in their circulation compared to healthy controls in contrast to patients with AS or PsA, who had only weakly elevated COMP-C3b levels. This shows that the tissue degradation process and complement activity are different in these diseases. It also confirms that complement may not have such a prominent role in the pathology of AS and PsA as compared to RA.

Complement in Cancer

The main function of the immune system is to protect the host against possible threats, both from the surroundings and from within the body. Cells that become malignant undergo changes, such as alteration of surface protein expression, which make them prone to be recognized by the immune system and therefore cancer cells need to find ways to escape this surveillance. Complement has been implicated in the defense against tumor development and growth in several ways. Certain cancer cells can activate complement directly through the alternative or lectin pathways (*250, 251*) whereas cancerous cells recognized by antibodies might activate the classical complement pathway. In accordance, complement deposition has been demonstrated in breast cancer tissue, whereas no such deposition was seen on corresponding benign tissue (*252*). Similar complement deposition was found in papillary thyroid carcinoma (*253*) and on several tumor cell lines studied *in vitro*. Reducing cell surface expression of complement inhibitors CD59 and CD55 on cancer cells diminished tumor burden in mice models of ovarian cancer and prostate cancer, showing a direct effect of complement on tumor suppression *in vivo* (*254, 255*).

Complement can act to control tumor growth by either inducing MAC-formation on the cell surface or by inducing C3b-deposition on the cancer cell, which in the context of pro-inflammatory signaling may trigger cell death through an interaction with CR3 on effector cells. Tumor cells may counteract this complement attack by up-regulating the cell surface expression of complement inhibitors or by recruiting soluble complement inhibitors such as factor H to their surface (*256*). Also elimination of surface deposited MAC and inhibition of MAC-induced cellular signaling serve to minimize complement-induced cellular damage. Since most tumors are not on their own immunogenic enough to lead to tumor killing by the immune system, therapeutic interventions stimulating immune recognition and clearance of tumor cells have been implicated. Monoclonal antibodies specifically targeting tumor cells have proven successful in the induction of both antibody-dependent cellular cytotoxicity as well as complement-dependent cytotoxicity. However, complement protection mechanisms utilized by tumor cells have been suggested to severely impair the efficiency such interventions.

Even though the original hypothesis has been that complement activation is beneficial in the battle against tumor growth, evidence for a dual role of complement has emerged. Markiewski and colleagues showed that C5a produced through complement activation actually enhances tumor growth in a model where cervical cancer cells were used to induce subcutaneous tumors in mice (*257*). This effect was mediated by the recruitment of

myeloid-derived suppressor cells into the tumor with the ability to suppress the anti-tumor effects of $CD8^+$ T-cells. Mice deficient in C3 or C4 showed a significantly decreased tumor burden whereas factor B deficiency had no effect, indicating that classical pathway mediated complement activation promoted the formation of C5a in this model (*257*). In order for a tumor to survive it requires vascularization of the tissue to maintain sufficient delivery of nutrients and growth factors to the proliferating tissue. C3a, C5a (*258*) and MAC (*259*) have been shown to stimulate the secretion of vascular endothelial growth factor (VEGF), which is essential for the development of novel blood vessels and may thereby contribute to sustain tumor growth.

It seems that the tumor sustaining effect of complement might be of importance especially in the context of solid tissue tumors whereas one may hypothesize that cancers affecting blood cells are more negatively affected by an increased complement attack. In this sense, up-regulation of complement inhibitors or expression of other non-classical complement modulators in leukemia or myeloma should promote cancer progression. In paper V we have studied a proteoglycan secreted by multiple myeloma cells, serglycin, and show that serglycin protects myeloma cells from complement attack, thereby possibly contributing to their survival.

The present investigation

The aim of this thesis was to characterize novel interactions between cartilage proteins and complement, which might be of importance for complement regulation in arthritis. It has been shown that joint inflammation resides upon joint replacement surgery, where the majority of the cartilage is removed. This led to the hypothesis that components within the cartilage tissue might stimulate the immune system thereby feeding the vicious circle of chronic inflammation. Indeed, it has been shown that several members of the SLRP-family interact with complement to either stimulate or inhibit complement activation. In this thesis I have studied the binding of certain SLRPs to the complement inhibitor C4BP (Paper III) as well as explored the complement inhibiting properties of one of the SLRP-members, PRELP (Paper IV). We also found a new complement-regulating molecule, COMP, which complexes with C3b during joint inflammation, thereby providing new possibilities for diagnostics in inflammatory joint diseases (Papers I and II). Paper V is related complement in cancer. In this paper we have studied the proteoglycan serglycin, which is secreted by multiple myeloma cells, and its ability to protect cancer cells from complement attack.

COMP-C3b as a molecular marker for RA

COMP is a structural molecule in the cartilage where it serves both as a catalyst of collagen fibrillogenesis in the young (*232, 260*) as well as a tissue fortifier through interactions with other extracellular matrix proteins in the adult. 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 (*261*). When cartilage explants are stimulated by IL-1β, COMP is released from the tissue through a process mediated by MMP-1, -3, -9, 13 (*122*) and ADAMTS-4 (*129*), -7 and -12 (*131, 132*) and possibly other, yet unrecognized, proteases. This leads to the release of COMP-fragments just above 100 kDa in size, corresponding to almost full monomeric chains lacking a part of the N-terminus. COMP-release can also be seen *in vivo* in patients with different types of arthritides, where elevated COMP concentration can be detected both in the synovial fluid and in the serum during active disease. However, 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 (*262, 263*). Interestingly, 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 seems 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 (*263*).

Carlsen and colleagues have shown that injection of rat COMP into rats or mice induces the development of chronic arthritis (*264, 265*). They were also able to induce arthritis in mice using anti-COMP antibodies in a serum transfer model (*265*). This shows that, at least in rodents, COMP is arthritogenic. Since we are constantly exposed to low amounts of COMP from regenerating tissue, some sort of tolerance must build up towards circulating COMP. This tolerance may be broken during disease by exposure of new epitopes generated by protease cleavage or unfolding of the molecule.

We set out to investigate whether COMP is able to interact with the complement system and whether this may be one of the pathological events behind arthritis development. We found that COMP has a dual role in complement regulation; it can both inhibit the classical and lectin pathways as well as activate the alternative pathway of complement.

We found that COMP binds to the stalks of C1q, but not to the intact C1-complex. The C1-complex has a dynamic character, it dissociates and re-associates in circulation with approximately 10% of circulating C1q being unbound (*266*). We showed that COMP is able to bind free C1q and thereby prevent the association of $C1r_2C1s_2$ with C1q. This would lead to a local decrease of functional C1-complexes and thereby downregulate classical pathway activity. COMP had only a weak ability to dissociate pre-formed C1-complexes, indicating that it binds to overlapping sites with the $C1r_2C1s_2$-tetramer. We also showed that COMP interacts with MBL and inhibits lectin pathway activation. Since deposition of MBL onto mannan was not inhibited, COMP most likely affects the integrity of the MBL-MASP-complex or inhibits C4-cleavage by the complex. Both C1q and MBL were able to bind monomeric arms of COMP although this binding was weaker than for intact pentameric COMP.

The COMP monomers lack most of the polymer forming N-terminus, and are composed of four epidermal growth factor (EGF) domains, eight thrombospondin type 3 (TSP3) domains and a globular C-terminus. We studied the interaction between complement proteins and COMP using truncated recombinant constructs representing individual domains of COMP with ELISA as well as with electron microscopy. C1q was found to interact with all C-terminus-containing constructs and in electron microscopy it was evident that the binding site lies in the C-terminal domain of COMP. By electron microscopy we could also see that COMP-seems to bind the C1q stalks, quite close to the head-region as we had hypothesized based on the competition studies with $C1r_2C1s_2$. A similar pattern of interaction was observed between MBL and COMP.

We were also able to show that COMP can trigger alternative pathway activation through an interaction with properdin or C3b. Properdin was found to bind both

to the TSP3-region as well as the globular C-terminus whereas C3 bound only to the C-terminus. C9 deposition was only observed on the C-terminal domain indicating that the complement-activating domain resides here. Since the main complement-binding site of COMP seems to be the globular-C-terminus, the availability of complement factors might direct whether COMP activates or inhibits complement. Interestingly, recombinantly produced human COMP and tissue purified COMP from bovine cartilage both had the ability to activate complement. This indicates that not only disease specific cleavage fragments of COMP can regulate complement. We also observed that recombinantly produced monomeric arms of COMP were able to induce C9-deposition showing that an intact pentameric conformation is not needed to activate complement.

We were able to confirm the complement activating properties of COMP observed *in vitro* also *in vivo* by studying serum and synovial fluid from patients with different rheumatic disorders. If a complement activating protein circulates in serum it should rapidly induce deposition of C3b onto its surface. Since the deposition of C3b involves the formation of a covalent bond between the two proteins, the complex between the complement activating protein and C3b should be stable and measurable from bodily fluids. We therefore set up an ELISA for measuring COMP-C3b complexes based on an immobilized antibody that captures COMP and a detector antibody recognizing C3b/d. In our first pilot study (Paper I) we included patients with RA, OA and healthy controls. We found that RA patients have high levels of COMP-C3b in their serum compared to healthy controls that were mainly negative for such complexes. In this study cohort the OA patients were negative for COMP-C3b complexes in their serum. However, we later on observed that in the serum samples from the OA patients all C3 was degraded into small fragments as observed by western blotting. What actually has driven this C3 degradation is still unclear but it might be an effect of blood sampling, storage or excessive freezing/thawing. Therefore no conclusions can be drawn from the results obtained with these samples. However, both the RA and OA patients had COMP-C3b in their synovial fluids indicating local COMP-induced complement activation within the joint. Since the OA patients had much higher total COMP in their synovial fluid than RA patients but equal amounts of COMP-C3b, it seems that either only a fraction of the released COMP in OA was able to activate complement or that other competing molecules were present.

Since the pilot study was carried out with less than 20 patients in each group, we set out to confirm our results in a larger patient cohort also including patients with other rheumatic disorders to investigate the specificity of serum COMP-C3b for RA (Paper II). Also in the new patient cohort including 98 RA patients and 97 age and gender matched healthy controls the RA patients showed to have increased levels of COMP-C3b in their serum. The levels of these complexes correlated weakly to several inflammatory parameters indicating that COMP-C3b formation is partially dependent on synovial inflammation. Unfortunately we did not have information about the ACPA-status of these patients so we could not determine whether there is a difference in the amount of COMP-C3b between ACPA positive and negative patients. Since TNF-α inhibitors have shown to

both ameliorate disease activity in RA (*211*) and also reduce serum COMP-levels (*267*) we measured the COMP-C3b levels in patients receiving TNF-α inhibitors at baseline, after 6 weeks of treatment or after 3 months of treatment to see whether a reduced disease activity is reflected in a decrease in serum COMP-C3b. Indeed, serum COMP-C3b was significantly reduced at 3 months compared to baseline. Notably, COMP-C3b decreased dramatically in some patients whereas the levels remained unchanged in others. However, this decrease followed different kinetics than the general improvement as measured by a decrease in CRP or the disease activity score (DAS); CRP and DAS were decreased already by week 6 after which no further improvement was seen whereas there were only mild alternations in COMP-C3b by week 6 compared to baseline. This shows that the release of COMP and formation of COMP-C3b is a complex process that most likely is regulated by an altered turnover of the cartilage and dependent on other factors within the joint microenvironment. It has been shown that TNF-α can regulate the expression of proteases cleaving COMP so therefore one might speculate that a different type of COMP is being released from the tissue upon TNF-α inhibition. Another option is that the proteolytic degradation of COMP affects the folding of the COMP arms. Normally, the C-terminal domain, which is responsible for activating complement, is partially covered by the TSP3-region due to a back folding of the COMP arm. During disease an altered proteolysis of COMP might affect this folding and expose new epitopes within the C-terminus not available in native COMP.

In this patient cohort, elevated levels of COMP-C3b were also seen in patients with OA, systemic sclerosis (SSc), ReA and SLE. There was no difference between serum COMP-C3b in SLE patients during active disease or during flare, but SLE patients with arthritis had higher serum COMP-C3b than patients without arthritis. Weakly elevated COMP-C3b levels were also seen in patients with AS or PsA, however, in these patients serum COMP-C3b did not correlate with disease activity. Surprisingly, TNF-α inhibition therapy increased the COMP-C3b levels in AS and PsA patients already by week 6, possibly by a tissue repair mechanism increasing the synthesis of cartilage components. CRP-levels were decreased in both patient groups already by week 6. This shows that the disease process and COMP-release is different in RA compared to AS and PsA.

We could conclude from this study that serum COMP-C3b is not a unique feature of RA, but can also be found in patients with other rheumatic disorders. Due to the presence of COMP-C3b in several diseases, it is not on its own applicable as a disease marker, but in combination with other markers, such as RF and ACPA, it might upon further optimization provide enhanced sensitivity to the diagnostics of RA. Furthermore, the presence of especially high levels of COMP-C3b in certain patients indicates that they have severe pathological complement activation and therefore measuring COMP-C3b in serum might provide valuable information on which patients would benefit from complement inhibition therapy.

It would be highly interesting to see whether COMP is subjected to any modifications, such as citrullination, during disease. This might contribute to the complement activating properties of COMP and also possibly lead to the production of ACPA, which are known to have complement stimulating properties. It would also be of interest to study whether patients with rheumatologic diseased have autoantibodies directly against COMP that might further drive complement activation or alter the complement regulatory functions of COMP.

SLRPs regulating complement

SLRPs are small proteoglycans found in a variety of extracellular matrices where they stabilize tissue structure by cross-linking different components of the matrix. Several novel SLRPs have been characterized the past decade, contributing today to a protein family of 17 members. Not all SLRPs fulfill the traditional requirements for proteoglycans, i.e. containing at least one glycosaminoglycan side chain, but are still considered to be a part of this family due to structural and functional similarities. Traditionally SLRPs were divided into three subfamilies, but recently a new division with five subfamilies was suggested (*268*). 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 (for review on SLRP structures, see (*269*)). Many of the SLRPs participate in the regulation of collagen fiber synthesis, both regulating the rate of assembly and termination of fibrillation to maintain proper thickness of the intact fibers (*270, 271*). SLRP-deficient mice present with diseases such as osteoporosis, osteoarthritis, muscular dystrophy, Ehlers-Danlos syndrome and corneal diseases, primarily due to abnormal collagen fiber formation (*272*).

Most SLRPs are enriched within the cartilage although a wide tissue distribution can be found for many SLRP-members. Fibromodulin and PRELP are found in addition to cartilage in tendon, sclera and aorta as well as less abundantly in several other tissues (*273*). PRELP is furthermore quite uniquely expressed in basement membranes, Bowman's capsule and testis. Osteoadherin is only expressed in bone (*274*) whereas chondroadherin is found in different types of cartilage, in bone and to a low extent in tendon (*275*). Decorin and biglycan are of the most abundantly expressed SLRPs, found among other tissues in cartilage, bone, skin, tendon, smooth muscle and cornea (*276, 277*).

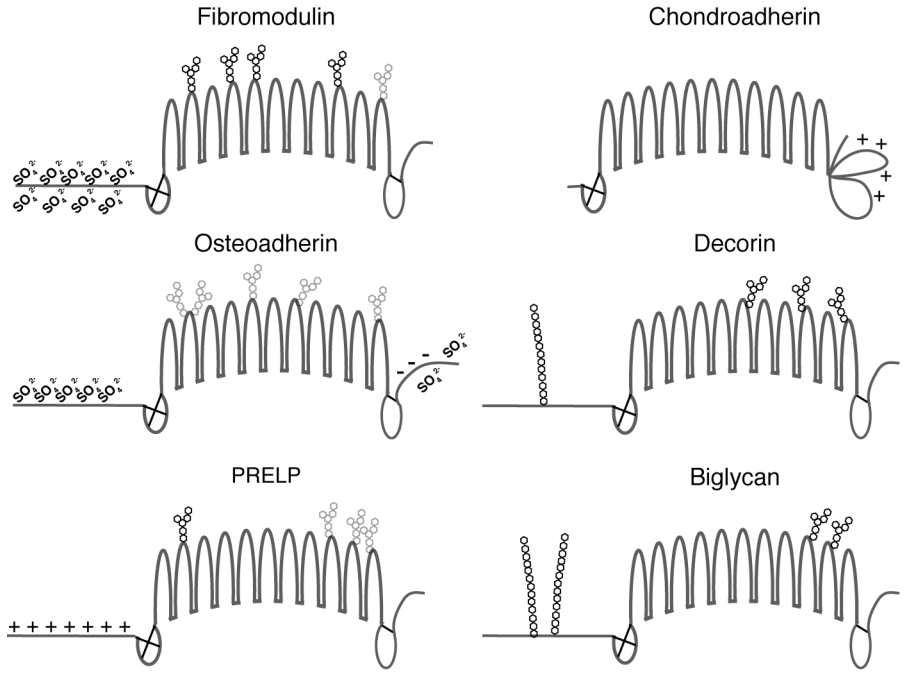

Figure 5. *The SLRPs.* Members of the SLRP-family contain a characteristic LRR-region, which is flanked in both the N- and C-termini by cystein loops. The main collagen-binding region lies within the LRR. The SLRPs have unique N- and C-terminal regions, which provide the molecules with their respective ligand-binding properties. Fibromodulin contains keratan sulfate chains in the LRR and 7-9 tyrosine sulfates in the N-terminal extension making this region highly acidic. Osteoadherin carries tyrosine sulfates in both the N- and C-terminal extensions. PRELP has a basic N-terminus, which is rich in proline and arginine residues. Chondroadherin lacks a N-terminal extension but has instead a bulky, positively charged C-terminus. Decorin and biglycan have one and two chondroitin sulfate or dermatan sulfate chains in their N-termini, respectively. Verified glycosylation sites are depicted in black, whereas glycosylation sites implicated by protein sequence are depicted in grey.

In addition to promoting proper assembly of extracellular matrices, novel functions have recently been designated SLRPs in regulating cellular responses. Studies on tumor cells showed that decorin binds the epidermal growth factor receptor thereby preventing receptor dimerization and induces receptor internalization. This is thought to lead to enhanced apoptosis and retarded growth of tumor cells. Decorin-deficient mice accordingly develop diverse tumors. However, on normal cells decorin may interact with the insulin-like growth factor receptor 1 to stimulate proliferation (reviewed in (*278*)). Biglycan binds on the other hand TLR2 and 4 on macrophages and triggers a robust inflammatory response (*279*). Therefore release of biglycan from the tissue has been suggested to represent a danger signal for tissue destruction. In addition, decorin, biglycan

and fibromodulin were shown to bind transforming growth factor-β (*280*) and are though to modulate its biological activity.

Some members of the SLRP family have in previous studies shown to also regulate complement activation; decorin and biglycan bind C1q and MBL and thereby inhibit the classical and lectin pathways (*207, 208*) whereas fibromodulin, osteoadherin and chondroadherin bind C1q and induce classical pathway complement activation (*11, 12*). The complement activators also bind factor H and thereby SLRP-induced complement activity downstream of C3-convertase is limited (*12*). In Paper III we studied the ability of different SLRPs to bind C4BP and found an interaction between C4BP and fibromodulin, osteoadherin, chondroadherin and PRELP. The main binding site for these SLRPs, as studied using deletion mutants lacking individual domains of C4BP as well as electron microscopy, was found to reside within the core of C4BP close to the polymerization site of the individual chains. This is highly interesting since the complement-regulating site of C4BP lies within the four outermost CCP-domains on the α-chains. Therefore we hypothesized that C4BP would maintain its complement regulatory function even in the presence of the SLRPs. Indeed, we found that C4BP still acted as a cofactor for factor I in the cleavage of C4b in the presence of the SLRPs in conditions where over 95% of C4BP should be bound to the SLRPs as calculated based on their specific dissociation coefficients. We also found that the difference between C9-deposition from C4BP-depleted serum compared to normal serum was much more pronounced on the SLRPs than on aggregated IgG, indicating that binding of C4BP to the SLRPs limit their ability to stimulate terminal pathway activation. Even though all of these SLRPs contain post-transcriptional modifications, also bacterially expressed counterparts wee found to bind C4BP and C1q. Therefore it seems that the actual polypeptide chain and not the glycosylations mediate the interactions.

Studies are currently undertaken to explore the binding sites of these complement proteins on the SLRPs. It would be of high interest to see whether the released SLRP fragments have the same complement regulating properties as the intact proteins and also whether binding sites for both complement initiators and inhibitors can be found on the same released fragment. Whether complement activation on the SLRPs is a factor mediating further joint inflammation or instead promoting the clearance of these released proteins is still unknown. Further studies are also needed to elucidate whether SLRP-mediated complement activation actually occurs *in vivo*. One approach would be to measure complexes between classical pathway-activating SLRPs and C4b since this interaction should be of covalent nature. This approach would be homologous to that of measuring COMP-C3b complexes.

In Paper IV we studied more closely the SLRP-member PRELP, a 58 kDa protein expressed in a variety of tissues but is also found abundantly in cartilage. PRELP is though to act as a linker molecule connecting different components of the extracellular matrix to basement membranes since it binds perlecan (*281*) or to cells since PRELP also

binds and heparin and heparan sulfate present on cell surfaces (*282*). PRELP differs from the other members of the SLRP-family in that it has an N-terminus rich in proline and arginine, which gives this region highly basic properties (*283*). Much attention has been given to the charged N-terminal region prior to the LRR-domain. The N-terminus mediates the interaction between PRELP and heparin and heparan sulfate (*282*) and peptides derived from this region also have bactericidal effects against certain pathogens (*284, 285*). Furthermore, this domain was shown to be a cell-type specific NF-κB inhibitor and thereby inhibit osteoclastogenesis (*286*).

In paper IV we could show that PRELP binds C9 and therefore inhibits its polymerization and the formation of a functional MAC. The binding of C9 to C5b-8 does not seem to be impaired, but the interaction between C9 and the neoepitope exposed on activated C9 is inhibited. We also found that PRELP inhibits the alternative pathway C3-convertase, either by binding directly to C3 or to the forming convertase itself. Therefore it seems that PRELP can inhibit complement in two ways, either by inhibiting the alternative pathway C3-convertase and thereby also the amplification loop of the classical and lectin pathways or by inhibiting formation of MAC.

The degradation and release of PRELP during normal cartilage turnover or during disease has not been studied in detail and whether fragments with complement regulatory properties actually are released from the tissue is still unknown. However, several studies have indicated that inhibition of MAC-formation would be protective for RA. $CD59^{-/-}$ mice show a more pronounce disease phenotype than their wild type counterparts in an antigen-induced arthritis (AIA) model (*287*). Furthermore, rats injected with a membrane-targeted rat CD59 derivative (sCD59-APT542) had a milder disease course in an AIA model than rats injected with PBS alone (*288*). Therefore the release of PRELP from cartilage might also have a protective effect and serve to downregulate pathological MAC-formation. A similar scenario could be occurring during the formation of atherosclerotic plaques. Recent studies have shown that additional deletion of CD59 in $ApoE^{-/-}$ mice accelerated the formation of atherosclerotic lesions and death in mice, an effect that could be attenuated by overexpression of CD59 in the endothelium or administration of C5-blocking antibodies (*289, 290*). PRELP has been found in atherosclerotic lesions together with several other extracellular matrix components, where it has been speculated to stabilize the plaque due to its collagen binding properties and regulate cell migration and proliferation (*291*). Possibly the presence of PRELP also limit the amount of deposited MAC in these lesions. It would be interesting to see whether PRELP and ApoE double knock-out mice have bigger plaques or more MAC deposition in their atherosclerotic lesions compared to PRELP-sufficient $ApoE^{-/-}$ mice.

Serglycin as a complement inhibitor

The expression of proteoglycans is abundant in various extracellular matrices and on cell surfaces and is by no means restricted to cartilage. In this thesis I have also studied a chondroitin sulfate proteoglycan, serglycin, and evaluated its effect on the complement system. Serglycin is a proteoglycan expressed by several hematopoietic cells, mast cells and endothelial cells (*292*). The GAG-chains linked to the core protein can vary depending on cell type and the degree of differentiation and extracellular stimulus, the main types being chondroitin-6-sulfate (CS-6), chondroitin-4-sulfate (CS-4), chondroitin sulfate-E (CS-E), dermatan sulfate (DS) or heparin (*293*). Stimulation of macrophages or monocytes with phorbol 12-myristate 13-acetate induces the expression of more highly sulfated CS (*294*) showing that inflammation and activation of these cells might influence the sulfation pattern of GAG-chains. Variations in GAG-substitution and the sulfation pattern of the GAG chains give the proteoglycan the possibility to interact with a plethora of ligands.

Serglycin is abundant intracellularly and has an important role in the maturation of secretory granules. Serglycin knock-out mice were shown to be viable and fertile but the generation of mast cell secretory granules was severely compromised, likewise to the secretory granules of cytotoxic T-cells (*295, 296*). Thereby it has been suggested that the biological function of serglycin is to direct and cluster molecules into secretory vesicles and upon release of these granules concentrate the secreted molecules in the extracellular environment (*293*). Regarding cytotoxic T-cells, it was shown that serglycin plays a role in the delivery of granzyme B into target cells thereby facilitating induction of target cell apoptosis (*297*).

Multiple myeloma (MM) is a disease characterized by a clonal proliferation of malignant plasma cells. The development of the disease relies both on multiple chromosomal changes as well as on alterations in the bone marrow microenvironment. Multiple myeloma is associated with an excess of plasma cells in the bone marrow, which produce non-functional immunoglobulins as well as with osteolytic bone lesions, renal disease and immunodeficiency. In most developing countries, the disease occurrence is around 4 cases per 100 000 people and it is more frequent in men than women, in afro-Americans than in Caucasians (*298*).

Serglycin is constitutively expressed by multiple myeloma (MM) cells, in which it can be found both intracellularly as well as bound to the cell surface. Also benign plasma cells secrete serglycin, but due to the increased proliferation of myeloma plasma cells and their constant secretion of serglycin, serum and bone marrow concentration of serglycin is elevated in patients with MM compared to healthy controls (*299, 300*). In paper V we studied the effect of serglycin on the complement system to see whether secretion of serglycin might protect MM cells from complement attack.

We found that serglycin, by binding C1q and MBL, inhibits both the classical and lectin pathways of complement. Interestingly, the interaction between C1q

and serglycin was severely diminished by the removal of GAG-chains linked to the protein core. The interaction could also be completely abolished by pre-incubating C1q with SG-derived GAG chains before adding the mixture to serglycin-coated plates. This shows that serglycin binds C1q mainly with its GAG-moieties. Serglycin can carry up to eight GAG chains, which in MM cells are mainly CS-4 (92.5%), CS-6 (4.5%) and low amounts of disulfated CS (1.5%) or non-sulfated CS (1.5%) (*299*). There seems to be a very high specificity in the GAG-C1q interaction, since isolated CS-4 and CS-6 on their own had non or a very weak impact on the binding of C1q to serglycin, whereas both serglycin-derived GAGs, heparin and CS-E inhibited the interaction readily. Therefore specific charge patterns formed by highly sulfated CS-units on the serglycin-attached GAGs are needed for an interaction to occur.

The interaction between MBL and SG was mediated both by the protein core of serglycin as well as the GAG-chains. In this case only serglycin-derived GAGs and CS-E were able to inhibit the interaction between MBL and serglycin whereas CS-4 and CS-6 had no inhibitory effect. The interaction could, however, not be completely abolished, not by adding excess GAGs nor by deglycosylating serglycin, which showed that also the protein core of serglycin binds MBL.

Since the GAG-substitutions on the serglycin molecules might vary depending on cell type and degree of differentiation or activation, it would be of high interest to see whether serglycin from other cell-types inhibits complement as well. So far only one study has been carried out with serglycin isolated from a breast cancer cell line. This serglycin also has the ability to bind both C1q and MBL and thereby inhibit the classical and lectin pathways (unpublished observation). Comparing serglycin secreted by malignant cells to that secreted by benign cells might give us insights to whether the complement inhibitory effect is gained upon cellular pathological changes or whether it is a normally occurring phenomena.

Other studies have also reported C1q-binding and complement inhibition by chondroitin sulfate proteoglycans. In 1997, Kirschfink and colleagues isolated a chondroitin sulfate proteoglycan from the U266 myeloma cell line, which showed the ability to bind and inhibit C1q via its GAG-chains (*301*). It is quite likely that this, at that time unidentified, proteoglycan was serglycin. Another C1q-inhibitor present in serum that was demonstrated to be a proteoglycan with mainly CS-4 (*302*) also bound and inhibited C1q, but this interaction was mediated by the protein core of the proteoglycan (*303*). Chondroitin sulfate secreted by platelets upon stimulation with thrombin receptor-activating peptide-6 on the other hand binds C1q and stimulates classical pathway activation (*304*). This shows that there is a high specificity for only particular chondroitin sulfates to actually bind and inhibit C1q.

Attempts to produce monoclonal antibodies targeting MM cells for therapeutic use have been disappointing due to the lack of specific antigens only found on malignant cells, and stem cell transplantation in combination with immunomodulatory

drugs still remains the most efficient treatment. T-cell depletion by anti-thymocyte globulins (ATG) can be used in the process of allogenic stem cell transplantation in MM patients to increase engraftment and reduce the risk of graft-versus-host disease (*305*). However, these ATG also cross react with B-cells and plasma cells since the cell preparation used for immunization contains low amounts of $CD20^+$and $CD138^+$ cells (*306*). Importantly, ATG has been shown to induce both complement independent and complement dependent death also in MM plasma cells (*307*).

Since serglycin is both present on the cell surface of MM cells as well as secreted to the surrounding matrix, we hypothesized that serglycin might partially protect these MM cells against ATG-induced classical pathway complement activation. Indeed, we found that extracellular serglycin inhibited ATG-induced complement deposition onto three different MM cell lines in a dose-dependent manner. We could also correlate the amount of cell-surface expression of serglycin to the complement resistance of the three different cell lines; U266-cells, which do not have cell surface serglycin, obtained high amounts of deposited C3b on their surface, whereas CAG and JJN3 cells, with strong staining for serglycin on the surface, were more resistant to C3b-deposition. This classical pathway down-regulation might influence the efficacy of future monoclonal antibody therapies against MM, which are under development.

Taken together, these data show that secretion of serglycin by MM cells might protect them from therapeutic inventions involving antibody-mediated complement activation and limit treatment outcome. This protection might be due to either higher local amounts of serglycin or secretion of a cell-type specific serglycin with the ability to regulate complement, but further investigations are needed to shed light on this matter.

Immune deficiency is commonly associated with MM and infections following a poor immunological response to a bacterial burden are often the cause of mortality. Numerous immunological defects have been found in MM but very little is known about the involvement of complement in the disease. A limited amount of studies have showed abnormalities in classical or alternative pathway complement components in the sera of patients with MM (*308, 309*). Furthermore, two cases of acquired C1q deficiency have been found in patients with multiple myeloma, one of them associated with a meningococcal infection (*310, 311*). One study also showed defective C3b-opsonization of various strains of *Streptococcus pneumoniae* in MM patients (*312*). How these complement abnormalities relate to MM or why they occur is not known, but defects in complement mediated pathogen recognition might contribute to the increased risk of infection in MM patients. If present at abnormally high amounts, serglycin might, by inhibiting complement, further promote immunodeficiency in the already immunocompromized MM patients.

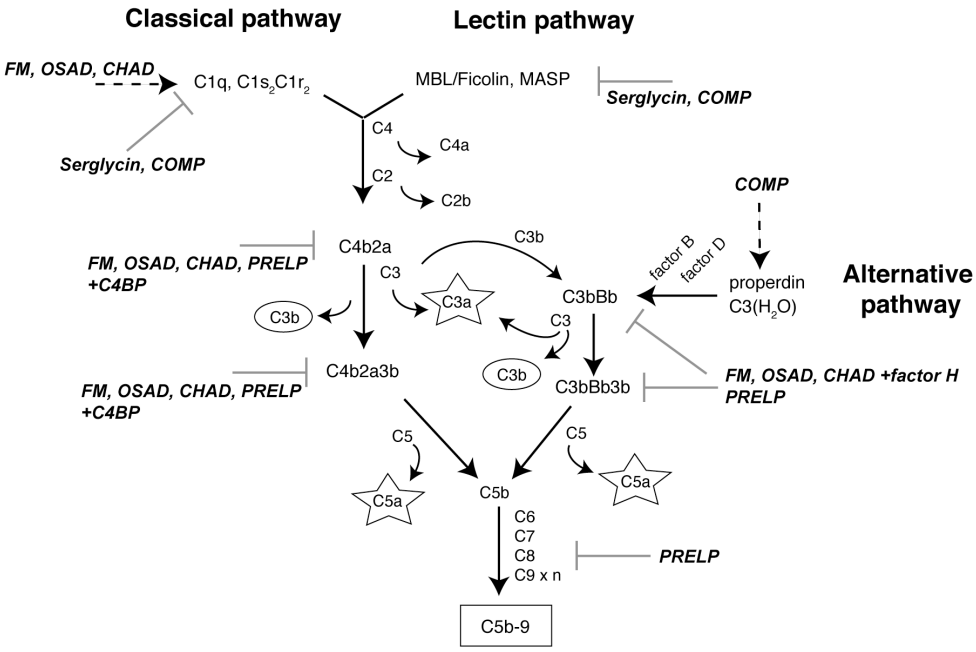

Figure 6. Summary of studied complement regulators. Fibromodulin, osteoadherin and chondroadherin bind C1q and activate the classical pathway, whereas they downregulate the classical and alternative C3-convertases by recruiting C4BP and factor H. PRELP inhibits directly the alternative C3-convertase as well as MAC-formation and binds C4BP. COMP binds C1q and MBL and inhibits the classical and lectin pathways whereas it activates the alternative pathway. Serglycin binds C1q and MBL and inhibits the classical and lectin pathways. CHAD, chondroadherin; FM, fibromodulin; OSAD, osteoadherin.

Major findings

- COMP inhibits the classical and lectin pathways of complement by binding MBL and C1q. COMP also activates the alternative pathway by binding properdin and C3.

- COMP-C3b complexes can be found in the circulation of patients with various rheumatic diseases, as an indication of COMP-induced complement activation *in vivo*.

- Fibromodulin, osteoadherin, chondroadherin and PRELP bind C4BP without disturbing its complement regulatory properties. This downregulates SLRP-induced complement activation downstream of the C3-convertases.

- PRELP inhibits complement by interfering with alternative pathway C3-convertase assembly and MAC-formation.

- Serglycin inhibits the classical and lectin pathways by binding C1q and MBL and thereby protects MM cells from complement attack.

Future perspectives

The fact that cartilage resident proteins are able to regulate complement is highly intriguing and the main question that arises is how this contributes to pathological joint inflammation. Since we have seen that full length purified SLRPs and COMP can bind various complement components it is clear that these effects are not only mediated by specific protein degradation fragments released during disease. To clarify the *in vivo* relevance of these findings it would be of outmost interest to see whether also fragments that are released by the activity of matrix proteases have the same complement modulatory function as the full-length proteins. Since we are constantly exposed to a certain degree of cartilage proteins due to tissue regeneration, some sort of tolerance need to have been built up towards these possibly pathogenic ligands. It is possible that this tolerance is broken upon an increased burden or by a changed microenvironment or alternatively by the exposition of epitopes normally hidden on the released proteins.

It is not clear yet whether SLRP-mediated complement activation drives inflammation or serves as a means to increase the clearance of these released protein fragments since complement activation downstream of C3-convertases is limited. SLRP-mediated complement activation should not result in a pronounced release of the anaphylatoxins C3a or C5a, which have and important role in several inflammatory conditions. Whether complement activation actually happens *in vivo* could be investigated by measuring complexes between complement activation products, such as C4b or C3b, and the relevant cartilage proteins. Since we have already demonstrated the presence of complexes between COMP and C3b in the serum and synovial fluid there is evidence that at least COMP does activate complement *in vivo*, however, the contribution of this to the actual pathology of joint inflammation is still unknown. Knowledge about the detailed complement binding sites on the different SLRPs or COMP would allow for the development of small inhibitory peptides that could specifically inhibit SLRP/COMP-mediated complement activation. Testing whether these kinds of peptides actually ameliorate disease would give valuable information on whether released SLRPs or COMP drive complement activation also in a clinical setting and in that case would provide new tools for complement directed therapy in RA. Using SLRP knock-out mice to study the role of specific SLRP proteins for complement activation in RA would most likely give misleading information since any modifications altering the structural stability of the cartilage might predispose to joint diseases. Furthermore, SLRPs are often functionally

redundant and single knock-out mice compensate the loss of one SLRP by upregulating another, thereby minimizing alterations in phenotype.

A further topic to study would be how the release of the SLRPs and COMP is related to different rheumatc diseases. We have already seen the presence of COMP-C3b complexes in several rheumatic diseases, albeit at various concentrations, indicating that the release or complement fixation of COMP is not solemnly related to the pathology of rheumatoid arthritis. Whether SLRP-C4b complexes would follow a similar pattern of prevalence in the different diseases could tell us something about the process of cartilage breakdown in various conditions. A highly interesting observation was the finding of COMP-C3b complexes also in the circulation of patients with OA. Even though OA has been shown to present with a low degree of inflammation, it has generally been considered to be a non-inflammatory joint disease. However, with accumulating evidence of several inflammatory components, the future might also bring novel anti-inflammatory therapies for the treatment of OA.

Autoantibodies are a major feature of RA. Whether any autoantibodies recognizing COMP or SLRPs are present in the sera of RA patients would be interesting to study. This would enhance the suspicion that these proteins are recognized by the immune system as danger signals. In rodent-models of COMP-induced arthritis a strong and specific antibody response is raised against the injected antigen, suggesting that this might also be the case in humans. Several proteins have also been found to be citrullinated during RA and antibodies against such proteins can be found in a highly specific manner in the circulation of a subset of RA patients. It would be interesting to see if SLRPs or COMP are citrullinated as a process of disease development, since this might influence their pathogenicity.

Most cancer cells have developed ways to protect themselves against complement attack. In addition to expressing membrane-bound complement inhibitors, such as CD59, MM cells express serglycin on their surface. Since we showed that serglycin inhibits antibody-mediated complement attack on myeloma cells, this would offer an extra means of protection against any antibody-mediated therapeutic approaches. However, since expression of serglycin is not restricted to malignant cells, it would be highly interesting to see whether serglycin expressed by benign plasma cells or mast cells have the same abilities. Due to the lower amount of serglycin-expression in normal cells it is unfortunately not trivial to purify serglycin from these sources. The contribution of an increased amount of circulating serglycin for the weakened immune response in MM would also be interesting to study. However, in a multi-component disease like MM, it is unlikely that a single deciding factor is present; instead the observed immune dysfunction is a combined effect of several parameters possibly including aberrant complement function.

Populärvetenskaplig sammanfattning

Immunförsvaret är främst till för att skydda oss mot utomstående hot som bakterier och virus men har även många viktiga roller i andra kroppsfunktioner. Immunförsvaret kan indelas i två grenar, det medfödda och det förvärvade. Det förvärvade immunförsvaret utvecklas under hela livstiden och har en minnesfunktion. Till denna gren tillhör bland annat celler som producerar antikroppar gentemot strukturer som kroppen känner igen som främmande och på så sätt markerar dessa strukturer så att andra immunceller kan oskadliggöra dem. Till det medfödda immunsystemet tillhör olika fagocyterande celler, det vill säga celler som specialiserar sig på att äta upp och på så sätt förstöra möjliga hot. Hit hör även molekyler som känner igen oföränderliga strukturer på bakterier eller andra ytor och kan signalera andra delar av immunförsvaret om fara. Ett av dessa molekylära system är komplementsystemet.

Komplementsystemet består av närmare 40 proteiner som cirkulerar i blodet som inaktiva molekyler och som efter rätt stimulans aktiveras i en kaskad, där ett aktiverat protein klyver det nästa som i och med detta aktiveras o.s.v. Komplement kan aktiveras genom tre olika vägar, den klassiska vägen, lektinvägen och den alternativa vägen. Den klassiska vägen aktiveras då C1q binder till bland annat antikroppar, vissa bakterier eller kroppens egna döende celler. Lektinvägen aktiveras då MBL känner igen vissa kolhydratstrukturer på bakterier. Den alternativa vägen aktiveras konstant till en låg grad men kan rikta sig till speciella ytor med hjälp av properdin. Komplementaktivering leder till att nya klyvningsfragment av komplementkomponenter med olika biologiska funktioner frigörs. Vissa fragment stimulerar inflammation, andra märker in den igenkända ytan och förstärker därmed antikroppsproduktionen mot denna komplementaktiverande struktur. Komplement kan även direkt döda celler genom att bilda en por i cellmembranen.

Kroppens egna vävnader skyddas mot komplement med hjälp av olika lösliga och cellbundna komplementhämmare. Till dessa hör bl.a. C4b-bindande protein (C4BP) och faktor H som cirkulerar i blodet. Ofullständigt reglerad komplementaktivering kan leda till sjukdomar såsom ledgångsreumatism eller blodförgiftning. I denna avhandling har jag studerat vad som bidrar till komplementaktivering i lederna i reumatism och även i relaterade ledsjukdomar såsom osteoartrit.

Benytorna i lederna är täckta med ett tunt lager brosk som är till för att ge en glapp yta så benen kan röra sig mot varandra och fungerar framförallt som en stötdämpare i leden. I vissa sjukdomar förstörs detta brosklager och molekyler som normalt funnits skyddade i brosket frigörs i ledvätskan där det finns komplementproteiner. Många studier har påvisat att komplementaktivering sker i lederna hos patienter med reumatism och detta tros bidra till den starka ledinflammationen som patienterna har. Två tidigare studier har visat att vissa broskproteiner tillhörande den s.k. SLRP-familjen kan aktivera komplement genom att binda C1q. Detta tros bidra till komplementaktiveringen i lederna även hos patienter. Därutöver kan samma SLRPar även binda komplementhämmaren faktor H och i denna avhandling har vi nu påvisat att samma SLRPar även binder C4BP. Ifall detta leder till att minimera den inflammation som proteinerna orsakar i kroppen är fortfarande oklart.

Vi har även studerat ett annat broskprotein, COMP, och observerat att COMP kan aktivera komplement genom den alternativa vägen. Vi kunde dessutom påvisa att detta händer i lederna hos patienter med olika reumatologiska sjukdomar och första gången påvisas därmed en *in vivo*-relevans av broskprotein-inducerad komplementaktivering.

En annan studie tillhörande denna avhandling undersökte SLRP-proteinet PRELP och dess komplementreglerande effekt. Vi kunde påvisa att PRELP hämmar komplement från att bilda porer i cellmembraner och skyddar därmed celler från komplementinducerad celldöd. Därutöver hämmar PRELP den alternativa vägen även på ett tidigare stadium i komplementkaskaden. Eftersom det har visat sig att många faktorer som kan bidra till komplementreglering finns i lederna och frigörs under sjukdomstillstånd verkar det som om den slutliga effekten på immunreglering beror på ett samspel mellan alla dessa komponenter.

Den sista studien i avhandlingen handlar om komplement och cancer. Då vanliga celler förändras och blir cancerceller ändrar de på sin ytstruktur. Detta gör att immunförsvaret, däri även komplementsystemet, känner igen dessa celler som främmande och vill oskadliggöra dem. Cancerceller har däremot flera sätt att skydda sig mot immunförsvaret och komplement, bl.a. kan de uttrycka extra höga nivåer av komplementhämmare på sin yta. I ett av delarbetena i denna avhandling har vi studerat ett protein, serglycin, vilket utsöndras av myelomceller som är en typ av blodcancer. Vi visade att serglycin kan hämma komplement genom att binda C1q och MBL och förhindra dem från att starta komplementaktiveringskaskaden. Vi kunde påvisa att myelomceller inte attackeras lika mycket av komplement i närvaro av serglycin och såg att de myelomceller som hade mest serglycin på sin yta var i sig själva mycket mer skyddade mot komplement än celler med mindre serglycin på ytan. En del nya läkemedel mot cancer syftar till att förstärka komplementaktivering specifikt på cancerceller, och vi tror att serglycin som myelomcellerna utsöndrar försvagar denna typ av terapi just för behandlingen av myelom.

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References

1. Bordet, J. (1896) Sur le mode d'action des sérums préventifs., *Ann Inst Pasteur 10*, 193-219.
2. Nuttal, G. H. F. (1888) Experimente übey die bakterienfeindlichen Einflüsse des Tierischen Körpers., *Z Hyg Infectionskr 4*, 353-356.
3. Buchner, H. (1889) Über die bakterientötende Wirkung des zellfreien Blutserums, *Zentralbl Bakterial 6*, 1-11.
4. 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 (New York, N.Y 271*, 348-350.
5. Wolbink, G. J., Brouwer, M. C., Buysmann, S., ten Berge, I. J., and Hack, C. E. (1996) CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes, *J Immunol 157*, 473-479.
6. Arlaud, G. J., Gaboriaud, C., Thielens, N. M., Rossi, V., Bersch, B., Hernandez, J. F., and Fontecilla-Camps, J. C. (2001) Structural biology of C1: dissection of a complex molecular machinery, *Immunological reviews 180*, 136-145.
7. Sellar, G. C., Cockburn, D., and Reid, K. B. (1992) Localization of the gene cluster encoding the A, B, and C chains of human C1q to 1p34.1-1p36.3, *Immunogenetics 35*, 214-216.
8. MacKenzie, M. R., Creevy, N., and Heh, M. (1971) The interaction of human IgM and C1q, *J Immunol 106*, 65-68.
9. Linscott, W. D. (1970) Complement fixation: the effects of IgG and IgM antibody concentration on C1-binding affinity, *J Immunol 105*, 1013-1023.
10. Volanakis, J. E. (1982) Complement activation by C-reactive protein complexes, *Annals of the New York Academy of Sciences 389*, 235-250.
11. 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, *The Journal of biological chemistry 280*, 32301-32308.
12. Sjöberg, A. P., Manderson, G. A., Mörgelin, M., Day, A. J., Heinegård, D., and Blom, A. M. (2009) Short leucine-rich glycoproteins of the extracellular matrix

display diverse patterns of complement interaction and activation, *Molecular immunology 46*, 830-839.

13. 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.

14. Naff, G. B., and Ratnoff, O. S. (1968) The enzymatic nature of C'1r. Conversion of C'1s to C'1 esterase and digestion of amino acid esters by C'1r, *The Journal of experimental medicine 128*, 571-593.

15. Lepow, I. H., Naff, G. B., Todd, E. W., Pensky, J., and Hinz, C. F. (1963) Chromatographic resolution of the first component of human complement into three activities, *The Journal of experimental medicine 117*, 983-1008.

16. Ziccardi, R. J., and Cooper, N. R. (1978) Modulation of the antigenicity of C1r and C1s by C1 inactivator, *J Immunol 121*, 2148-2152.

17. Law, S. K., Lichtenberg, N. A., Holcombe, F. H., and Levine, R. P. (1980) Interaction between the labile binding sites of the fourth (C4) and fifth (C5) human complement proteins and erythrocyte cell membranes, *J Immunol 125*, 634-639.

18. Ip, W. K., Takahashi, K., Ezekowitz, R. A., and Stuart, L. M. (2009) Mannose-binding lectin and innate immunity, *Immunological reviews 230*, 9-21.

19. Nauta, A. J., Raaschou-Jensen, N., Roos, A., Daha, M. R., Madsen, H. O., Borrias-Essers, M. C., Ryder, L. P., Koch, C., and Garred, P. (2003) Mannose-binding lectin engagement with late apoptotic and necrotic cells, *European journal of immunology 33*, 2853-2863.

20. Matsushita, M. (2009) Ficolins: complement-activating lectins involved in innate immunity, *Journal of innate immunity 2*, 24-32.

21. Matsushita, M., Thiel, S., Jensenius, J. C., Terai, I., and Fujita, T. (2000) Proteolytic activities of two types of mannose-binding lectin-associated serine protease, *J Immunol 165*, 2637-2642.

22. Dahl, M. R., Thiel, S., Matsushita, M., Fujita, T., Willis, A. C., Christensen, T., Vorup-Jensen, T., and Jensenius, J. C. (2001) MASP-3 and its association with distinct complexes of the mannan-binding lectin complement activation pathway, *Immunity 15*, 127-135.

23. Takahashi, M., Endo, Y., Fujita, T., and Matsushita, M. (1999) A truncated form of mannose-binding lectin-associated serine protease (MASP)-2 expressed by alternative polyadenylation is a component of the lectin complement pathway, *International immunology 11*, 859-863.

24. Degn, S. E., Hansen, A. G., Steffensen, R., Jacobsen, C., Jensenius, J. C., and Thiel, S. (2009) MAp44, a human protein associated with pattern recognition

molecules of the complement system and regulating the lectin pathway of complement activation, *J Immunol 183*, 7371-7378.

25. Tack, B. F., Harrison, R. A., Janatova, J., Thomas, M. L., and Prahl, J. W. (1980) Evidence for presence of an internal thiolester bond in third component of human complement, *Proceedings of the National Academy of Sciences of the United States of America 77*, 5764-5768.

26. Pangburn, M. K., and Muller-Eberhard, H. J. (1983) Initiation of the alternative complement pathway due to spontaneous hydrolysis of the thioester of C3, *Annals of the New York Academy of Sciences 421*, 291-298.

27. Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., and Wardlaw, A. C. (1954) The properdin system and immunity. I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena, *Science (New York, N.Y 120*, 279-285.

28. Hourcade, D. E. (2006) The role of properdin in the assembly of the alternative pathway C3 convertases of complement, *The Journal of biological chemistry 281*, 2128-2132.

29. Spitzer, D., Mitchell, L. M., Atkinson, J. P., and Hourcade, D. E. (2007) Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly, *J Immunol 179*, 2600-2608.

30. Fearon, D. T., and Austen, K. F. (1975) Properdin: binding to C3b and stabilization of the C3b-dependent C3 convertase, *The Journal of experimental medicine 142*, 856-863.

31. 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, *Proceedings of the National Academy of Sciences of the United States of America 105*, 9023-9028.

32. 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.

33. Pangburn, M. K. (1989) Analysis of the natural polymeric forms of human properdin and their functions in complement activation, *J Immunol 142*, 202-207.

34. Ferreira, V. P., Cortes, C., and Pangburn, M. K. (2010) Native polymeric forms of properdin selectively bind to targets and promote activation of the alternative pathway of complement, *Immunobiology 215*, 932-940.

35. Matsushita, M., and Okada, H. (1986) Alternative complement pathway activation by C4b deposited during classical pathway activation, *J Immunol 136*, 2994-2998.

36. May, J. E., and Frank, M. M. (1973) A new complement-mediated cytolytic mechanism--the C1-bypass activation pathway, *Proceedings of the National Academy of Sciences of the United States of America 70*, 649-652.

37. Takahashi, M., Ishida, Y., Iwaki, D., Kanno, K., Suzuki, T., Endo, Y., Homma, Y., and Fujita, T. (2010) Essential role of mannose-binding lectin-associated serine protease-1 in activation of the complement factor D, *The Journal of experimental medicine 207*, 29-37.

38. Podack, E. R., Esser, A. F., Biesecker, G., and Muller-Eberhard, H. J. (1980) Membrane attack complex of complement: a structural analysis of its assembly, *The Journal of experimental medicine 151*, 301-313.

39. Podack, E. R., Tschoop, J., and Muller-Eberhard, H. J. (1982) Molecular organization of C9 within the membrane attack complex of complement. Induction of circular C9 polymerization by the C5b-8 assembly, *The Journal of experimental medicine 156*, 268-282.

40. Huber-Lang, M., Sarma, J. V., Zetoune, F. S., Rittirsch, D., Neff, T. A., McGuire, S. R., Lambris, J. D., Warner, R. L., Flierl, M. A., Hoesel, L. M., Gebhard, F., Younger, J. G., Drouin, S. M., Wetsel, R. A., and Ward, P. A. (2006) Generation of C5a in the absence of C3: a new complement activation pathway, *Nature medicine 12*, 682-687.

41. Köhl, J. (2006) Self, non-self, and danger: a complementary view, *Advances in experimental medicine and biology 586*, 71-94.

42. Fällman, M., Andersson, R., and Andersson, T. (1993) Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles, *J Immunol 151*, 330-338.

43. Bohlson, S. S., Fraser, D. A., and Tenner, A. J. (2007) Complement proteins C1q and MBL are pattern recognition molecules that signal immediate and long-term protective immune functions, *Molecular immunology 44*, 33-43.

44. Köhl, J. (2001) Anaphylatoxins and infectious and non-infectious inflammatory diseases, *Molecular immunology 38*, 175-187.

45. Peng, Q., Li, K., Anderson, K., Farrar, C. A., Lu, B., Smith, R. A., Sacks, S. H., and Zhou, W. (2008) Local production and activation of complement up-regulates the allostimulatory function of dendritic cells through C3a-C3aR interaction, *Blood 111*, 2452-2461.

46. Peng, Q., Li, K., Wang, N., Li, Q., Asgari, E., Lu, B., Woodruff, T. M., Sacks, S. H., and Zhou, W. (2009) Dendritic cell function in allostimulation is modulated by C5aR signaling, *J Immunol 183*, 6058-6068.

47. Lalli, P. N., Strainic, M. G., Yang, M., Lin, F., Medof, M. E., and Heeger, P. S. (2008) Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis, *Blood 112*, 1759-1766.

48. Strainic, M. G., Liu, J., Huang, D., An, F., Lalli, P. N., Muqim, N., Shapiro, V. S., Dubyak, G. R., Heeger, P. S., and Medof, M. E. (2008) Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells, *Immunity 28*, 425-435.

49. Sacks, S. H. Complement fragments C3a and C5a: the salt and pepper of the immune response, *European journal of immunology 40*, 668-670.

50. Johswich, K., and Klos, A. (2007) C5L2--an anti-inflammatory molecule or a receptor for acylation stimulating protein (C3a-desArg)?, *Advances in experimental medicine and biology 598*, 159-180.

51. Würzner, R., Orren, A., and Lachmann, P. J. (1992) Inherited deficiencies of the terminal components of human complement, *Immunodeficiency reviews 3*, 123-147.

52. Podack, E. R., Deyev, V., and Shiratsuchi, M. (2007) Pore formers of the immune system, *Advances in experimental medicine and biology 598*, 325-341.

53. Morgan, B. P., Dankert, J. R., and Esser, A. F. (1987) Recovery of human neutrophils from complement attack: removal of the membrane attack complex by endocytosis and exocytosis, *J Immunol 138*, 246-253.

54. Parker, C. J. (2007) The pathophysiology of paroxysmal nocturnal hemoglobinuria, *Experimental hematology 35*, 523-533.

55. Fosbrink, M., Niculescu, F., and Rus, H. (2005) The role of c5b-9 terminal complement complex in activation of the cell cycle and transcription, *Immunologic research 31*, 37-46.

56. Fosbrink, M., Niculescu, F., Rus, V., Shin, M. L., and Rus, H. (2006) C5b-9-induced endothelial cell proliferation and migration are dependent on Akt inactivation of forkhead transcription factor FOXO1, *The Journal of biological chemistry 281*, 19009-19018.

57. Gigli, I., Fujita, T., and Nussenzweig, V. (1979) Modulation of the classical pathway C3 convertase by plasma proteins C4 binding protein and C3b inactivator, *Proceedings of the National Academy of Sciences of the United States of America 76*, 6596-6600.

58. Rawal, N., and Pangburn, M. K. (2007) Role of the C3b-binding site on C4b-binding protein in regulating classical pathway C5 convertase, *Molecular immunology 44*, 1105-1114.

59. Scharfstein, J., Ferreira, A., Gigli, I., and Nussenzweig, V. (1978) Human C4-binding protein. I. Isolation and characterization, *The Journal of experimental medicine 148*, 207-222.

60. Blom, A. M., Kask, L., and Dahlbäck, B. (2003) CCP1-4 of the C4b-binding protein alpha-chain are required for factor I mediated cleavage of complement factor C3b, *Molecular immunology 39*, 547-556.

61. Dahlbäck, B., and Stenflo, J. (1981) High molecular weight complex in human plasma between vitamin K-dependent protein S and complement component C4b-binding protein, *Proceedings of the National Academy of Sciences of the United States of America 78*, 2512-2516.

62. Garcia de Frutos, P., Alim, R. I., Härdig, Y., Zoller, B., and Dahlbäck, B. (1994) Differential regulation of alpha and beta chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S, *Blood 84*, 815-822.

63. Blom, A. M., Kask, L., and Dahlbäck, B. (2001) Structural requirements for the complement regulatory activities of C4BP, *The Journal of biological chemistry 276*, 27136-27144.

64. Blom, A. M., Villoutreix, B. O., and Dahlback, B. (2004) Complement inhibitor C4b-binding protein-friend or foe in the innate immune system?, *Molecular immunology 40*, 1333-1346.

65. Trouw, L. A., Bengtsson, A. A., Gelderman, K. A., Dahlback, 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, *The Journal of biological chemistry 282*, 28540-28548.

66. 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, *The Journal of experimental medicine 201*, 1937-1948.

67. Sjöberg, A. P., Trouw, L. A., McGrath, F. D., Hack, C. E., and Blom, A. M. (2006) Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein, *J Immunol 176*, 7612-7620.

68. 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, *J Immunol 182*, 1518-1525.

69. Schmidt, C. Q., Herbert, A. P., Hocking, H. G., Uhrin, D., and Barlow, P. N. (2008) Translational mini-review series on complement factor H: structural and functional correlations for factor H, *Clinical and experimental immunology 151*, 14-24.

70. Pangburn, M. K., Schreiber, R. D., and Muller-Eberhard, H. J. (1977) Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein beta1H for cleavage of C3b and C4b in solution, *The Journal of experimental medicine 146*, 257-270.

71. Zipfel, P. F., and Skerka, C. (1999) FHL-1/reconectin: a human complement and immune regulator with cell-adhesive function, *Immunology today 20*, 135-140.

72. Heinen, S., Hartmann, A., Lauer, N., Wiehl, U., Dahse, H. M., Schirmer, S., Gropp, K., Enghardt, T., Wallich, R., Halbich, S., Mihlan, M., Schlotzer-Schrehardt, U., Zipfel, P. F., and Skerka, C. (2009) Factor H-related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation, *Blood 114*, 2439-2447.

73. Eberhardt, H. E., Chen, Q., Zipfel, P. F., and Skerka, C. (2011) Human complement factor H-related protein 2 (CFHR2) represents a novel complement regulator, which is reduced in a patient with MPGN I, *Molecular Immunlogy 48*, 1674.

74. Hellwage, J., Jokiranta, T. S., Koistinen, V., Vaarala, O., Meri, S., and Zipfel, P. F. (1999) Functional properties of complement factor H-related proteins FHR-3 and FHR-4: binding to the C3d region of C3b and differential regulation by heparin, *FEBS letters 462*, 345-352.

75. McRae, J. L., Duthy, T. G., Griggs, K. M., Ormsby, R. J., Cowan, P. J., Cromer, B. A., McKinstry, W. J., Parker, M. W., Murphy, B. F., and Gordon, D. L. (2005) Human factor H-related protein 5 has cofactor activity, inhibits C3 convertase activity, binds heparin and C-reactive protein, and associates with lipoprotein, *J Immunol 174*, 6250-6256.

76. Goicoechea de Jorge, E., Harris, C. L., Patel, M. P., and Pickering, M. C. (2011) Differential binding of CFHR5 and factor H to C3 metabolites suggests that these two proteins regulate complement activation at different stages, *Molecular Immunlogy 48*, 1675.

77. Medof, M. E., Kinoshita, T., and Nussenzweig, V. (1984) Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes, *The Journal of experimental medicine 160*, 1558-1578.

78. Seya, T., Turner, J. R., and Atkinson, J. P. (1986) Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b, *The Journal of experimental medicine 163*, 837-855.

79. Ahearn, J. M., and Fearon, D. T. (1989) Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21), *Advances in immunology 46*, 183-219.

80. Ross, G. D., Lambris, J. D., Cain, J. A., and Newman, S. L. (1982) Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR1 cofactor activity, *J Immunol 129*, 2051-2060.

81. Medof, M. E., Iida, K., Mold, C., and Nussenzweig, V. (1982) Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes, *The Journal of experimental medicine 156*, 1739-1754.

82. Rollins, S. A., and Sims, P. J. (1990) The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9, *J Immunol 144*, 3478-3483.

83. Ninomiya, H., and Sims, P. J. (1992) The human complement regulatory protein CD59 binds to the alpha-chain of C8 and to the "b"domain of C9, *The Journal of biological chemistry 267*, 13675-13680.

84. Tschopp, J., Chonn, A., Hertig, S., and French, L. E. (1993) Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 beta, and the b domain of C9, *J Immunol 151*, 2159-2165.

85. Podack, E. R., Kolb, W. P., and Muller-Eberhard, H. J. (1978) The C5b-6 complex: formation, isolation, and inhibition of its activity by lipoprotein and the S-protein of human serum, *J Immunol 120*, 1841-1848.

86. Dahlbäck, B., and Podack, E. R. (1985) Characterization of human S protein, an inhibitor of the membrane attack complex of complement. Demonstration of a free reactive thiol group, *Biochemistry 24*, 2368-2374.

87. Daha, M. R., Fearon, D. T., and Austen, K. F. (1976) C3 nephritic factor (C3NeF): stabilization of fluid phase and cell-bound alternative pathway convertase, *J Immunol 116*, 1-7.

88. Appel, G. B., Cook, H. T., Hageman, G., Jennette, J. C., Kashgarian, M., Kirschfink, M., Lambris, J. D., Lanning, L., Lutz, H. U., Meri, S., Rose, N. R., Salant, D. J., Sethi, S., Smith, R. J., Smoyer, W., Tully, H. F., Tully, S. P., Walker, P., Welsh, M., Wurzner, R., and Zipfel, P. F. (2005) Membranoproliferative glomerulonephritis type II (dense deposit disease): an update, *J Am Soc Nephrol 16*, 1392-1403.

89. Sim, R. B., Arlaud, G. J., and Colomb, M. G. (1979) C1 inhibitor-dependent dissociation of human complement component C1 bound to immune complexes, *The Biochemical journal 179*, 449-457.

90. Ziccardi, R. J. (1985) Demonstration of the interaction of native C1 with monomeric immunoglobulins and C1 inhibitor, *J Immunol 134*, 2559-2563.

91. Folkerd, E. J., Gardner, B., and Hughes-Jones, N. C. (1980) The relationship between the binding ability and the rate of activation of the complement component C1, *Immunology 41*, 179-185.

92. Davis, A. E., 3rd, Mejia, P., and Lu, F. (2008) Biological activities of C1 inhibitor, *Molecular immunology 45*, 4057-4063.

93. Kaplan, A. P., and Ghebrehiwet, B. (2010) The plasma bradykinin-forming pathways and its interrelationships with complement, *Molecular immunology 47*, 2161-2169.

94. Campbell, W., Okada, N., and Okada, H. (2001) Carboxypeptidase R is an inactivator of complement-derived inflammatory peptides and an inhibitor of fibrinolysis, *Immunological reviews 180*, 162-167.

95. Pickering, M. C., and Walport, M. J. (2000) Links between complement abnormalities and systemic lupus erythematosus, *Rheumatology (Oxford, England) 39*, 133-141.

96. Turner, M. W. (2003) The role of mannose-binding lectin in health and disease, *Molecular immunology 40*, 423-429.

97. Fijen, C. A., van den Bogaard, R., Schipper, M., Mannens, M., Schlesinger, M., Nordin, F. G., Dankert, J., Daha, M. R., Sjoholm, A. G., Truedsson, L., and Kuijper, E. J. (1999) Properdin deficiency: molecular basis and disease association, *Molecular immunology 36*, 863-867.

98. Pettigrew, H. D., Teuber, S. S., and Gershwin, M. E. (2009) Clinical significance of complement deficiencies, *Annals of the New York Academy of Sciences 1173*, 108-123.

99. Pickering, M. C., and Cook, H. T. (2008) Translational mini-review series on complement factor H: renal diseases associated with complement factor H: novel insights from humans and animals, *Clinical and experimental immunology 151*, 210-230.

100. Noris, M., and Remuzzi, G. (2009) Atypical hemolytic-uremic syndrome, *The New England journal of medicine 361*, 1676-1687.

101. Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, K. L., Kwan, S. Y., Noureddine, M., Gilbert, J. R., Schnetz-Boutaud, N., Agarwal, A., Postel, E. A., and Pericak-Vance, M. A. (2005) Complement factor H variant increases the risk of age-related macular degeneration, *Science (New York, N.Y 308*, 419-421.

102. Kaplan, A. P. (2010) Enzymatic pathways in the pathogenesis of hereditary angioedema: the role of C1 inhibitor therapy, *The Journal of allergy and clinical immunology 126*, 918-925.

103. Blom, A. M., Bergström, F., Edey, M., Diaz-Torres, M., Kavanagh, D., Lampe, A., Goodship, J. A., Strain, L., Moghal, N., McHugh, M., Inward, C., Tomson, C., Fremeaux-Bacchi, V., Villoutreix, B. O., and Goodship, T. H. (2008) A novel non-synonymous polymorphism (p.Arg240His) in C4b-binding protein is associated with atypical hemolytic uremic syndrome and leads to impaired alternative pathway cofactor activity, *J Immunol 180*, 6385-6391.

104. Hart, M. L., Walsh, M. C., and Stahl, G. L. (2004) Initiation of complement activation following oxidative stress. In vitro and in vivo observations, *Molecular immunology 41*, 165-171.

105. Okroj, M., Heinegård, D., Holmdahl, R., and Blom, A. M. (2007) Rheumatoid arthritis and the complement system, *Annals of medicine 39*, 517-530.

106. Oksjoki, R., Kovanen, P. T., Meri, S., and Pentikainen, M. O. (2007) Function and regulation of the complement system in cardiovascular diseases, *Front Biosci 12*, 4696-4708.

107. Speidl, W. S., Kastl, S. P., Huber, K., and Wojta, J. (2011) Complement in atherosclerosis: friend or foe?, *J Thromb Haemost 9*, 428-440.

108. Zhang, X., and Köhl, J. (2010) A complex role for complement in allergic asthma, *Expert review of clinical immunology 6*, 269-277.

109. Sjöberg, A. P., Trouw, L. A., and Blom, A. M. (2009) Complement activation and inhibition: a delicate balance, *Trends in immunology 30*, 83-90.

110. Mabbott, N. A. (2004) The complement system in prion diseases, *Current opinion in immunology 16*, 587-593.

111. Islander, U., Jochems, C., Lagerquist, M. K., Forsblad-d'Elia, H., and Carlsten, H. (2010) Estrogens in rheumatoid arthritis; the immune system and bone, *Molecular and cellular endocrinology 335*, 14-29.

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

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

114. Heinegård, D. (2009) Proteoglycans and more--from molecules to biology, *International journal of experimental pathology 90*, 575-586.

115. Burrage, P. S., Mix, K. S., and Brinckerhoff, C. E. (2006) Matrix metalloproteinases: role in arthritis, *Front Biosci 11*, 529-543.

116. Yoshihara, Y., Nakamura, H., Obata, K., Yamada, H., Hayakawa, T., Fujikawa, K., and Okada, Y. (2000) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis, *Ann Rheum Dis 59*, 455-461.

117. Cawston, T. E., and Young, D. A. (2010) Proteinases involved in matrix turnover during cartilage and bone breakdown, *Cell and tissue research 339*, 221-235.

118. Mitchell, P. G., Magna, H. A., Reeves, L. M., Lopresti-Morrow, L. L., Yocum, S. A., Rosner, P. J., Geoghegan, K. F., and Hambor, J. E. (1996) Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage, *The Journal of clinical investigation 97*, 761-768.

119. 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, *The Journal of biological chemistry 282*, 36933-36941.

120. Fosang, A. J., Last, K., Knauper, V., Murphy, G., and Neame, P. J. (1996) Degradation of cartilage aggrecan by collagenase-3 (MMP-13), *FEBS letters 380*, 17-20.

121. 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, *The Journal of biological chemistry 279*, 6286-6295.

122. Ganu, V., Goldberg, R., Peppard, J., Rediske, J., Melton, R., Hu, S. I., Wang, W., Duvander, C., and Heinegard, 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 and rheumatism 41*, 2143-2151.

123. Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J. P., and Fahmi, H. (2010) Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, *Nature reviews 7*, 33-42.

124. Saklatvala, J. (1986) Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage, *Nature 322*, 547-549.

125. Tyler, J. A. (1985) Articular cartilage cultured with catabolin (pig interleukin 1) synthesizes a decreased number of normal proteoglycan molecules, *The Biochemical journal 227*, 869-878.

126. Asgari, E., Sacks, S., Perucha, E., Köhl, J., and Kemper, C. (2011) C3a drives Th17 lineage decisions in humans via induction of IL-1beta production in monocytes, *Molecular Immunlogy 48*, 1671.

127. Aikawa, Y., Morimoto, K., Yamamoto, T., Chaki, H., Hashiramoto, A., Narita, H., Hirono, S., and Shiozawa, S. (2008) Treatment of arthritis with a selective inhibitor of c-Fos/activator protein-1, *Nature biotechnology 26*, 817-823.

128. Song, R. H., Tortorella, M. D., Malfait, A. M., Alston, J. T., Yang, Z., Arner, E. C., and Griggs, D. W. (2007) Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5, *Arthritis and rheumatism 56*, 575-585.

129. 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.

130. Melching, L. I., Fisher, W. D., Lee, E. R., Mort, J. S., and Roughley, P. J. (2006) The cleavage of biglycan by aggrecanases, *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society 14*, 1147-1154.

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

132. Liu, C. J., Kong, W., Xu, K., Luan, Y., Ilalov, K., Sehgal, B., Yu, S., Howell, R. D., and Di Cesare, P. E. (2006) ADAMTS-12 associates with and degrades cartilage oligomeric matrix protein, *The Journal of biological chemistry 281*, 15800-15808.

133. Lin, E. A., and Liu, C. J. (2010) The role of ADAMTSs in arthritis, *Protein & cell 1*, 33-47.

134. Imada, K., Lin, N., Liu, C., Lu, A., Chen, W., Yano, M., Sato, T., and Ito, A. (2008) Nobiletin, a citrus polymethoxy flavonoid, suppresses gene expression and production of aggrecanases-1 and -2 in collagen-induced arthritic mice, *Biochemical and biophysical research communications 373*, 181-185.

135. Majumdar, M. K., Askew, R., Schelling, S., Stedman, N., Blanchet, T., Hopkins, B., Morris, E. A., and Glasson, S. S. (2007) Double-knockout of ADAMTS-4 and ADAMTS-5 in mice results in physiologically normal animals and prevents the progression of osteoarthritis, *Arthritis and rheumatism 56*, 3670-3674.

136. Sandy, J. D., Gamett, D., Thompson, V., and Verscharen, C. (1998) Chondrocyte-mediated catabolism of aggrecan: aggrecanase-dependent cleavage induced by interleukin-1 or retinoic acid can be inhibited by glucosamine, *The Biochemical journal 335 (Pt 1)*, 59-66.

137. Chan, P. S., Caron, J. P., and Orth, M. W. (2006) Short-term gene expression changes in cartilage explants stimulated with interleukin beta plus glucosamine and chondroitin sulfate, *The Journal of rheumatology 33*, 1329-1340.

138. Yatabe, T., Mochizuki, S., Takizawa, M., Chijiiwa, M., Okada, A., Kimura, T., Fujita, Y., Matsumoto, H., Toyama, Y., and Okada, Y. (2009) Hyaluronan inhibits expression of ADAMTS4 (aggrecanase-1) in human osteoarthritic chondrocytes, *Ann Rheum Dis 68*, 1051-1058.

139. Ghosh, P., and Guidolin, D. (2002) Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent?, *Seminars in arthritis and rheumatism 32*, 10-37.

140. Yamaguchi, K., Sakiyama, H., Matsumoto, M., Moriya, H., and Sakiyama, S. (1990) Degradation of type I and II collagen by human activated C1-s, *FEBS letters 268*, 206-208.

141. Sakiyama, H., Inaba, N., Toyoguchi, T., Okada, Y., Matsumoto, M., Moriya, H., and Ohtsu, H. (1994) Immunolocalization of complement C1s and matrix metalloproteinase 9 (92kDa gelatinase/type IV collagenase) in the primary ossification center of the human femur, *Cell and tissue research 277*, 239-245.

142. van der Woude, D., Houwing-Duistermaat, J. J., Toes, R. E., Huizinga, T. W., Thomson, W., Worthington, J., van der Helm-van Mil, A. H., and de Vries, R. R.

(2009) Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis, *Arthritis and rheumatism 60*, 916-923.

143. MacGregor, A. J., Snieder, H., Rigby, A. S., Koskenvuo, M., Kaprio, J., Aho, K., and Silman, A. J. (2000) Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins, *Arthritis and rheumatism 43*, 30-37.

144. Gregersen, P. K., Silver, J., and Winchester, R. J. (1987) The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis, *Arthritis and rheumatism 30*, 1205-1213.

145. van der Helm-van Mil, A. H., Verpoort, K. N., Breedveld, F. C., Huizinga, T. W., Toes, R. E., and de Vries, R. R. (2006) The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis, *Arthritis and rheumatism 54*, 1117-1121.

146. Hinks, A., Worthington, J., and Thomson, W. (2006) The association of PTPN22 with rheumatoid arthritis and juvenile idiopathic arthritis, *Rheumatology (Oxford, England) 45*, 365-368.

147. Burr, M. L., Naseem, H., Hinks, A., Eyre, S., Gibbons, L. J., Bowes, J., Wilson, A. G., Maxwell, J., Morgan, A. W., Emery, P., Steer, S., Hocking, L., Reid, D. M., Wordsworth, P., Harrison, P., Thomson, W., Worthington, J., and Barton, A. (2010) PADI4 genotype is not associated with rheumatoid arthritis in a large UK Caucasian population, *Ann Rheum Dis 69*, 666-670.

148. Lee, Y. H., Rho, Y. H., Choi, S. J., Ji, J. D., and Song, G. G. (2007) PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis, *Rheumatology international 27*, 827-833.

149. Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., and Yamamoto, K. (2003) Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis, *Nature genetics 34*, 395-402.

150. Nepom, G. T., and Erlich, H. (1991) MHC class-II molecules and autoimmunity, *Annual review of immunology 9*, 493-525.

151. Silman, A. J., Newman, J., and MacGregor, A. J. (1996) Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins, *Arthritis and rheumatism 39*, 732-735.

152. Stolt, P., Bengtsson, C., Nordmark, B., Lindblad, S., Lundberg, I., Klareskog, L., and Alfredsson, L. (2003) Quantification of the influence of cigarette smoking on

rheumatoid arthritis: results from a population based case-control study, using incident cases, *Ann Rheum Dis 62*, 835-841.

153. Linn-Rasker, S. P., van der Helm-van Mil, A. H., van Gaalen, F. A., Kloppenburg, M., de Vries, R. R., le Cessie, S., Breedveld, F. C., Toes, R. E., and Huizinga, T. W. (2006) Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles, *Ann Rheum Dis 65*, 366-371.

154. Makrygiannakis, D., Hermansson, M., Ulfgren, A. K., Nicholas, A. P., Zendman, A. J., Eklund, A., Grunewald, J., Skold, C. M., Klareskog, L., and Catrina, A. I. (2008) Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells, *Ann Rheum Dis 67*, 1488-1492.

155. Willemze, A., van der Woude, D., Ghidey, W., Levarht, E. W., Stoeken-Rijsbergen, G., Verduyn, W., de Vries, R. R., Houwing-Duistermaat, J. J., Huizinga, T. W., Trouw, L. A., and Toes, R. E. (2011) The interaction between HLA shared epitope alleles and smoking and its contribution to autoimmunity against several citrullinated antigens, *Arthritis and rheumatism 63*, 1823-1832.

156. Carty, S. M., Snowden, N., and Silman, A. J. (2003) Should infection still be considered as the most likely triggering factor for rheumatoid arthritis?, *The Journal of rheumatology 30*, 425-429.

157. Symmons, D. P., Bankhead, C. R., Harrison, B. J., Brennan, P., Barrett, E. M., Scott, D. G., and Silman, A. J. (1997) Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care-based incident case-control study in Norfolk, England, *Arthritis and rheumatism 40*, 1955-1961.

158. Cutolo, M., and Straub, R. H. (2006) Stress as a risk factor in the pathogenesis of rheumatoid arthritis, *Neuroimmunomodulation 13*, 277-282.

159. Merlino, L. A., Curtis, J., Mikuls, T. R., Cerhan, J. R., Criswell, L. A., and Saag, K. G. (2004) Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study, *Arthritis and rheumatism 50*, 72-77.

160. Pattison, D. J., Harrison, R. A., and Symmons, D. P. (2004) The role of diet in susceptibility to rheumatoid arthritis: a systematic review, *The Journal of rheumatology 31*, 1310-1319.

161. Duskin, A., and Eisenberg, R. A. The role of antibodies in inflammatory arthritis, *Immunological reviews 233*, 112-125.

162. Whiting, P. F., Smidt, N., Sterne, J. A., Harbord, R., Burton, A., Burke, M., Beynon, R., Ben-Shlomo, Y., Axford, J., and Dieppe, P. (2010) Systematic review: accuracy of anti-citrullinated Peptide antibodies for diagnosing rheumatoid arthritis, *Annals of internal medicine 152*, 456-464; W155-466.

163. Mageed, R. A., Borretzen, M., Moyes, S. P., Thompson, K. M., and Natvig, J. B. (1997) Rheumatoid factor autoantibodies in health and disease, *Annals of the New York Academy of Sciences 815*, 296-311.

164. Sato, Y., Sato, R., Watanabe, H., Kogure, A., Watanabe, K., Nishimaki, T., Kasukawa, R., Kuraya, M., and Fujita, T. (1993) Complement activating properties of monoreactive and polyreactive IgM rheumatoid factors, *Ann Rheum Dis 52*, 795-800.

165. Tanimoto, K., Cooper, N. R., Johnson, J. S., and Vaughan, J. H. (1975) Complement fixation by rheumatoid factor, *The Journal of clinical investigation 55*, 437-445.

166. Bhatia, A., Blades, S., Cambridge, G., and Edwards, J. C. (1998) Differential distribution of Fc gamma RIIIa in normal human tissues and co-localization with DAF and fibrillin-1: implications for immunological microenvironments, *Immunology 94*, 56-63.

167. Pope, R. M., Teller, D. C., and Mannik, M. (1975) The molecular basis of self-association of IgG-Rheumatoid factors, *J Immunol 115*, 365-373.

168. Edwards, J. C., Cambridge, G., and Abrahams, V. M. (1999) Do self-perpetuating B lymphocytes drive human autoimmune disease?, *Immunology 97*, 188-196.

169. Vossenaar, E. R., Zendman, A. J., van Venrooij, W. J., and Pruijn, G. J. (2003) PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease, *Bioessays 25*, 1106-1118.

170. Lundberg, K., Nijenhuis, S., Vossenaar, E. R., Palmblad, K., van Venrooij, W. J., Klareskog, L., Zendman, A. J., and Harris, H. E. (2005) Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity, *Arthritis research & therapy 7*, R458-467.

171. Wegner, N., Lundberg, K., Kinloch, A., Fisher, B., Malmstrom, V., Feldmann, M., and Venables, P. J. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis, *Immunological reviews 233*, 34-54.

172. Avouac, J., Gossec, L., and Dougados, M. (2006) Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review, *Ann Rheum Dis 65*, 845-851.

173. Kokkonen, H., Mullazehi, M., Berglin, E., Hallmans, G., Wadell, G., Ronnelid, J., and Rantapaa-Dahlqvist, S. (2011) Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis, *Arthritis research & therapy 13*, R13.

174. Klaasen, R., Cantaert, T., Wijbrandts, C. A., Teitsma, C., Gerlag, D. M., Out, T. A., de Nooijer, M. J., Baeten, D., and Tak, P. P. (2011) The value of rheumatoid factor and anti-citrullinated protein antibodies as predictors of response to

infliximab in rheumatoid arthritis: an exploratory study, *Rheumatology (Oxford, England)*.

175. Klareskog, L., Ronnelid, J., Lundberg, K., Padyukov, L., and Alfredsson, L. (2008) Immunity to citrullinated proteins in rheumatoid arthritis, *Annual review of immunology 26*, 651-675.

176. 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 and rheumatism 60*, 1923-1931.

177. Clavel, C., Nogueira, L., Laurent, L., Iobagiu, C., Vincent, C., Sebbag, M., and Serre, G. (2008) Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen, *Arthritis and rheumatism 58*, 678-688.

178. Schuerwegh, A. J., Ioan-Facsinay, A., Dorjee, A. L., Roos, J., Bajema, I. M., van der Voort, E. I., Huizinga, T. W., and Toes, R. E. (2010) Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis, *Proceedings of the National Academy of Sciences of the United States of America 107*, 2586-2591.

179. McGraw, W. T., Potempa, J., Farley, D., and Travis, J. (1999) Purification, characterization, and sequence analysis of a potential virulence factor from Porphyromonas gingivalis, peptidylarginine deiminase, *Infection and immunity 67*, 3248-3256.

180. Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K. A., Lundberg, K., Kinloch, A., Culshaw, S., Potempa, J., and Venables, P. J. (2010) Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis, *Arthritis and rheumatism 62*, 2662-2672.

181. 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.

182. 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.

183. 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.

184. 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.

185. Kimura, Y., Zhou, L., Miwa, T., and Song, W. C. (2010) Genetic and therapeutic targeting of properdin in mice prevents complement-mediated tissue injury, *The Journal of clinical investigation 120*, 3545-3554.

186. 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.

187. 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, *Molecular immunology 45*, 2808-2819.

188. 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, *Proceedings of the National Academy of Sciences of the United States of America 92*, 8955-8959.

189. 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 and rheumatism 56*, 1187-1197.

190. 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.

191. 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.

192. 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, *Clinical rheumatology 6*, 350-357.

193. 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.

194. 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 and rheumatism 54*, 1143-1150.

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

196. 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.

197. Morgan, B. P., Daniels, R. H., and Williams, B. D. (1988) Measurement of terminal complement complexes in rheumatoid arthritis, *Clinical and experimental immunology 73*, 473-478.

198. 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 and rheumatism 34*, 1531-1537.

199. Daha, N. A., Kurreeman, F. A. S., Böhringer, S., Stoeken-Rijsbergen, G., Houwing-Duistermaat, J. J., Huizinga, T. W., Toes, R. E. M., and Trouw, L. A. (2011) Genetic variants of C1q are a risk for rheumatoid arthritis, *Molecular immunology 48*, 1667.

200. 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, England) 46*, 1773-1778.

201. Woodruff, T. M., Nandakumar, K. S., and Tedesco, F. (2011) Inhibiting the C5-C5a receptor axis, *Molecular immunology 48*, 1631-1642.

202. 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, *Nature medicine 1*, 237-243.

203. 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, *The Journal of experimental medicine 194*, 781-795.

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

205. Firestein, G. S., Yeo, M., and Zvaifler, N. J. (1995) Apoptosis in rheumatoid arthritis synovium, *The Journal of clinical investigation 96*, 1631-1638.

206. 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 and rheumatism 41*, 1-9.

207. 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.

208. 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.

209. 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, *The Journal of biological chemistry*.

210. 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.

211. Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J. S., Leeb, B., Breedveld, F. C., Macfarlane, J. D., Bijl, H., and et al. (1994) Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis, *Lancet 344*, 1105-1110.

212. Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., Ghrayeb, J., and et al. (1993) Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha, *Arthritis and rheumatism 36*, 1681-1690.

213. Maini, R. N., Breedveld, F. C., Kalden, J. R., Smolen, J. S., Davis, D., Macfarlane, J. D., Antoni, C., Leeb, B., Elliott, M. J., Woody, J. N., Schaible, T. F., and Feldmann, M. (1998) Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis, *Arthritis and rheumatism 41*, 1552-1563.

214. Klareskog, L., van der Heijde, D., de Jager, J. P., Gough, A., Kalden, J., Malaise, M., Martin Mola, E., Pavelka, K., Sany, J., Settas, L., Wajdula, J., Pedersen, R., Fatenejad, S., and Sanda, M. (2004) Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial, *Lancet 363*, 675-681.

215. Bondeson, J., Blom, A. B., Wainwright, S., Hughes, C., Caterson, B., and van den Berg, W. B. (2010) The role of synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis, *Arthritis and rheumatism 62*, 647-657.

216. Perlmutter, D. H., Dinarello, C. A., Punsal, P. I., and Colten, H. R. (1986) Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression, *The Journal of clinical investigation 78*, 1349-1354.

217. Di Muzio, G., Perricone, C., Ballanti, E., Kroegler, B., Greco, E., Novelli, L., Conigliaro, P., Cipriani, P., Giacomelli, R., and Perricone, R. (2011) Complement system and rheumatoid arthritis: relationships with autoantibodies, serological, clinical features, and anti-TNF treatment, *International journal of immunopathology and pharmacology 24*, 357-366.

218. Familian, A., Voskuyl, A. E., van Mierlo, G. J., Heijst, H. A., Twisk, J. W., Dijkmans, B. A., and Hack, C. E. (2005) Infliximab treatment reduces complement activation in patients with rheumatoid arthritis, *Ann Rheum Dis 64*, 1003-1008.

219. Woodruff, T. M., Strachan, A. J., Dryburgh, N., Shiels, I. A., Reid, R. C., Fairlie, D. P., and Taylor, S. M. (2002) Antiarthritic activity of an orally active C5a receptor antagonist against antigen-induced monarticular arthritis in the rat, *Arthritis and rheumatism 46*, 2476-2485.

220. Hedbom, E., and Hauselmann, H. J. (2002) Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation, *Cell Mol Life Sci 59*, 45-53.

221. Felson, D. T., Lawrence, R. C., Dieppe, P. A., Hirsch, R., Helmick, C. G., Jordan, J. M., Kington, R. S., Lane, N. E., Nevitt, M. C., Zhang, Y., Sowers, M., McAlindon, T., Spector, T. D., Poole, A. R., Yanovski, S. Z., Ateshian, G., Sharma, L., Buckwalter, J. A., Brandt, K. D., and Fries, J. F. (2000) Osteoarthritis: new insights. Part 1: the disease and its risk factors, *Annals of internal medicine 133*, 635-646.

222. Nevitt, M. C., Cummings, S. R., Lane, N. E., Hochberg, M. C., Scott, J. C., Pressman, A. R., Genant, H. K., and Cauley, J. A. (1996) Association of estrogen replacement therapy with the risk of osteoarthritis of the hip in elderly white women. Study of Osteoporotic Fractures Research Group, *Archives of internal medicine 156*, 2073-2080.

223. Zhang, Y., McAlindon, T. E., Hannan, M. T., Chaisson, C. E., Klein, R., Wilson, P. W., and Felson, D. T. (1998) Estrogen replacement therapy and worsening of radiographic knee osteoarthritis: the Framingham Study, *Arthritis and rheumatism 41*, 1867-1873.

224. de Klerk, B. M., Schiphof, D., Groeneveld, F. P., Koes, B. W., van Osch, G. J., van Meurs, J. B., and Bierma-Zeinstra, S. M. (2009) No clear association between female hormonal aspects and osteoarthritis of the hand, hip and knee: a systematic review, *Rheumatology (Oxford, England) 48*, 1160-1165.

225. Davis, M. A., Ettinger, W. H., Neuhaus, J. M., Cho, S. A., and Hauck, W. W. (1989) The association of knee injury and obesity with unilateral and bilateral osteoarthritis of the knee, *American journal of epidemiology 130*, 278-288.

226. Kannus, P., and Jarvinen, M. (1989) Posttraumatic anterior cruciate ligament insufficiency as a cause of osteoarthritis in a knee joint, *Clinical rheumatology 8*, 251-260.

227. Verrouil, E., and Mazieres, B. (1995) Etiologic factors in finger osteoarthritis, *Revue du rhumatisme (English ed 62*, 9S-13S.

228. Sowers, M. R., and Karvonen-Gutierrez, C. A. (2010) The evolving role of obesity in knee osteoarthritis, *Current opinion in rheumatology 22*, 533-537.

229. Heinegård, D., and Saxne, T. (2010) The role of the cartilage matrix in osteoarthritis, *Nature reviews 7*, 50-56.

230. Rothwell, A. G., and Bentley, G. (1973) Chondrocyte multiplication in osteoarthritic articular cartilage, *The Journal of bone and joint surgery 55*, 588-594.

231. Kim, H. A., Lee, Y. J., Seong, S. C., Choe, K. W., and Song, Y. W. (2000) Apoptotic chondrocyte death in human osteoarthritis, *The Journal of rheumatology 27*, 455-462.

232. Halasz, K., Kassner, A., Mörgelin, M., and Heinegård, D. (2007) COMP acts as a catalyst in collagen fibrillogenesis, *The Journal of biological chemistry 282*, 31166-31173.

233. 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, *The Journal of rheumatology 24*, 365-371.

234. 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.

235. 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, *Rheumatology international 16*, 191-196.

236. 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, England) 42*, 903-904.

237. 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 and rheumatism 40*, 723-727.

238. Cooke, T. D., Richer, S., Hurd, E., and Jasin, H. E. (1975) Localization of antigen-antibody complexes in intraarticular collagenous tissues, *Annals of the New York Academy of Sciences 256*, 10-24.

239. Cantini, F., Niccoli, L., Nannini, C., Kaloudi, O., Bertoni, M., and Cassara, E. (2010) Psoriatic arthritis: a systematic review, *International journal of rheumatic diseases 13*, 300-317.

240. Rosenberg, E. W., Noah, P. W., Wyatt, R. J., Jones, R. M., and Kolb, W. P. (1990) Complement activation in psoriasis, *Clinical and experimental dermatology 15*, 16-20.

241. Partsch, G., Bauer, K., Broll, H., Petera, P., Dunky, A., and Meretey, K. (1991) Complement C3 cleavage product in synovial fluids detected by immunofixation, *Zeitschrift fur Rheumatologie 50*, 82-85.

242. Triolo, G., Accardo-Palumbo, A., Salli, L., Ciccia, F., Ferrante, A., Tedesco, L., Salli, S., Giardina, E., Pappalardo, A., and Licata, G. (2003) Impaired expression of erythrocyte glycosyl-phosphatidylinositol-anchored membrane CD59 in patients with psoriatic arthritis. Relation to terminal complement pathway activation, *Clinical and experimental rheumatology 21*, 225-228.

243. Rivas, D., Riestra-Noriega, J. L., Torre-Alonso, J. C., Rodriguez, A., and Gutierrez, C. (1994) Decrease in detectable complement receptor type 1 levels on erythrocytes from patients with psoriatic polyarthritis, *British journal of rheumatology 33*, 626-630.

244. Lories, R. J., and Baeten, D. L. (2009) Differences in pathophysiology between rheumatoid arthritis and ankylosing spondylitis, *Clinical and experimental rheumatology 27*, S10-14.

245. Krauledat, P. B., Krapf, F. E., Manger, B., and Kalden, J. R. (1985) Evaluation of plasma C3d and immune complex determinations in the assessment of disease activity of patients with rheumatoid arthritis, systemic lupus erythematosus, and spondylitis ancylopoetica, *Rheumatology international 5*, 97-101.

246. Sturrock, R. D., Barret, A. J., Versey, J., and Rennolds, P. (1975) Proceedings: Raised levels of complement inactivation products in ankylosing spondylitis, *Ann Rheum Dis 34*, 202-203.

247. Gabay, R., Zubler, R. H., Nydegger, U. E., and Lambert, P. H. (1977) Immune complexes and complement catabolism in ankylosing spondylitis, *Arthritis and rheumatism 20*, 913-916.

248. Coremans, I. E., Daha, M. R., van der Voort, E. A., Siegert, C. E., and Breedveld, F. C. (1995) Subclass distribution of IgA and IgG antibodies against Clq in patients with rheumatic diseases, *Scandinavian journal of immunology 41*, 391-397.

249. Wu, I. B., and Schwartz, R. A. (2008) Reiter's syndrome: the classic triad and more, *Journal of the American Academy of Dermatology 59*, 113-121.

250. Matsumoto, M., Takeda, J., Inoue, N., Hara, T., Hatanaka, M., Takahashi, K., Nagasawa, S., Akedo, H., and Seya, T. (1997) A novel protein that participates in nonself discrimination of malignant cells by homologous complement, *Nature medicine 3*, 1266-1270.

251. Fujita, T., Taira, S., Kodama, N., Matsushita, M., and Fujita, T. (1995) Mannose-binding protein recognizes glioma cells: in vitro analysis of complement activation on glioma cells via the lectin pathway, *Jpn J Cancer Res 86*, 187-192.

252. Niculescu, F., Rus, H. G., Retegan, M., and Vlaicu, R. (1992) Persistent complement activation on tumor cells in breast cancer, *The American journal of pathology 140*, 1039-1043.

253. Yamakawa, M., Yamada, K., Tsuge, T., Ohrui, H., Ogata, T., Dobashi, M., and Imai, Y. (1994) Protection of thyroid cancer cells by complement-regulatory factors, *Cancer 73*, 2808-2817.

254. Shi, X. X., Zhang, B., Zang, J. L., Wang, G. Y., and Gao, M. H. (2009) CD59 silencing via retrovirus-mediated RNA interference enhanced complement-mediated cell damage in ovary cancer, *Cellular & molecular immunology 6*, 61-66.

255. Loberg, R. D., Day, L. L., Dunn, R., Kalikin, L. M., and Pienta, K. J. (2006) Inhibition of decay-accelerating factor (CD55) attenuates prostate cancer growth and survival in vivo, *Neoplasia (New York, N.Y 8*, 69-78.

256. Fishelson, Z., Donin, N., Zell, S., Schultz, S., and Kirschfink, M. (2003) Obstacles to cancer immunotherapy: expression of membrane complement regulatory proteins (mCRPs) in tumors, *Molecular immunology 40*, 109-123.

257. Markiewski, M. M., DeAngelis, R. A., Benencia, F., Ricklin-Lichtsteiner, S. K., Koutoulaki, A., Gerard, C., Coukos, G., and Lambris, J. D. (2008) Modulation of the antitumor immune response by complement, *Nature immunology 9*, 1225-1235.

258. Nozaki, M., Raisler, B. J., Sakurai, E., Sarma, J. V., Barnum, S. R., Lambris, J. D., Chen, Y., Zhang, K., Ambati, B. K., Baffi, J. Z., and Ambati, J. (2006) Drusen complement components C3a and C5a promote choroidal neovascularization, *Proceedings of the National Academy of Sciences of the United States of America 103*, 2328-2333.

259. Liu, J., Jha, P., Lyzogubov, V. V., Tytarenko, R. G., Bora, N. S., and Bora, P. S. (2011) Relationship between Complement Membrane Attack Complex, Chemokine (C-C Motif) Ligand 2 (CCL2) and Vascular Endothelial Growth Factor in Mouse Model of Laser-induced Choroidal Neovascularization, *The Journal of biological chemistry 286*, 20991-21001.

260. Rosenberg, K., Olsson, H., Mörgelin, M., and Heinegård, D. (1998) Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen, *The Journal of biological chemistry 273*, 20397-20403.

261. Oldberg, A., Antonsson, P., Lindblom, K., and Heinegård, D. (1992) COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins, *The Journal of biological chemistry 267*, 22346-22350.

262. Saxne, T., and Heinegård, D. (1992) Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood, *British journal of rheumatology 31*, 583-591.

263. 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, *British journal of rheumatology 36*, 1151-1160.

264. Carlsen, S., Hansson, A. S., Olsson, H., Heinegård, D., and Holmdahl, R. (1998) Cartilage oligomeric matrix protein (COMP)-induced arthritis in rats, *Clinical and experimental immunology 114*, 477-484.

265. 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 and rheumatism 58*, 2000-2011.

266. Ziccardi, R. J., and Tschopp, J. (1982) The dissociation properties of native C1, *Biochemical and biophysical research communications 107*, 618-623.

267. Crnkic, M., Månsson, B., Larsson, L., Geborek, P., Heinegård, D., and Saxne, T. (2003) Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept, *Arthritis research & therapy 5*, R181-185.

268. Schaefer, L., and Iozzo, R. V. (2008) Biological functions of the small leucine-rich proteoglycans: from genetics to signal transduction, *The Journal of biological chemistry 283*, 21305-21309.

269. 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, *Journal of structural biology 155*, 294-305.

270. Hedbom, E., and Heinegård, D. (1993) Binding of fibromodulin and decorin to separate sites on fibrillar collagens, *The Journal of biological chemistry 268*, 27307-27312.

271. 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.

272. Ameye, L., and Young, M. F. (2002) Mice deficient in small leucine-rich proteoglycans: novel in vivo models for osteoporosis, osteoarthritis, Ehlers-Danlos syndrome, muscular dystrophy, and corneal diseases, *Glycobiology 12*, 107R-116R.

273. 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, *The Journal of biological chemistry 261*, 13866-13872.

274. 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, *The Journal of biological chemistry 273*, 16723-16729.

275. 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, *The Journal of biological chemistry 266*, 20428-20433.

276. 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.

277. 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 research 14*, 9861-9876.

278. Iozzo, R. V., and Schaefer, L. (2010) Proteoglycans in health and disease: novel regulatory signaling mechanisms evoked by the small leucine-rich proteoglycans, *The FEBS journal 277*, 3864-3875.

279. 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, *The Journal of clinical investigation 115*, 2223-2233.

280. Hildebrand, A., Romaris, M., Rasmussen, L. M., Heinegard, D., Twardzik, D. R., Border, W. A., and Ruoslahti, E. (1994) Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta, *The Biochemical journal 302 (Pt 2)*, 527-534.

281. Bengtsson, E., Mörgelin, M., Sasaki, T., Timpl, R., Heinegård, D., and Aspberg, A. (2002) The leucine-rich repeat protein PRELP binds perlecan and collagens and may function as a basement membrane anchor, *The Journal of biological chemistry 277*, 15061-15068.

282. Bengtsson, E., Aspberg, A., Heinegård, D., Sommarin, Y., and Spillmann, D. (2000) The amino-terminal part of PRELP binds to heparin and heparan sulfate, *The Journal of biological chemistry 275*, 40695-40702.

283. Bengtsson, E., Neame, P. J., Heinegård, D., and Sommarin, Y. (1995) The primary structure of a basic leucine-rich repeat protein, PRELP, found in connective tissues, *The Journal of biological chemistry 270*, 25639-25644.

284. Malmsten, M., Davoudi, M., and Schmidtchen, A. (2006) Bacterial killing by heparin-binding peptides from PRELP and thrombospondin, *Matrix Biol 25*, 294-300.

285. Malmsten, M., Kasetty, G., Pasupuleti, M., Alenfall, J., and Schmidtchen, A. (2011) Highly selective end-tagged antimicrobial peptides derived from PRELP, *PloS one 6*, e16400.

286. Rucci, N., Rufo, A., Alamanou, M., Capulli, M., Del Fattore, A., Ahrman, E., Capece, D., Iansante, V., Zazzeroni, F., Alesse, E., Heinegård, D., and Teti, A. (2009) The glycosaminoglycan-binding domain of PRELP acts as a cell type-specific NF-kappaB inhibitor that impairs osteoclastogenesis, *The Journal of cell biology 187*, 669-683.

287. 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 and rheumatism 50*, 3035-3044.

288. 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, *The Journal of biological chemistry 278*, 48921-48927.

289. Wu, G., Hu, W., Shahsafaei, A., Song, W., Dobarro, M., Sukhova, G. K., Bronson, R. R., Shi, G. P., Rother, R. P., Halperin, J. A., and Qin, X. (2009) Complement regulator CD59 protects against atherosclerosis by restricting the formation of complement membrane attack complex, *Circulation research 104*, 550-558.

290. Lewis, R. D., Jackson, C. L., Morgan, B. P., and Hughes, T. R. (2010) The membrane attack complex of complement drives the progression of atherosclerosis in apolipoprotein E knockout mice, *Molecular immunology 47*, 1098-1105.

291. Ström, A., Ahlqvist, E., Franzen, A., Heinegård, D., and Hultgårdh-Nilsson, A. (2004) Extracellular matrix components in atherosclerotic arteries of Apo E/LDL receptor deficient mice: an immunohistochemical study, *Histology and histopathology 19*, 337-347.

292. Kolset, S. O., Prydz, K., and Pejler, G. (2004) Intracellular proteoglycans, *The Biochemical journal 379*, 217-227.

293. Kolset, S. O., and Tveit, H. (2008) Serglycin--structure and biology, *Cell Mol Life Sci 65*, 1073-1085.

294. Uhlin-Hansen, L., Eskeland, T., and Kolset, S. O. (1989) Modulation of the expression of chondroitin sulfate proteoglycan in stimulated human monocytes, *The Journal of biological chemistry 264*, 14916-14922.

295. Abrink, M., Grujic, M., and Pejler, G. (2004) Serglycin is essential for maturation of mast cell secretory granule, *The Journal of biological chemistry 279*, 40897-40905.

296. Grujic, M., Braga, T., Lukinius, A., Eloranta, M. L., Knight, S. D., Pejler, G., and Abrink, M. (2005) Serglycin-deficient cytotoxic T lymphocytes display defective secretory granule maturation and granzyme B storage, *The Journal of biological chemistry 280*, 33411-33418.

297. Raja, S. M., Metkar, S. S., Honing, S., Wang, B., Russin, W. A., Pipalia, N. H., Menaa, C., Belting, M., Cao, X., Dressel, R., and Froelich, C. J. (2005) A novel mechanism for protein delivery: granzyme B undergoes electrostatic exchange from serglycin to target cells, *The Journal of biological chemistry 280*, 20752-20761.

298. Raab, M. S., Podar, K., Breitkreutz, I., Richardson, P. G., and Anderson, K. C. (2009) Multiple myeloma, *Lancet 374*, 324-339.

299. Theocharis, A. D., Seidel, C., Borset, M., Dobra, K., Baykov, V., Labropoulou, V., Kanakis, I., Dalas, E., Karamanos, N. K., Sundan, A., and Hjerpe, A. (2006) Serglycin constitutively secreted by myeloma plasma cells is a potent inhibitor of bone mineralization in vitro, *The Journal of biological chemistry 281*, 35116-35128.

300. 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, *European journal of immunology 41*, 437-449.

301. Kirschfink, M., Blase, L., Engelmann, S., and Schwartz-Albiez, R. (1997) Secreted chondroitin sulfate proteoglycan of human B cell lines binds to the complement protein C1q and inhibits complex formation of C1, *J Immunol 158*, 1324-1331.

302. Silvestri, L., Baker, J. R., Roden, L., and Stroud, R. M. (1981) The C1q inhibitor in serum is a chondroitin 4-sulfate proteoglycan, *The Journal of biological chemistry 256*, 7383-7387.

303. Ghebrehiwet, B., and Galanakis, D. K. (1993) C1q inhibitor (chondroitin-4-sulfate proteoglycan): structure and function, *Behring Institute Mitteilungen*, 214-223.

304. Hamad, O. A., Ekdahl, K. N., Nilsson, P. H., Andersson, J., Magotti, P., Lambris, J. D., and Nilsson, B. (2008) Complement activation triggered by chondroitin sulfate released by thrombin receptor-activated platelets, *J Thromb Haemost 6*, 1413-1421.

305. Kröger, N., Sayer, H. G., Schwerdtfeger, R., Kiehl, M., Nagler, A., Renges, H., Zabelina, T., Fehse, B., Ayuk, F., Wittkowsky, G., Schmitz, N., and Zander, A. R. (2002) Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality, *Blood 100*, 3919-3924.

306. Zand, M. S., Vo, T., Huggins, J., Felgar, R., Liesveld, J., Pellegrin, T., Bozorgzadeh, A., Sanz, I., and Briggs, B. J. (2005) Polyclonal rabbit antithymocyte globulin triggers B-cell and plasma cell apoptosis by multiple pathways, *Transplantation 79*, 1507-1515.

307. Zand, M. S., Vo, T., Pellegrin, T., Felgar, R., Liesveld, J. L., Ifthikharuddin, J. J., Abboud, C. N., Sanz, I., and Huggins, J. (2006) Apoptosis and complement-mediated lysis of myeloma cells by polyclonal rabbit antithymocyte globulin, *Blood 107*, 2895-2903.

308. Kraut, E. H., and Sagone, A. L., Jr. (1981) Alternative pathway of complement in multiple myeloma, *American journal of hematology 11*, 335-345.

309. Zurlo, J. J., Schechter, G. P., and Fries, L. F. (1989) Complement abnormalities in multiple myeloma, *The American journal of medicine 87*, 411-420.

310. Schifferli, J. A., Pascual, M., Deleamont, P., Hirschel, B., and Perrin, L. (1984) Meningococcemia in a patient with acquired complement deficiency and myeloma, *The New England journal of medicine 311*, 1381-1382.

311. Tokumitsu, S., Furuta, Y., Kobayashi, T., Amano, T., Suzuki, S., and Ota, Z. (1989) Complete Clq deficiency associated with IgG multiple myeloma, *Japanese journal of medicine 28*, 216-218.

312. Cheson, B. D., Walker, H. S., Heath, M. E., Gobel, R. J., and Janatova, J. (1984) Defective binding of the third component of complement (C3) to Streptococcus pneumoniae in multiple myeloma, *Blood 63*, 949-957.