Review

Complement activation and inhibition: a delicate balance

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Complement is part of the innate immune defence and not only recognizes microbes but also unwanted host molecules to enhance phagocytosis and clearance. This process of opsonisation must be tightly regulated to prevent immunopathology. Endogenous ligands such as dying cells, extracellular matrix proteins, pentraxins, amyloid deposits, prions and DNA, all bind the complement activator C1q, but also interact with complement inhibitors C4b-binding protein and factor H. This contrasts to the interaction between C1q and immune complexes, in which case no inhibitors bind, resulting in full complement activation. Disturbances to the complement regulation on endogenous ligands can lead to diseases such as age-related macular degeneration, neurological and rheumatic disorders. A thorough understanding of these processes might be crucial to developing new therapeutic strategies.

Introduction

The complement system (Box 1) was initially recognized as a defence system against infections, and this is certainly one of its main functions. However, it is now clear that complement also functions as an important humoral system to sense danger, which in addition to conserved molecular patterns on pathogens, also includes damaged or altered self tissues. After sensing these danger signals, complement is able to respond to them directly but also indirectly by activating cellular innate and adaptive immune responses via several complement receptors [1]. These complement receptors can interact directly with danger sensors such as C1q (Box 2), but they can also interact with products that are generated via the proteolytic complement activation cascade, for example fragments of C3 and anaphylatoxins (C5a, C3a). This complex pattern of responses enables the body to react in a different manner according to the various types of danger. For some types of danger signal, the complement system initiates a strong inflammatory response, whereas for others it merely flags the target molecules and/or cells for enhanced phagocytosis. Furthermore, under physiological conditions, limited complement activation on host material can occur not just as a result of danger sensing to promote clearance, as exemplified by the restricted complement activation on acrosome-reacted spermatozoa [2]. Complement is an aggressive proteolytic cascade working under the tight control of inhibitors (Figure 1 and Box 3) and the final outcome in each case depends on a tipping of the balance between activation and inhibition [3]. We propose that one important mechanism determining the nature of the complement-mediated response relies on the fact that many endogenous targets that are recognized by C1q and initiate the classical pathway, also interact with the complement inhibitors factor H (FH) and C4b-binding protein (C4BP). Here, we focus on mechanisms of complement regulation by endogenous molecules and their implications in several human diseases. We provide several examples of such mechanisms to illustrate the importance of balanced complement regulation on endogenous ligands, the skewing of which in one direction or the other can result in deleterious effects.

Endogenous ligands that bind both complement activating molecules and complement inhibitors

Extracellular matrix proteins

The extracellular matrix (ECM) consists mainly of large molecules such as proteoglycans and collagens but it is structurally organized and stabilized by members of the family of small leucine-rich repeat proteins (SLRPs). Several of these proteins interact directly with C1q (Table 1). The SLRPs fibromodulin and osteoadherin bind C1q via its globular head domains (Box 2) and activate the classical pathway as efficiently as IgM at the level of C4 and C3 [4,5]. Surprisingly, the terminal pathway is poorly triggered by fibromodulin and osteoadherin, which has been attributed to the fact that they also bind FH and C4BP [4–6]. This indicates that opsonisation via both C1q and fragments of C3b can take place without generating large amounts of C5a and membrane attack complex (MAC) and hence an inflammatory environment. C4BP and FH always regulate complement activation but they can act much more efficiently when bound to the same ligand that activated the complement cascade. For example, we showed that C4BP in solution was a very poor inhibitor of the alternative pathway convertase [7], whereas it was able to strongly inhibit deposition of C3b when bound to the same surface as zymosan, which was used for activation of the alternative pathway [8]. This phenomenon is actually utilized as an evasion strategy by many pathogens, which

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Box 1. Activation and functions of the complement system

The complement system is an important part of innate immunity but also participates in antibody responses of adaptive immunity. The main biological functions of complement include defence against infections, clearance of unwanted debris such as apoptotic and necrotic cells, in addition to guiding adaptive immunity [3]. Complement is a system consisting of more than 35 proteins, which participate in three main pathways of activation; (i) the classical, (ii) the lectin and (iii) the alternative pathway, which can all be triggered by various stimuli (Figure 1). The classical pathway is initiated by interaction between C1q from the C1 complex and immune complexes and many other molecules such as CRP, DNA and misfolded proteins. The lectin pathway is triggered by the recognition of particular saccharides (e.g. those associated with bacteria) by MBL or ficolins. The alternative pathway is initiated either by autoactivation of unstable complement factor C3, via binding of properdin or as an amplification loop to the other two pathways that generate C3b. Each pathway is a cascade of proteolytic cleavages that lead to formation of crucial enzymatic complexes (C3 and C5 convertases), opsonisation of the target with C3b/iC3b (allowing phagocytosis), release of pro-inflammatory anaphylatoxins (C5a, C3a) that attract white blood cells and finally formation of the lytic MAC, which forms a pore in the target membrane. Except for a few membrane bound receptors and regulators, all complement proteins circulate in the blood. Nearly all components can, however, also be found relocated to tissues. In addition to the liver, which is the main source of most complement proteins, there are many local sources of production.

Box 2. The C1q and C1 complex

C1, the initiator of the classical pathway, is a large Ca^{2+}-dependent complex of 790 kDa, consisting of one C1q molecule and two molecules each of the smaller catalytic subunits C1r and C1s [71]. C1q, which is the recognition subunit of the C1 complex, consists of 18 polypeptide chains; six A, six B and six C chains, which are encoded by three different genes on chromosome 1. The globular head domains of C1q comprise its recognition unit and display a remarkable capacity of versatile ligand identification [67]. The globular head of C1q is a heterotrimeric association of globular C1q domains that can also be found at the C-terminal end of other proteins such as types VIII and X collagens, precerebellin and multimerin. Each of the three subunits of the C1q head exhibits particular surface patterns in terms of charged and hydrophobic residues, but the compact trimeric structure of the C1q head enables ligand recognition through residues contributed by two or even three subunits, thereby broadening the recognition spectrum of C1q [72]. Usually, endogenous or pathogen derived ligands, which bind the head domain, trigger activation of the C1 complex and the following complement cascade, whereas molecules with affinity for the collagenous tail region do not. The C1 complex is triggered by the simultaneous binding of ligands to several globular heads of C1q, leading to conformational changes that allow for encounters between proteases C1r and C1s. These then undergo activation, allowing C1s to proteolytically activate C4 and C2 to form a C3-convertase. Binding of C1q to multivalent IgG or IgM, the model ligands of C1q, results in strong activation of the classical pathway.

protect themselves from full complement attack by capturing C4BP and FH. Interestingly, the binding sites for C1q, FH and C4BP on SLRPs do not overlap with one another. Furthermore, the binding sites of the complement proteins are confined to the polypeptide chain of the SLRPs, although SLRPs are heavily modified by large glycosaminoglycan chains. These, if anything, interfere with some of the interactions with complement and its inhibitors. For example, FH binds fibromodulin stronger when carbohydrate modifications are removed [5]. Interestingly, the SLRPs decorin and biglycan also bind C1q, but do so mainly via the collagen-like tail domains of C1q and actually inhibit activation of the classical pathway [9]. C1q also interacts with two other ECM proteins unrelated to the SLRP family: fibronectin [10] and laminin [11]. These interactions do not lead to activation of the classical pathway, most likely because the binding sites for fibronectin and laminin lie outside the globular heads of C1q.

Pentraxins

The acute phase protein C-reactive protein (CRP), a member of the pentraxin family, is an important activator of the classical pathway [12] because it binds to the globular heads of C1q [13]. CRP consists of five identical β-sheet rich subunits arranged in the presence of Ca^{2+} in a flattened donut-like shape. CRP not only recognizes several bacterial pathogens but also binds several host molecules, damaged membranes and nuclear antigens originating from dying cells [14].

Several interactions between complement proteins and CRP have been studied but currently these are a matter of some, yet unresolved controversy. C1q-interaction with CRP is believed to involve one globular head domain of C1q and all five subunits of CRP [15]. Despite a strong affinity between CRP and C1q, the terminal complement pathway is poorly activated by CRP and it is thus reasonable to argue that CRP also interacts directly with complement inhibitors. Such a mechanism could help in controlling disproportionate complement attack and tissue damage during the initial stages of inflammation (Figure 2). Indeed, interaction between FH and CRP has been observed in several studies [16,17]. FH binds CRP by its CCP7 domain (Box 3), which is the same domain bound by heparin and streptococcal M protein [18]. More recently, a direct interaction between CRP and C4BP was demonstrated [19]. The interaction between CRP and FH has recently been questioned and suggested to occur only when CRP is denatured [20] or in a monomeric state [21]. It is unclear at the moment, which form of CRP interacts with C1q and C4BP and if monomeric CRP can be found *in vivo*.

Serum amyloid P-component (SAP), a pentraxin and thus structural analogue to CRP, has also been reported to bind both the activator C1q [22] and the inhibitor C4BP [23]. Most plausibly, strong binding of C1q will occur when SAP is bound to its ligands such as histones upon which activation of the classical pathway will occur [24]. Firm binding of C1q and C4BP to SAP was shown to require that SAP is either oligomeric or presented on a solid phase [25,26]. However, there are also contradictory reports which imply that all C4BP in serum circulates in complex with SAP [27].

Amyloid and prion proteins

C1q has been linked to several neurodegenerative diseases including Alzheimer's disease (AD) because of its ability to recognize misfolded proteins such as amyloid Aβ peptide found in the brains of AD patients [28]. Aβ binds C1q head domains via its N-terminal region and, thus, activates complement. C1q is found in plaques and extracellular tangles in the AD-affected brain [29] to a similar degree as C4 and C3, but there is much less deposition of MAC components. This discrepancy might be explained by the local deposition of complement inhibitors such as C4BP,

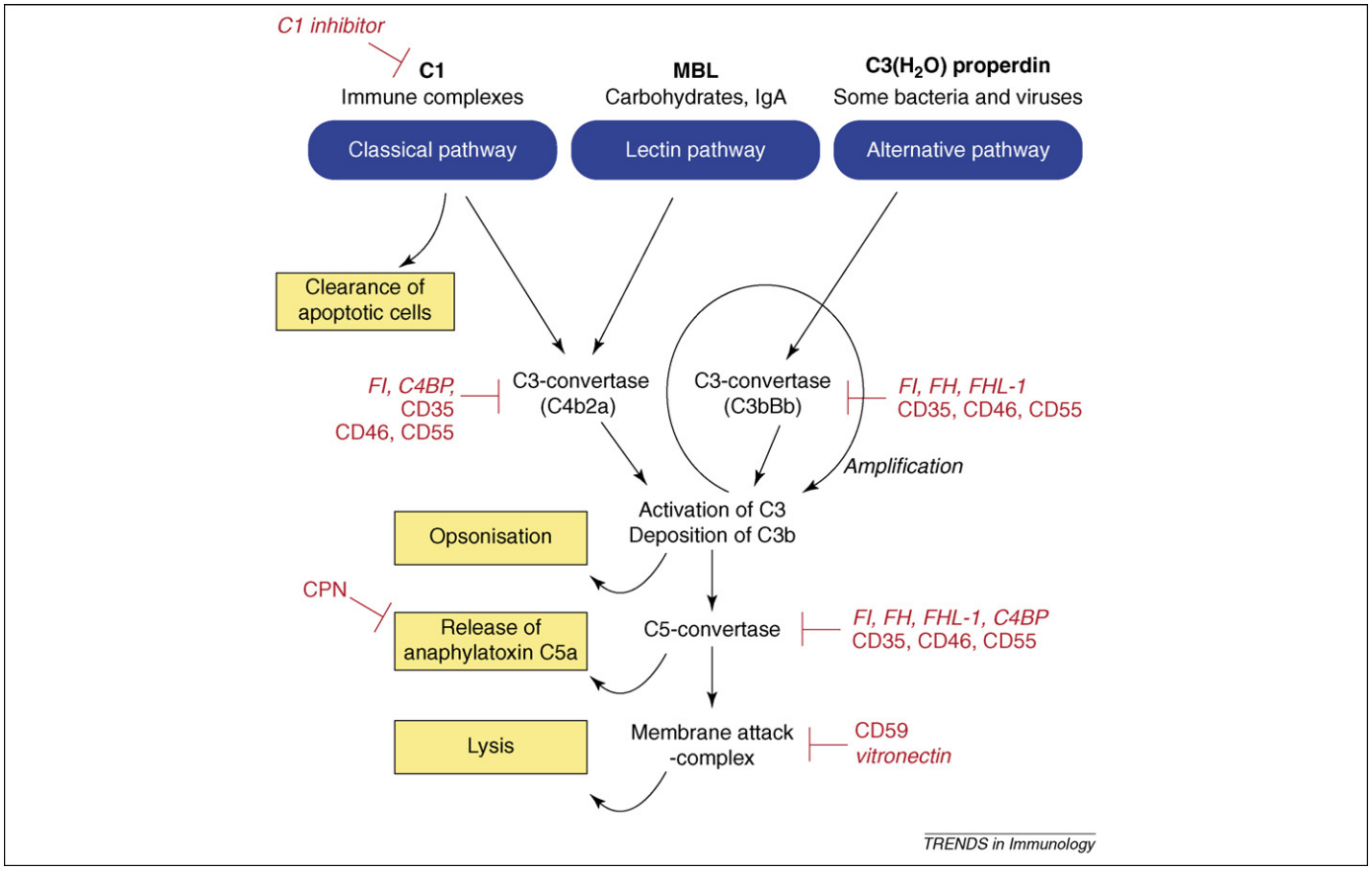

Figure 1. Three pathways of the human complement system and their physiological effects. The three pathways are activated by sensory molecules such as the C1 complex, mannose-binding lectin (MBL), properdin and the active form of C3 with H_2O bound to the active thiolester [C3(H_2O)]. Yellow boxes indicate the main effects of complement: clearance of apoptotic cells, opsonisation of pathogens and immune complexes for phagocytosis, release of anaphylatoxins and lysis. Furthermore, sites of action of soluble (italics) and membrane-bound (regular font) complement inhibitors are indicated in red. Soluble inhibitors are factor I (FI), C4b-binding protein (C4BP), factor H (FH), FH-like protein (FHL-1), carboxypeptidase N (CPN) and vitronectin. Membrane bound inhibitors include: CD35, CD46, CD55 and CD59.

allowing complement mediated opsonisation but preventing excessive complement activation. Indeed, C4BP has been reported to co-localize in the brain with Aβ plaques [29,30], not only because C4BP has affinity for SAP and CRP but also because of direct binding of C4BP to Aβ [30].

Prion protein (PrP) is an endogenous protein involved in the pathogenesis of transmissible spongiform encephalopathies. PrP can misfold and assemble into oligomers and amyloid fibrils. These β-sheet rich aggregates, resistant to degradation by proteases, are denoted PrP^{Sc}, pathogenic conformational isoforms of PrP, where Sc is short for 'scrapie'. Misfolded murine PrP binds C1q [31] and thereby activates the classical pathway [32]. The C-terminal domain of human PrP also activates C1q [33] but it can, in addition, also trigger the alternative pathway. The activation potential is apparent both for native PrP and fibrillated PrP^{Sc} but it is particularly strong for soluble β-oligomers. Furthermore, human PrP^{Sc} and especially β-oligomeric human PrP^{Sc} bind both FH [32,33] and C4BP [33]. Again this shows that both the activator C1q and the inhibitors C4BP and FH bind simultaneously. A functional role for C4BP was indeed shown because PrP^{Sc}-triggered complement activation was increased in C4BP-depleted serum.

Apoptotic and necrotic cells

During the process of apoptosis, the dying cell changes its surface composition to decrease immunogenicity and enhance its uptake (Figure 2). These changes enable apoptotic cells to bind the complement initiators C1q [34] and mannose binding lectin (MBL) [35]. C1q binds directly to apoptotic cells via the globular head domains [36] and activates the classical pathway [37]. Several cellular ligands have been proposed to mediate this interaction such as DNA [38] and phosphatidylserine [39] but it is likely that other ligands are involved as well. Furthermore, C1q can be localized to apoptotic cells via IgM, IgG, CRP and SAP [40]. Recently, properdin was shown to interact with dying cells and to promote activation of complement [41]. Interestingly the fluid-phase inhibitors C4BP and FH also bind to apoptotic and necrotic cells [42,43]. This seems to be a general mechanism not limited to blood cells but also occurs in tissues, for example in the brain and myocardium [30,44]. C4BP binds to these dead or dying cells via a high affinity interaction with phosphatidylserine [43], whereas the ligands for FH binding are currently unknown [43] although CRP was suggested to facilitate FH binding to apoptotic cells [45]. Importantly, C4BP and FH do not require deposited C3b or C4b to bind to apoptotic cells [43].

DNA is exposed on both apoptotic and necrotic cells and it binds C1q and MBL, which leads to a basal level of complement activation. Yet again, excessive complement activation by the C1q–DNA interaction is prevented by the simultaneous binding of inhibitors C4BP [42] and FH [46]. The C4BP–DNA interaction [42,47] not only seems to limit

Box 3. Inhibition of the complement system

Without strict regulation, complement would wreak havoc in the body. Several kinds of soluble and membrane-bound inhibitors limit complement activation to protect host tissues and prevent systemic depletion. Most inhibitors act on complement convertases by accelerating their decay or through promoting enzymatic degradation of C3b and C4b by a serine proteinase, factor I (FI). These inhibitors are mainly composed of complement control protein (CCP) domains. The constitutively active serine protease FI has a key role because it cleaves both C4b and C3b to attenuate activation of all complement pathways [73]. To prevent non-specific activity, direct binding of the substrate to a cofactor is necessary for the proteolytic reaction to take place. C4BP, CD35 and CD46 bind C4b and present it in a cleavable fashion to FI, thus diminishing activity via the classical and lectin pathway. Factor H (FH), CD35, CD46 and C4BP can perform the same role with C3b to inhibit the alternative pathway. CD55 is able to decay convertases of all pathways but it has no FI-cofactor activity. C1-inhibitor controls activation of the C1 complex, whereas CD59 and vitronectin inhibit assembly of MAC. Carboxypeptidase N (CPN) inhibits anaphylatoxins C5a and C3a by removing their C-terminal arginine residues. C4BP, a multimeric 570 kDa protein with an octopus-like structure can be found in plasma. The most abundant isoform of C4BP consists of seven identical α-chains and one β-chain, composed of 8 and 3 CCP domains, respectively, and circulates in complex with anticoagulant protein S. C4BP binds C4b thereby preventing assembly of the classical C3-convertase, accelerating its decay and presenting C4b to FI [74]. C4BP also binds C3b thus affecting the alternative pathway, albeit to lower extent than FH [7].

A single chain of 20 uninterrupted CCPs with a total weight of 150 kDa makes up FH, the most important fluid-phase inhibitor of the alternative pathway [75]. FH binds multiple ligands and its complement regulatory activities depend on three C3b-interaction sites with CCP1–4 being the most important one. FH accelerates decay of the alternative C3-convertase and presents C3b to FI for degradation. FH also affects complement activation initiated by the classical pathway because it inhibits the alternative pathway serving as an amplification loop.

complement activation on the DNA but also to inhibit the further release of DNA from the dead cells [42].

The binding of fluid-phase complement inhibitors to dying cells is particularly important because apoptotic cells down-regulate membrane-bound complement inhibitors such as CD46, which in a similar manner to CD47 down-regulation [48], might signal for phagocytosis [38]. However, this down-regulation of membrane-bound inhibitors would also leave the cells vulnerable to complement mediated lysis and inflammation [43]. We propose the following model [49]: during apoptosis, the cells first down-regulate membrane-bound complement inhibitors, which by itself serves as an 'eat me' signal, but does not lead to complement activation and is complement-independent (Figure 2). At this stage, most cells are removed via phagocytosis mediated by several bridging molecules unrelated to complement. The remaining dying cells (i.e. late apoptotic cells) acquire the ability to bind complement activators such as C1q, MBL and properdin and initiate complement activation leading to opsonisation with C4 and C3 fragments. The presence of these fragments will further stimulate phagocytosis and is beneficial to the host. Concomitant with this, these cells start to bind the complement inhibitors C4BP and FH, allowing the low level of complement activation needed for enhanced clearance but not overt inflammation because of the release of C5a and assembly of MAC [43]. Necrotic cells are cleared via a similar mechanism [42]. Unfortunately, there are large discrepancies in the literature regarding the definition of early and late apoptotic cells making interpretation of some results difficult. For our studies, we have defined the early apoptotic cells as annexin V positive but propidium iodide negative and late apoptotic cells as double positive for these markers.

Diseases involving dysregulation of the balance between complement activation and inhibition on endogenous ligands

Rheumatoid arthritis

Joint inflammation in rheumatoid arthritis (RA) is a very complex process, but evidence from both clinical studies measuring many different products resulting from complement activation [50] and experiments using animal

Table 1. Various ligands of the complement recognition molecule C1q and potential disease associations of complement dysregulation

Ligand	Binding site on C1q	Activates classical pathway	Binding of C4BP	Binding of FH	Potential consequences of complement dysregulation	Refs
IgG, IgM	Heads[a]	Yes	No	No	–	[67]
SAP	?[b]	Yes	+++[c]	?	Alzheimer's disease	[22]
CRP	Heads	Yes	+++	++	Age-related macular degeneration? cardiovascular diseases	[15]
amyloid Aβ peptide	Heads	Yes	+++	?	Alzheimer's disease, Age-related macular degeneration	[68]
Prions	Heads/Tails	Yes	+++	+++	Transmissible spongiform encephalopathies	[33]
DNA	Heads	Yes	+++	++	Systemic lupus erythematosus	[69]
Late apoptotic and necrotic cells	Heads	Yes	+++	+++	Systemic lupus erythematosus	[36]
Fibromodulin	Heads	Yes	++	+++	Joint diseases	[5]
Chondroadherin	Heads/Tails	Weakly	+++	++	Joint diseases	[4]
Osteoadherin	Heads	Yes	+++	+	Joint diseases	[4]
Decorin and biglycan	Tails	No	No	No	Joint diseases	[9]
Laminin and fibronectin	Tails	No	?	?	Joint diseases	[11,70]

?, indicates that it is unknown where or if the interaction occurs.

[a]Determination of the binding sites is often done by using proteolytic degradation fragments of C1q, which display small overlap, therefore most often a binding signal is obtained for both regions of C1q. However, if the binding was mediated more strongly by head domains of C1q it is defined here as 'Heads' and similarly the ligands mainly binding tails are classified as 'Tails'. In cases when the binding to both regions was comparable, we define it as 'Heads/Tails'.

[b]Binding site on C1q not determined.

[c]+, ++ and +++ indicate estimated strength of binding based on published data, affinity constants for the majority of these interactions are not determined.

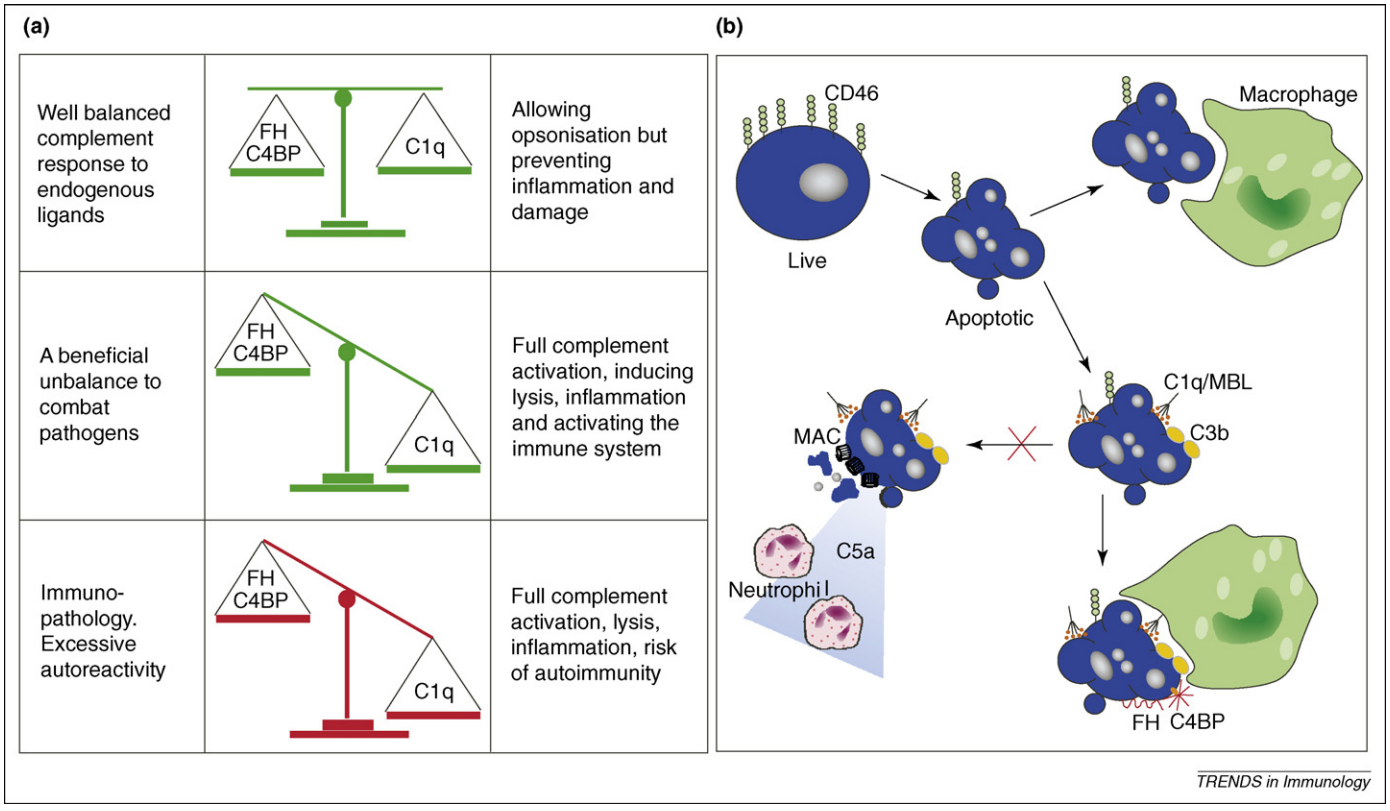

Figure 2. **(a)** Proposed model displaying three possible outcomes of complement activation depending on the level of inhibition and involvement of complement activator C1q and two complement inhibitors: C4b-binding protein (C4BP) and factor H (FH). **(b)** Complement and apoptotic cells. Apoptosis is accompanied by many changes on cellular surfaces that serve as phagocytosis signals for neighbouring cells and macrophages. Early during the process of apoptosis, cells downregulate membrane bound complement inhibitors such as CD46. Most cells are taken up efficiently before they express phenotypic changes allowing for the binding of C1q and mannose-binding lectin (MBL). However, late apoptotic cells are recognized by C1q and MBL, which leads to a certain level of complement activation. If complement activation were left uncontrolled, the complement cascade would proceed to membrane attack complex (MAC) assembly and release of the pro-inflammatory, chemoattractant anaphylatoxin C5a. This, however, is not observed *in vivo*. Instead, apoptotic cells gain the ability to bind soluble complement inhibitors C4BP and FH. This enables enhancement of phagocytosis by bound C1q and C3b/iC3b without substantial activation of the terminal complement pathway and inflammation.

models [51] show that complement contributes to the development of the disease (see Ref. [52]). Traditionally, complement is considered to be a serum component, but in fact all its components can also be found at high concentrations in the synovial fluid of joints. During cartilage injury, complement in synovial fluid is exposed to components of the ECM, which are liberated from cartilage by proteinases [53]. A well-established clinical observation, pertinent to the understanding of mechanisms underlying joint diseases, is that joint replacement surgery ameliorates inflammation. It seems that molecules present in, or released from, cartilage might have a role in propagating this inflammation by activating complement [4,5]. A well-balanced response from the complement system to these ECM components [6] might be sufficient to maintain a non-inflammatory condition during normal physiological ECM turnover or minor damage. Yet during arthritis, when many ECM entities become exposed and there are other complement activators present including dying cells and autoantibodies, the balance between complement activation and inhibition can be tipped to the activation side now resulting in release of C5a and generation of the MAC, contributing to a strong inflammatory milieu. However, the situation in the joint is rather complex because some ECM proteins activate complement [5] (Table 1), whereas others act as local complement inhibitors [9] and the final outcome will depend on many local parameters, such as the extent of fragmentation of the proteoglycans during tissue destruction and binding site availability. It is not clear if C1q-SLRP interactions leading to complement activation have a physiologically beneficial function or if they only contribute to joint disease pathology.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune disease involving multiple organs and is characterized by the presence of auto-antibodies directed against nuclear components. Impaired clearance of dying cells is suggested to drive the autoimmune response in SLE. The importance of complement for the development of SLE is clearly shown by the fact that over 90% of individuals with genetic deficiency of C1q develop severe SLE [54]. Deficiencies of C4 and C2 are also associated with SLE but these three genetic deficiencies are rare, and therefore cannot explain the majority of SLE cases [54]. Opsonisation of targets with C4 and C3 is important for the maintenance of immune tolerance because they facilitate the trapping of immune complexes containing foreign but also self-antigens on follicular dendritic cells (DCs). This prolonged trapping of antigen is important both to induce immune responses against foreign particles and also to prevent autoimmunity against self-antigens. C4BP and FH diminish but do not completely block or prevent complement activation, again allowing opsonisation without the risk of stimulating

autoimmunity. It is currently unknown if SLE patients commonly carry inherited defects in complement proteins or inhibitors that do not lead to full deficiency but to disruption of their function. One could speculate that a dysregulated complement response to dying cells could result in lysis and release of autoantigens triggering an autoimmune reaction in SLE prone individuals. Furthermore, complement has a double role in SLE. As mentioned earlier, rare complement deficiencies lead to SLE, presumably because of impaired clearance of dying cells and dysregulated processing of immune complexes. However, complement also contributes to organ damage because the tissue deposits of immune complexes activate complement (for review see Ref. [55]). In the tissue where complement is activated and potentially damaging for the host, both C4BP and FH are likely to have a protective role, however, the massive nature of the complement activation now establishes an imbalance between activation and inhibition, resulting in tissue damage. Further studies on the role of complement and its dysregulation in the initiation and pathophysiology of SLE are clearly merited.

Alzheimer's disease

AD is characterised by extracellular brain deposits of Aβ in plaques and vessel walls and a focal loss of neurons with reactive gliosis (proliferation of microglia). These amyloid deposits are associated with inflammation-related proteins, including complement factors [29]. The role of complement in AD and related diseases has been a matter of debate because it is hard to distinguish between pathological complement mediated damage and physiological complement mediated clearance of dead cells and misfolded proteins. The local inflammation and complement deposition can be found in human AD brains already in the early stages of disease, before microglial clustering and neurodegeneration. Mouse models provide a valuable tool to evaluate the contribution of individual complement proteins on the progression of AD. In mouse models, C1q and complement activation were found to contribute to AD pathology [56]. However, there are also indications for a protective role of C1q and complement activation in the early phase of disease [28]. *In vitro*, C4BP binds amyloid deposits in addition to apoptotic and necrotic but not viable brain cells (astrocytes, neurons and oligodendrocytes) and limits complement activation on dead brain cells [30]. C4BP has also been detected ex-vivo in AD brains. Therefore, it is plausible that binding of C1q to misfolded proteins in early AD, together with C4BP that decreases activation of MAC, are beneficial and enable clearance of the misfolded material. However, when the system is overwhelmed by amyloid, C1q binds in large amounts allowing full activation of complement and fuelling detrimental inflammation.

Transmissible spongiform encephalopathies

PrP^{Sc} is the assumed culprit in several transmissible spongiform encephalopathies (TSEs), such as scrapie in sheep and Creutzfeldt-Jakob disease in humans. In TSEs, amyloid fibrils consisting of PrP^{Sc}, localize to CNS-regions resulting in spongiformous deformation, neuronal loss and astrocytosis. Deposits of C1q, C3b and MAC have all been detected juxtaposed to PrP^{Sc} plaques in brains of patients with prion disease and correlate with the degree of pathology [57]. Thus, it seems that misfolded PrP^{Sc} present in the brain might activate complement and contribute to the pathology because of propagation of inflammation. This, however, does not seem to affect the kinetics of disease because PrP^{Sc} introduced intracerebrally into mice induces rapidly deteriorating and lethal disease independent of complement [58]. Importantly, in knockout mice, deficiencies of C1q, C3 and factor B (FB) inhibit PrP^{Sc} accumulation in the spleen after its oral administration [58,59], suggesting that complement enhances early TSE progression. One theory explaining the role of complement in the spreading of PrP^{Sc} via the gastrointestinal tract identifies DCs as key players [60]. Natural exposure to PrP^{sc} is mainly via oral ingestion, in which it penetrates the intestinal epithelium, propagates in lymphoid tissue and migrates to the CNS using the endings of sympathetic nerves innervating DC-rich immune centra. It is possible that PrP^{Sc} in the gut activates complement and becomes opsonized, which enables interaction with intestinal DCs via interaction with complement receptors leading to dissemination into host tissues. Within lymphoid tissue, follicular DCs naturally express PrP on their surface and are, hence, potential efficient target foci for conversion of endogenously expressed protein into the pathogenic PrP^{Sc} form. Follicular DCs also function as traps for complement-opsonized particles because of their high expression of complement receptors. Presence of C4BP and FH at the same site will greatly facilitate conversion of C3b into iC3b, which has higher affinity for complement receptors. Thus, follicular DCs constitute sites where complement-opsonized PrP^{Sc} could encounter PrP within an environment allowing PrP^{Sc} replication. It is unknown if interactions of correctly folded PrP with C1q, C4BP and FH under physiological conditions are of importance.

Age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of natural blindness in the elderly. Risk factors for developing AMD include genetic predisposition, such as a combination of common polymorphisms in the *CFH*-gene encoding FH [61]. A polymorphism resulting in an amino acid change in the CCP7 domain of FH was found to account for 50% of the attributable risk of AMD in Caucasians [61,62]. Furthermore, certain haplotypes of the genes encoding FB and C2 are also associated with AMD [63]. The effect of the observed FH polymorphisms on protein function is still not finally elucidated but the polymorphism in CCP7 was shown to affect the interaction between FH and glycosaminoglycans [64], CRP [46,65,66], fibromodulin [46] and chondroadherin [4], DNA [46] and necrotic cells [46]. Interestingly, the disease-associated FH variant bound weaker to CRP, fibromodulin and chondroadherin but stronger to DNA and necrotic cells. Binding to glycosaminoglycans was affected differentially depending on their type. One current hypothesis for the involvement of FH in AMD is that the risk-associated allele has an impaired ability to inhibit complement activation, leading to inflammation in macular tissue. However, in most situ-

ations the complement system acts as a double-edged sword; excessive complement activation generates inflammation and exacerbates tissue injury, yet at the same time, controlled, low-level activation of complement is necessary for the removal of debris. Therefore, the FH polymorphism could be associated with either a decrease or an increase in its ability to inhibit complement, with either scenario potentially resulting in immunopathology. These opposite effects on complement activation could occur simultaneously in different locations within the same tissue. Because the majority of ligands relevant for the AMD-related FH polymorphism are also binders and activators of C1q, it seems that AMD might be another disease in which the balance between simultaneous binding of C1q with FH or C4BP is disturbed.

Concluding remarks

In contrast to most pathogens, endogenous ligands of C1q also bind fluid-phase complement inhibitors. The binding of these inhibitors does not completely block complement activation, but enables opsonisation and is sufficient to prevent massive complement activation and lysis, which could trigger inflammation. It is clear that disruption of the balance between complement activation and inhibition contributes to several diseases. More examples will certainly become apparent in the near future. In some diseases, including neurological diseases and AMD, complement is implicated in the underlying pathology but it is not entirely clear by which mechanisms. It remains to be investigated if these disorders are related to overt complement activation or rather the opposite (i.e. too little complement activation resulting in uncleared debris). Importantly, artificial manipulation of the balance between complement activation and inhibition represents a very interesting therapeutic opportunity. However, it will be a challenge to develop molecules that will affect the balance between complement activation and inhibition in a specific condition without negatively affecting the beneficial functions of complement or interfering with other complex cascades that complement is linked to, such as Toll-like receptors and the coagulation system.

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