Chapter 1
Progress and Trends in Complement Therapeutics

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Abstract The past few years have proven to be a highly successful and exciting period for the field of complement-directed drug discovery and development. Driven by promising experiences with the first marketed complement drugs, increased knowledge about the involvement of complement in health and disease, and improvements in structural and analytical techniques as well as animal models of disease, the field has seen a surge in creative approaches to therapeutically intervene at various stages of the cascade. An impressive panel of compounds that show promise in clinical trials is meanwhile being lined up in the pipelines of both small biotechnology and big pharmaceutical companies. Yet with this new focus on complement-targeted therapeutics, important questions concerning target selection, point and length of intervention, safety, and drug delivery emerge. In view of the diversity of the clinical disorders involving abnormal complement activity or regulation, which include both acute and chronic diseases and affect a wide range of organs, diverse yet specifically tailored therapeutic approaches may be needed to shift complement back into balance. This chapter highlights the key changes in the field that shape our current perception of complement-targeted drugs and provides a brief overview of recent strategies and emerging trends. Selected examples of complement-related diseases and inhibitor classes are highlighted to illustrate the diversity and creativity in field.

1.1 Of Dogmas, Challenges, and Opportunities: The Changing Field of Complement Research

It is very rare that complement research in general, and complement-directed drug discovery in particular, finds itself in the spotlight of media attention. Yet success stories about the off-label use of the clinical anti-C5 antibody Eculizumab (Soliris, Alexion Pharmaceuticals) in the recent outbreak of enterohemorrhagic *E. coli* in Europe (Laursen 2011; Lapeyraque et al. 2011) or the promising results with a soluble form of complement receptor 1 (sCR1, Mirococept) in transplantation medicine (Sample 2010; Sacks and Zhou 2012) sparked a general interest in the field. While this attention may not persist at such a high level, it clearly underscores a new perception of the role of complement in health and disease and highlights the promise of therapeutic intervention in the complement cascade. Its upstream positioning in inflammatory processes and modulatory involvement in many (patho)

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physiological processes indeed render complement an attractive target system. Research in recent years has unraveled some of the mysteries about complement, shaken various dogmas, and revealed fascinating new insights that are of importance for work related to complement-directed drug discovery and beyond.

The most well-known function of complement is undoubtedly its role in microbial defense, where it recognizes, tags, and helps to eliminate intruders such as bacteria, viruses, fungi, or parasites. However, the surface recognition properties of complement are not restricted to pathogen-associated molecular patterns (PAMPs) but also include danger-, damage-, or disease-related patterns of host cells/tissues, immune complexes, or other foreign surfaces such as biomaterials. The severity and outcome of complement response to these distinct triggers have to be tuned carefully and may include opsonization, clearance, elimination, and/or danger signaling to inflammatory and adaptive systems. This tuning is dependent on the context-specific interplay of some 50 different proteins encompassing pattern recognition proteins, proteases and their complement component substrates, soluble and membrane-bound regulators, and various receptors (Ricklin and Lambris 2007a; Ricklin et al. 2010). While often organized in three distinct initiation pathways, that is, the classical, lectin, and alternative pathways (CP, LP, and AP, respectively; Fig. 1.1), it becomes increasingly evident that there are several interconnectivities and bypasses of the complement activation pathways; the involvement of these pathways may therefore greatly vary depending on the trigger, as well as other factors. Independent of the initiation route, amplification of the response by the AP, via formation of C3 convertases that cleave the central component C3 into an anaphylatoxin (C3a) and an opsonin (C3b) fragment, often causes the lion's share of overall complement activation. Opsonization with C3b and its degradation fragments iC3b and C3d facilitates both phagocytosis and adaptive immune signaling via complement receptors CR1 to CR4. Deposited C3b not only fuels amplification by forming additional C3 convertases but also induces the generation of C5 convertases. Cleavage of C5 generates a highly potent anaphylatoxin (C5a) with chemotactic and proinflammatory capacities as well as C5b, which initiates the formation of the terminal complement complex (TCC) that may induce lysis of susceptible cells or participate in signaling events. On host cells, a panel of "regulators of complement activation" (RCA) and other inhibitors tame amplification and the accumulation of effector molecules (Ricklin et al. 2010; Carroll and Sim 2011). While the underlying processes within the cascade during complement activation are highly complex and diverse, this level of complexity is not only essential for an adequate response to distinct triggers but also offers a wide panel of potential targets for therapeutic intervention. Likewise, it becomes apparent that complement is by far not an isolated system but heavily intertwined with other immune, defense, and inflammatory systems (e.g., toll-like receptors, the cytokine network, adaptive immunity, and coagulation) and involved in homeostatic, developmental, regenerative, or metabolic processes (Ricklin et al. 2010). For example, some proteases involved in coagulation (e.g., thrombin) or other networks (e.g., elastase) have been shown to directly activate C3 and C5 (Fig. 1.1) (Amara et al. 2010; Oikonomopoulou et al. 2012).

At the same time, we are getting to know the key players, and thereby potential drug targets, in this intricate complement network more closely than ever before. A wealth of high-resolution structural models and sensitive functional assays have offered unprecedented insight into the shape, conversions, and ligand interaction sites of complement components and revealed fascinating molecular mechanisms that help explain the ability of complement to direct and tune its activities to the specific task and target surface. While crystallography is an essential driving force in this development, orthogonal biophysical technologies like NMR, hydrogen/deuterium exchange mass spectrometry, small-angle x-ray spectroscopy, electron microscopy, or interaction analysis by surface plasmon resonance have filled many gaps and critically contributed to fully describe the structural and functional complexity of complement proteins (Arlaud et al. 2007; Ricklin and Lambris 2007b; Schuster et al. 2007; Perkins et al. 2002). Exemplary for this development is the structural characterization of C3, which progressed from describing the C3d fragment (Nagar et al. 1998) to the publication of full native C3 (Janssen et al. 2005) and provided critical insight into the dynamic activation process and

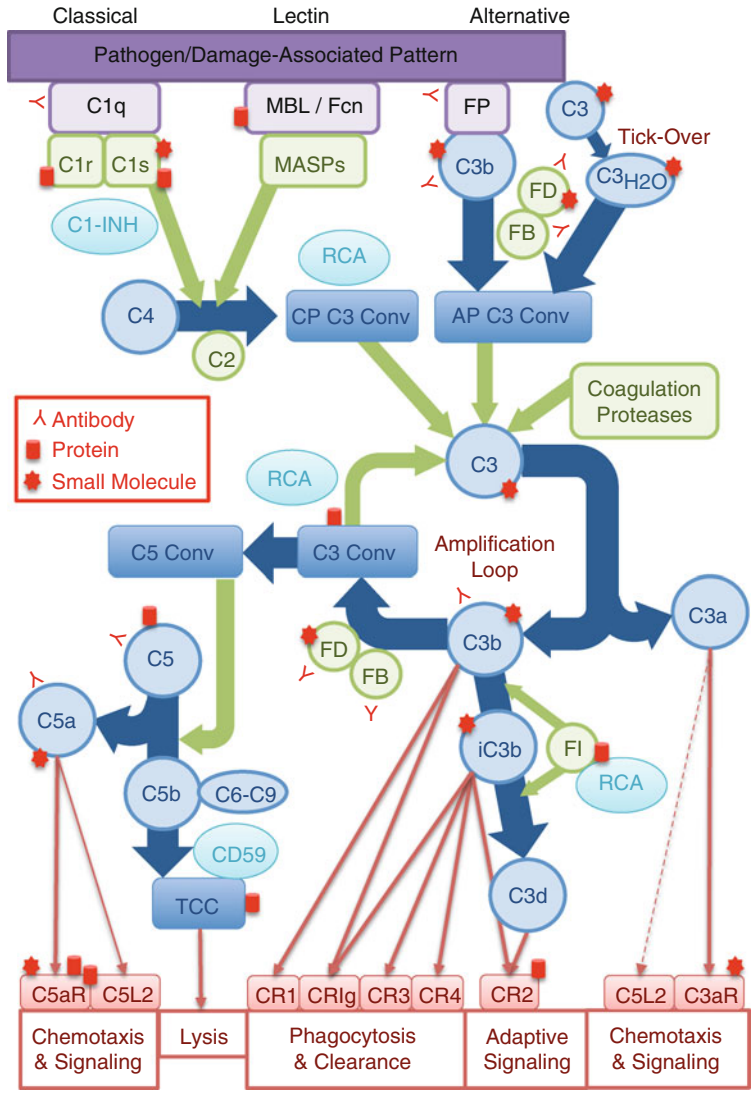

Fig. 1.1 Simplified scheme of the complement cascade and major points of therapeutic intervention. Pattern recognition molecules are colored in *purple*, proteases in *green*, complement components in *blue*, regulators in *cyan*, and receptors in *dark red*. *Red symbols* mark major therapeutic classes (small molecules, proteins, and antibodies; see legend) and are depicted next to their target protein. Abbreviations used: *C1–C9* complement components 1–9, *C1-INH* C1 esterase inhibitor, *C3aR* C3a receptor, *C5aR* C5a receptor, *Conv* convertase, *CR* complement receptor, *CRIg* complement receptor of the immunoglobulin family, *FB* factor B, *Fcn* ficolins, *FD* factor D, *FI* factor I, *FP* properdin, *MASPs* MBL-associated serine proteases, *MBL* mannose-binding lectin, *RCA* regulators of complement activation, *TCC* terminal complement complex

newly exposed binding sites in C3b (Janssen et al. 2006; Schuster et al. 2008; Wiesmann et al. 2006). Meanwhile, structural data of C3b-ligand complexes have included activators (Forneris et al. 2010; Torreira et al. 2009), regulators (Wu et al. 2009), and receptors (Wiesmann et al. 2006). More recently, the integration of structural and functional studies revealed mechanistic aspects of the C3 convertase (Forneris et al. 2010; Rooijakkers et al. 2009) and shed light into self-recognition and regulation by factor H (FH) (Morgan et al. 2011; Kajander et al. 2011), the interaction of C3d with CR2 (van den Elsen and Isenman 2011; Shaw et al. 2010), or the structure of iC3b (Alcorlo et al. 2011). Yet structural insight is hardly restricted to C3 and the AP, and important contributions have recently been

made in respect to the CP (e.g., pattern recognition by C1q (Garlatti et al. 2010)), the LP (e.g., MBL-MASP1 complex (Gingras et al. 2011)), or the terminal pathway (e.g., the structures of C5 or the C5b-C6 complex (Laursen et al. 2010, 2011; Hadders et al. 2012)). Importantly, and in addition to providing mechanistic insight, such structural studies also offer a highly valuable base for understanding disease-related effects of mutations and polymorphisms in complement proteins (Rodriguez de Cordoba et al. 2011). Clearly, this progress in providing molecular details of complement proteins will largely and beneficially influence complement-related drug discovery, either by understanding underlying processes or by allowing improved rational design. And there is a good chance that many more structures covering major drug targets will be released in the future.

Finally, genome-wide association studies (GWAS) and genomic/proteomic analyses have provided a steady flow of information that confirmed or extended many disease links and put new potential disease hot spots on the map. Whereas the link between polymorphisms in FH and the risk for developing AMD likely represents the most prominent case, strong connections have also been found for other complement components in AMD, for some RCA's in kidney diseases like atypical hemolytic uremic syndrome (aHUS) and membranoproliferative glomerulonephritis type II (MGPN II), or for several members of the CP in systemic lupus erythematosus (SLE) (Degn et al. 2011). More recently, important disease associations have also been revealed between MASP-1/3 and 3MC syndrome (Sirmaci et al. 2010) and between CR1 and the development of Alzheimer's disease and depression (Hamilton et al. 2012; Lambert et al. 2009).

1.2 Finding the Achilles' Heel of Complement, One Disease at a Time

1.2.1 A Broad Range of Diseases Warrant a Broad Range of Strategies

In view of its deep involvement in many key physiological processes and its complex interplay of several dozen specialized proteins, it is not surprising that any disruption in the balance of complement activation and regulation may have pathological consequences. Indeed, the list of diseases with contribution of complement has been steadily growing in the past few decades and encompasses autoimmune, inflammatory, hematological, and neurodegenerative disorders, as well as cancer, ischemia/reperfusion (I/R) injuries, and sepsis (Ricklin and Lambris 2007a; Ricklin et al. 2010; Lachmann and Smith 2009) (Fig. 1.2). In addition, and despite progress in material sciences, the foreign surfaces present in biomaterials ranging from medical implants and hemodialysis filters to drug delivery systems such as liposomes and micro-/nanoparticles may still trigger complement to a significant degree and contribute to clinical complications (Ekdahl et al. 2011; Nilsson et al. 2010). Finally, transplantation medicine faces multiple complement-related challenges that include antibody-mediated rejection of the allo- or xenotransplant, as well as I/R effects (Asgari et al. 2010). The reported diversity in involved components and pathways, affected organs, time courses, and case numbers for different diseases renders a "one-size-fits-all" complement treatment virtually impossible and suggests that therapeutic concepts have to be tailored to specific disorders.

The direct activation of complement by danger- and disease-associated patterns places it upstream of many inflammatory reactions that are triggered by foreign or damaged surfaces, thereby supporting the concept that complement inhibition should be considered in various inflammatory diseases. The same might be true for age-related diseases that are influenced by the slow accumulation of debris or plaques that may in turn trigger complement activation; such connections have, for example, been proposed for both age-related macular degeneration (AMD) and Alzheimer's disease, yet with distinct mechanisms (Anderson et al. 2010; Fonseca et al. 2011; Veerhuis et al. 2011; Proitsi et al. 2012). Again, complement inhibition could offer an early point of intervention that could retard, prevent, or

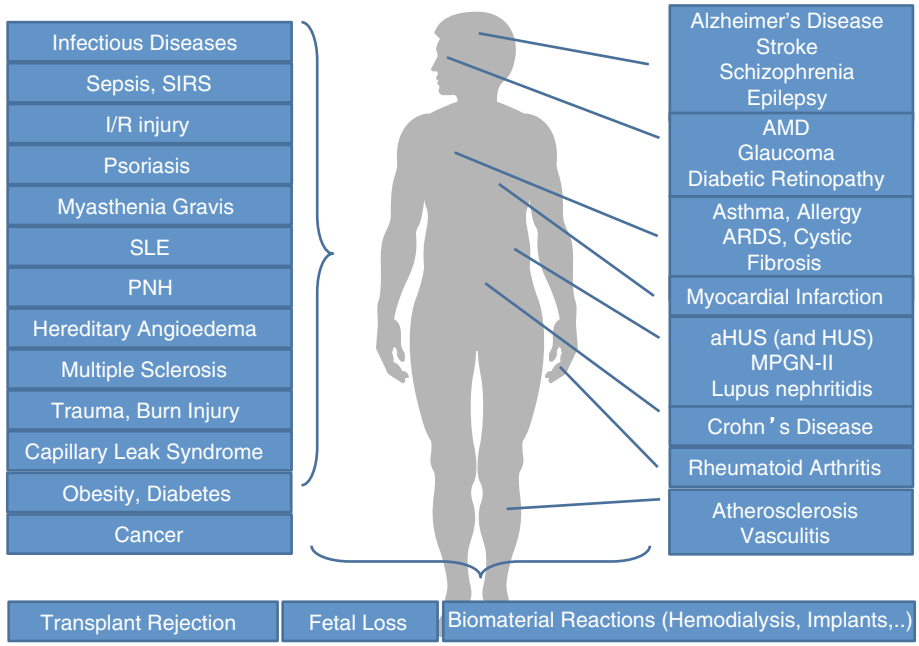

Fig. 1.2 Diseases and clinical disorders/complications with demonstrated or suspected involvement of complement. Abbreviations used: *aHUS* atypical hemolytic uremic syndrome, *AMD* age-related macular degeneration, *I/R* ischemia/reperfusion, *PNH* paroxysmal nocturnal hemoglobinuria, *SIRS* systemic inflammatory response syndrome, *SLE* systemic lupus erythematosus

even reverse disease progression. Especially in the case of such chronic and slowly accumulative diseases, we begin to recognize that the kinetics of complement turnover may be essential and that even small changes in the activity of individual complement proteins may add up to a significant effect over the progression of the disease (Heurich et al. 2011).

1.2.2 Is There an Optimal Point of Intervention?

In principle, the complement cascade offers points of intervention at almost any level ranging from initiation and primary activation to amplification, effector signaling, and lysis. While discussions about complement-related drug discovery occasionally center on the identification of the ideal target for "general" complement inhibition, the diversity of triggering patterns, pathomechanisms, and involved pathways and components more likely requires a careful and disease-specific selection of targets, treatment regimens, and delivery routes. The ideal compromise between sufficient blockage of disease-causing complement activation and preservation of the network's immune surveillance and homeostatic capacities therefore must be reassessed for each clinical disorder. In general, upstream intervention at the level of a specific initiation step, such as the inhibition of C1r/s by C1 esterase inhibitor (C1-INH), may effectively shut down activation and subsequent generation of effector molecules caused by an individual pathway without affecting the protective functions of the other pathways (Fig. 1.3a). However, this requires profound knowledge about the triggering mechanism and may not sufficiently work if more than one route contributes to the overall response. On the other hand, inhibiting at the level of C3 activation, either by blocking C3 directly (e.g., using compstatin) or by acting on the convertase, will efficiently block all activation, amplification, and effector routes independent of the disease mechanism (Fig. 1.3b) but may theoretically bear a higher risk of affecting

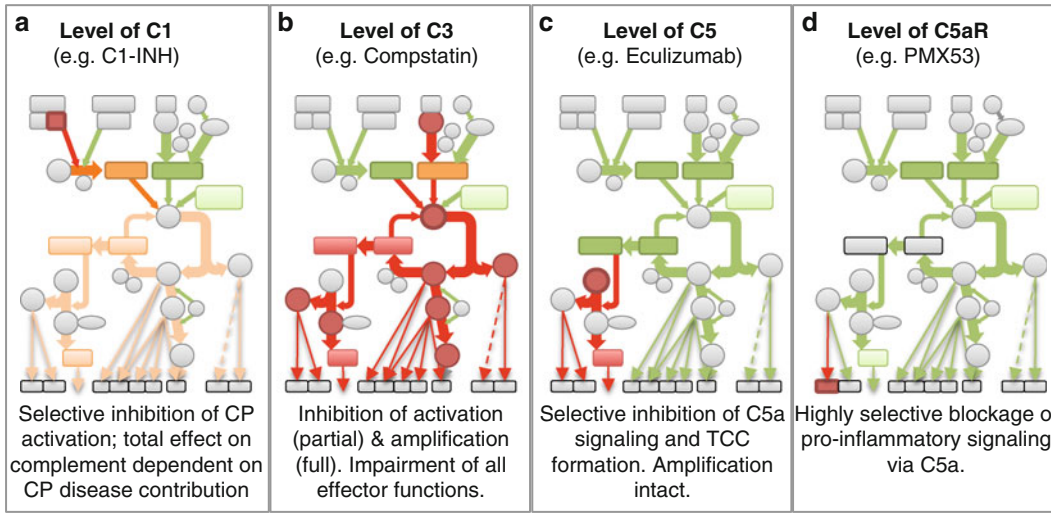

Fig. 1.3 Points of therapeutic intervention in the complement cascade and their theoretical effect on activation and effector mechanisms. The cascade organization in all panels corresponds to the one depicted in Fig. 1.1. *Green coloring* symbolizes unaffected functionality, whereas *orange* and *red* tones reflect partial or complete impairment, respectively

physiologically beneficial complement functions (see below). Finally, blockage at the terminal pathway level can often be tailored to eliminate one or several effector steps depending on the target. For example, blocking C5 (e.g., by Eculizumab) will impair both the formation of the TCC and inflammatory signaling by C5a (Fig. 1.3c), whereas only the latter, signaling function, is blocked when targeting the C5a receptor (C5aR) with antagonists like PMX53 (Fig. 1.3d). Such downstream interventions are often highly efficient in suppressing the major clinical manifestations of excessive complement activity while fully preserving key functions like opsonization. Yet, they also tend to be more "symptomatic" and do not eliminate the causative activation and amplification of the cascade. This dilemma is perhaps best illustrated in the case of paroxysmal nocturnal hemoglobinuria (PNH), where blockage of C5 by Eculizumab efficiently prevents erythrocyte lysis as the predominant clinical issue, thereby dramatically increasing the patient's quality of life. However, studies have also revealed that uninhibited amplification via the AP leads to an accumulation of C3-derived opsonins, which increases extravascular hemolysis of the resulting "ghost erythrocytes." Inhibition at the level of C3 or C3 convertases, for example, by anti-C3b antibodies, compstatin, or targeted regulators (TT30; see below), is therefore being evaluated as an alternative option (Risitano et al. 2011; Parker 2012; Luzzatto et al. 2010).

Besides the point of intervention, decisions about the type of administration (i.e., local vs. systemic) and treatment duration (i.e., acute/short term vs. chronic/continuous) are at least equally important and have to be critically evaluated for each disease (Fig. 1.4). In the case of AMD, for example, local injection of complement-targeted drugs into the eye combines several advantages concerning tissue targeting, pharmacokinetics, and safety (at least regarding preservation of systemic complement activation) but comes at the price of comparatively inconvenient, invasive, and costly intravitreal injection. As AMD has been considered to reflect a local manifestation of a more systemic complement imbalance, the potential of treating AMD by systemic administration of complement drugs has been evaluated with some promising results; however, careful evaluation of the therapeutic and economic benefits will certainly be necessary in such cases. On the other hand, the suppression of inflammatory effects and increased organ preservation by complement inhibitors in the case of severe sepsis, as recently demonstrated with compstatin treatment, will most likely be based on a short-term systemic administration in a hospital setting (e.g., via i.v. infusion) and less dependent on pharmacokinetic restrictions.

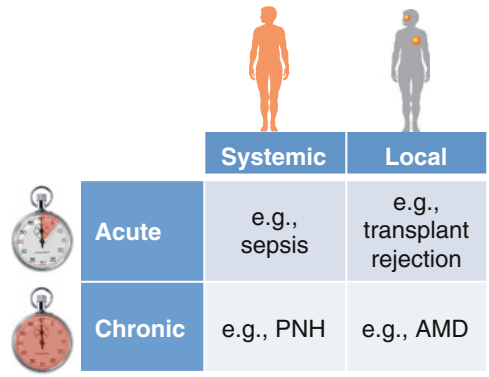

Fig. 1.4 Examples of complement-related disorders with different potential requirements concerning drug administration

1.2.3 Safe Ride or Tightrope Walk? A (Cautious) Risk Assessment

The strong involvement of complement in microbial defense and many other physiological processes intuitively raises questions about the safety and potential risks of therapeutic complement inhibition, especially in the case of systemic treatment over long periods. While long-term clinical data are still scarce, several clinical trials involving diverse complement inhibitors and the experiences of patients receiving complement-directed drugs (i.e., Eculizumab and C1-INH preparations) have largely shown beneficial safety profiles. Previous discontinuations of clinical trials with complement inhibitors were mostly caused by lower-than-expected efficacy or strategic business decisions rather than safety concerns. Importantly, in the case of Eculizumab, the long-term blockage of C5 activation in PNH patients did not only prove to be largely safe (Roth et al. 2011) but was also shown to normalize important immune parameters, such as the number of B lymphocytes, which are normally altered in patients suffering from PNH (Alfinito et al. 2011).

As evident from patients with primary deficiencies or total dysfunctions in specific complement proteins, the highest potential risk may be a higher susceptibility to bacterial infections. Whereas some individuals deficient in C5 have shown a higher rate of neisserial infections, the pathogen spectrum appears somewhat more extended in the case of C3 deficiencies (Skattum et al. 2011; Reis et al. 2006). Interestingly, though, these infections appear to be more frequent in childhood and tend to improve when deficient individuals become adults, and it has been speculated that complement-related bacterial defense becomes less important once our body gains full capacity to produce high IgG levels (Lachmann and Smith 2009). Importantly, across Eculizumab-related clinical PNH studies, the overall infection rate was not significantly increased between patients receiving the antibody drug or a placebo (three cases of *Neisseria meningitidis* were reported during these trials) (Dmytrijuk et al. 2008). In some cases, the risk or severity of potential infections can be further reduced by vaccination, as in the case of Eculizumab where treatment with a *N. meningitidis* vaccine is required prior to the start of therapy.

Of course, the question arises whether complete systemic complement blockage is actually necessary, or even achievable, in all cases. In many diseases, it is an imbalance between activation and regulation rather than a complete dysfunction that drives complement's contribution to pathological states. Even a partial or targeted inhibition strategy may therefore sufficiently help restore the balance of complement activity and potentially reverse disease symptoms. In the case of complement targets with high abundance (e.g., C3) or very rapid turnover in the body (e.g., FD), it may also prove technically difficult to achieve full complement inhibition over a long treatment period. Such partial inhibition may pose an even lower risk for infection; indeed, an assessment of patients who were positive for nephritic factor (i.e., antibodies that stabilize convertases and thereby largely deplete C3 stores)

indicated that residual C3 levels far below 10% of the normal range were sufficient for fighting intruders, as these patients did not display clinically significant infection rates (Skattum et al. 1997). Finally, many of the potential applications for complement-targeted drugs involve acute and/or time-restricted treatment (e.g., during hemodialysis or cardiopulmonary bypass surgery) or local administration to tissues with comparatively low risk of infection (e.g., vitreous in the case of AMD), thereby further reducing potential risks.

Clearly, more data from clinical trials and indicated or off-label use of the marketed drugs are needed to paint a more complete picture of complement therapeutics-related risks, and future studies still need to be carefully monitored and evaluated. Yet, the currently available data so far appear to indicate a rather beneficial and well-manageable risk profile.

1.3 A Handyman's Toolbox of Complement Inhibition

The points concerning disease diversity, pathway involvement, wealth of intervention points, safety and treatment regimens discussed above strongly support the notion that a one-size-fits-all approach will likely not suffice for managing a diverse set of complement-related disorders. Ideally, we should arrive at a panel of specific inhibitors that allow for the selective inhibition of initiation, amplification, and effector steps. Luckily, the field has experienced a high level of creativity and productivity over the past few years that have already produced an impressive panel of inhibitors, some of which have been evaluated in clinical trials. Similar to drug discovery in general, complement inhibitors have seen a shift from small molecules to biopharmaceuticals such as proteins, antibodies, aptamers, and peptides, though this trend seems to be much more pronounced for complement-related drugs. The following sections quickly summarize concepts and trends in the major drug classes and name a few examples of drugs in clinical development.

1.3.1 Towards More Selective Protease Inhibitors

Owing to complement's cascade-type architecture with strong involvement of several serine proteases, and due to the high drugability of proteases, enzymes such as C1s or FB have been among the earliest targets for complement-directed drugs. While several attempts have been undertaken to arrive at small molecule inhibitors of complement proteases, poor pharmacokinetic profiles and insufficient target specificity have so far prevented such drugs from entering clinical applications (Qu et al. 2009). For example, BCX-1470 (BioCryst) was found to inhibit both FD and C1s with high activity and has been used to prevent skin edema during Arthus reaction in rats (Szalai et al. 2000); however, no clinical development has been reported.

Today, human C1 esterase inhibitor (C1-INH) is the only complement-directed protease inhibitor in the clinic. This large glycoprotein that belongs to the SERPIN family is primarily used in connection with hereditary angioedema (HAE) (Tourangeau and Zuraw 2011), a genetic disease caused by a deficiency of functional C1-INH and characterized by a swelling of subcutaneous tissue. While C1-INH preparations have been available for the treatment of HAE for several years in Europe and other regions, the introduction of such drugs into the US market happened only recently. Whereas the C1-INH drug Cinryze (ViroPharma) was approved by the FDA for prophylactic treatment of HAE in late 2008, Berinert (CSL Behring) was approved in 2009 for the treatment of acute HAE attacks (Gompels and Lock 2011; Keating 2009). Although C1 is eponymous of the inhibitor, C1-INH is not C1s-specific but blocks additional proteases of the complement (C1r, MASP2), fibrinolytic (plasmin), coagulation (thrombin, factor Xa), and contact systems (plasma kallikrein, FXIIa) (Davis et al. 2010).

In addition, C1-INH may exert immunomodulatory activities that are not related to protease inhibition (Zeerleder 2011; Thorgersen et al. 2010). In fact, it is likely that the pathogenesis of HAE, and thereby the main pharmacological activity of the C1-INH treatment for this indication, is more closely related to the kallikrein-bradykinin system than to complement.

Despite the uncertain complement connection for the current use of C1-INH in HAE treatment, the availability of approved drugs with activity towards the classical pathway may largely facilitate applications in complement-related diseases (Beinrohr et al. 2008; Kirschfink and Mollnes 2001). Indeed, C1-INH has shown promising effects in various disease models ranging from I/R injury and transplantation (Banz and Rieben 2011; Tillou et al. 2010) to sepsis (Igonin et al. 2011). Whereas changes in the C1-INH status have also been observed in AMD, the therapeutic implications have to be further explored (Gibson et al. 2012).

Current efforts to modulate the action of complement proteases appear to primarily focus on the inhibition of substrate binding via monoclonal antibodies (e.g., against FB, FD, or MASP; see next chapter). In addition, peptide mimics of the scissile loop areas have been suggested for inhibiting C2 and FB (Ruiz-Gomez et al. 2009; Halili et al. 2009). More recently, a targeted phage-display library approach starting from the sequence of sunflower trypsin inhibitor has been used to arrive at peptidic inhibitors of MASP-1 and MASP-2 (Kocsis et al. 2010). Yet, the availability of improved structural models for activated protease states as in the case of FD (Forneris et al. 2010) may well revive the design and optimization of new small molecule protease inhibitors.

1.3.2 Block It: Protein Interaction Inhibitors from Big to Small

Structural studies of several target-ligand complexes of the complement system in recent years have impressively demonstrated the exceptionally high prevalence of large protein–protein interaction interfaces in complement activation and regulation. For example, the four regulatory domains of FH occupy an area of 4,500 Å^2 on the surface of C3b despite a comparatively weak interaction ($K_D \sim 10\ \mu M$) (Wu et al. 2009). In the case of the C3b-FB complex, the formation of additional contacts upon binding and extension of the interaction footprint largely drives convertase assembly and, thereby, complement activity (Forneris et al. 2010). Competitive or allosteric blockage of such interaction interfaces therefore offers a promising strategy for developing complement inhibitors.

Whereas the development of small molecule protein–protein interaction inhibitors has been gaining traction despite the inherent challenges, monoclonal antibodies and antibody fragments are undoubtedly the most rapidly growing class of complement inhibitors. Indeed, several complement-targeted antibodies are currently on the market (anti-C5, Eculizumab, Soliris, Alexion) or in clinical development (e.g., anti-FD Fab, Genentech; anti-FB, TA106, Alexion; anti-MASP2, Omeros; anti-properdin, NovelMed). In addition, minibodies (anti-C5, Mubodina, Adienne), aptamers (anti-C5, ARC1905, Ophthotech), and spiegelmers (anti-C5a, NOX-D19, Noxxon) are being developed as alternative macromolecular blocking entities. A majority of these new inhibitors in development are currently positioned as treatment options for AMD. In the case of Eculizumab, Alexion recently received FDA approval for the treatment of atypical hemolytic uremic syndrome (aHUS) in children and adults (Alexion 2011) after it was found to reduce complement-mediated thrombotic microangiopathy and other clinical parameters in this rare but severe genetic disease (Waters and Licht 2011; Nurnberger et al. 2009; Tschumi et al. 2011). In addition to PNH and aHUS, Alexion is currently evaluating Eculizumab in several other disorders ranging from AMD and *E. coli*-induced HUS to transplantation medicine.

Even though protein–protein interactions are more challenging to inhibit with small molecules, peptides and other low size entities have nevertheless proven successful in several cases and promise advantages in administration and cost when compared to therapeutic antibodies (Wells and McClendon

2007; Mullard 2012). In the case of complement, the limitation of low cell permeability, often seen for peptidic drugs (Mullard 2012), does not have to be taken into account since all potential targets are available in circulation or extracellularly. Compstatin, a cyclic 13-residue peptide that binds to C3 and prevents complement activation and amplification by all pathways (Ricklin and Lambris 2008; Sahu et al. 1996), is currently the only member of that class in clinical development. One compstatin analog (POT-4, Potentia; AL-78898A, Alcon) has successfully passed phase I clinical trials for AMD and is currently evaluated in a phase II trial (Yehoshua et al. 2011). In primates, this compstatin analog was shown to reverse the formation of drusen, which are a hallmark of early AMD (Chi et al. 2010). At the same time, compstatin has been further optimized and has shown promising effects in other disorders such as sepsis, transplantation, PNH, hemodialysis-associated complications, severe asthma, and chronic obstructive pulmonary disease (Kourtzelis et al. 2010; Silasi-Mansat et al. 2010; Qu et al. 2011; Qu et al. 2012) (currently developed by Amyndas Biotherapeutics and Apellis Pharmaceuticals). An alternative approach based on antisense homology has been explored to generate a peptide that is complementary to a region of C5a and binds to the anaphylatoxin (C5aIP (Fujita et al. 2004)). This peptide interfered with C5a-mediated activation of neutrophils and was later shown to attenuate cross talk between complement and coagulation in a model of islet transplantation; in addition, evaluation of C5aIP in a phase II clinical trial for sepsis has previously been announced (Fujita et al. 2004; Tokodai et al. 2010).

1.3.3 Build Your Own Regulator

Our body produces a natural panel of highly effective complement inhibitors that primarily act at the level of convertases or the TCC, and which can be exploited for therapeutic purposes. Among them, the "regulator of complement activation" (RCA) protein family has attracted particular interest. These modular proteins composed of 4–30 complement control protein (CCP) domains include FH and C4b-binding protein (C4BP) in circulation as well as membrane-associated CR1 (CD35), decay accelerating factor (DAF/CD55), and membrane cofactor protein (MCP/CD46); they either accelerate the decay of the C3 convertase or mediate the degradation of C3b by FI. With the strongest activity on both CP- and AP-mediated complement activations, soluble CR1 (sCR1; TP10, Avant) has been the most extensively developed RCA and used in a variety of disease models. Despite promising data in preclinical and phase I studies for preventing complement activation during cardiac surgery (Li et al. 2006), questions regarding gender specificity and efficacy in phase II trials affected its clinical progression (Lazar et al. 2007). More recently, Celldex has picked up development of sCR1 (CDX1135) and is assessing its use in rare renal diseases and antibody-mediated transplant rejection. In addition, recombinant FH (Optherion) has been evaluated for use in AMD and renal diseases that are often affected by polymorphisms in the FH gene. A key disadvantage of these RCAs is their large size (150–300 kDa), which may render production and therapy both challenging and costly.

The modular nature of RCA proteins has inspired various efforts to extract, combine, or extend selected domain modules to arrive at tailored or targeted regulators (Fig. 1.5). Early examples in clinical development include the combination of the short four CCP-domain regulators MCP and DAF into an inhibitor with extended regulatory functions (CAB-2, Millennium) or the truncation of CR1 into shorter fragments. More recently, targeting of regulatory modules to sites of activation or tissues of interest by addition of addressing moieties/modules has regained attention and led to the development of highly promising inhibitors and therapeutic approaches (e.g., TT30, Mirococept; Fig. 1.5; see Sect. 1.4).

Besides RCAs and C1-INH (see above), CD59 is another regulator with therapeutic potential. By intercalating with components C8 and C9, membrane-bound CD59 prevents formation of the TCC and, consequently, TCC-mediated lysis or signaling. While currently not clinically developed, soluble and targeted forms of CD59 have recently been explored for the prevention of choroidal neovascularization (CNV) as it occurs in the wet form of AMD (Cashman et al. 2011; Bora et al. 2010).

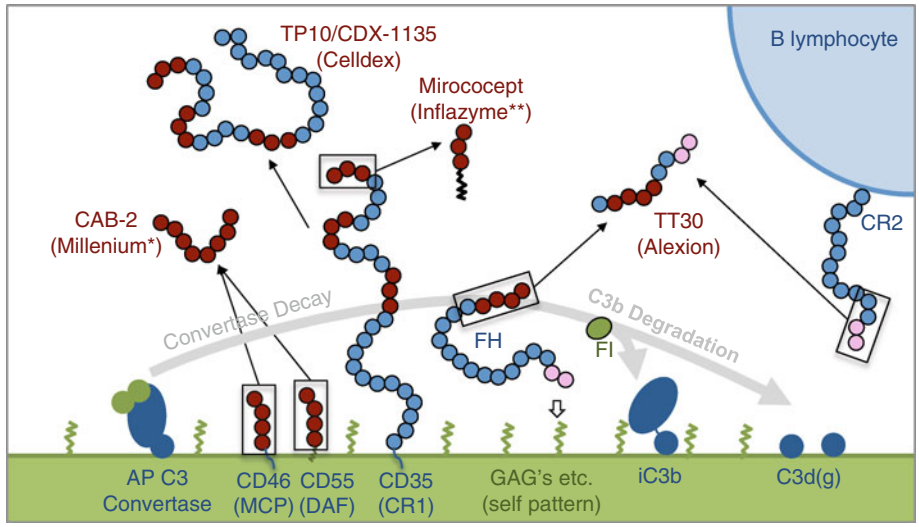

Fig. 1.5 Modular concept of complement regulators and receptors composed of CCP domains and their use for designing therapeutic complement inhibitors. *Colored circles* depict individual CCP domains, with regulatory and targeting entities marked in *red* and *pale magenta*, respectively. *CAB-2 is not currently listed in the pipeline of Millennium; **Even though Mirococept is not currently listed in the pipelines of pharmaceutical companies, it has recently been evaluated for transplant protection

1.3.4 A Friendly Reception: Prevention of Inflammatory Signaling

Anaphylatoxins (C3a, C5a) are among the strongest effectors generated by complement activation and induce chemotaxis, cell activation, and inflammatory signaling via binding to their respective G-protein-coupled receptors (GPCR), that is, C3aR and C5aR (CD88). While a third anaphylatoxin receptor (C5L2, GPR77) has been identified, its binding specificity, signaling pattern, and functional role have not yet been fully elucidated (Ricklin et al. 2010; Klos et al. 2009). In view of their strong inflammatory effects and the potential drugability as GPCRs, anaphylatoxin receptors have long been identified as attractive drug targets. However, the development of C3aR antagonists has proven to be considerably challenging, and a promising clinical candidate (SB 290157, Merck) was later shown to exert partial-agonistic activities (Mathieu et al. 2005). In contrast, the clinical development of antagonists for C5aR has been much more rewarding and has produced several low molecular weight candidates (e.g., PMX53 and PMX205, Cephalon/Teva; JPE-1375, Jerini; CCX168, ChemoCentryx; NGD-2000-1, former Neurogen; see also reviews in (Ricklin and Lambris 2007a; Qu et al. 2009; Woodruff et al. 2011; Monk et al. 2007)). Among them, the PMX family of molecules have been particularly well studied and successfully used in a variety of preclinical studies ranging from AMD and sepsis to trauma, transplantation, cancer, and Alzheimer's disease (Woodruff et al. 2011; Kohl 2006; Bosmann and Ward 2012; Fonseca et al. 2009; Recknagel et al. 2012; Lewis et al. 2008; Markiewski et al. 2008). Although both PMX53 and JPE-1375 have been evaluated in clinical trials and have shown beneficial safety profiles, none of these trials have yet been extended. In addition, C5aR agonists (e.g., EP54/EP67 (Kollessery et al. 2011)), inverse agonists (e.g., NDT 9513727, former Neurogen (Brodbeck et al. 2008)), or modified recombinant C5a with dual antagonistic activity for C5aR and C5L2 ($A8^{\Delta 71-73}$, (Otto et al. 2004)) have been explored. Clearly, anaphylatoxin receptor modulators remain a highly important class of drug candidates with high implication in inflammatory diseases.

1.3.5 Back to the Roots: Exploiting Natural Concepts

Microorganisms and parasites that enter circulation are exposed to the defensive action of complement and therefore have been required to develop powerful evasion strategies, many of which rely on the secretion of inhibitors against host complement components (Lambris et al. 2008). While they may not be used as therapeutic inhibitors directly due to immunogenicity concerns, these pathogen-derived inhibitors may nevertheless serve as templates for developing complement-targeted drugs. An example of a pathogen-derived inhibitor that is in clinical development is the chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS), which acts as an antagonist of the C5aR. A truncated and mutated form of CHIPS (ADC-1004, Alligator Bioscience) has been developed and successfully tested in a preclinical model of I/R injury during myocardial infarction (Gustafsson et al. 2009; van der Pals et al. 2010).

Another promising agent from natural sources is the tick-derived *Ornithodoros moubata* complement inhibitor (OmCI), which binds to C5 and potently inhibits its activation to C5a and C5b (Nunn et al. 2005; Roversi et al. 2007). OmCI has shown efficacy in a rodent model of myasthenia gravis in rats (Hepburn et al. 2007) as well as in an *ex vivo* model of *E. coli*-induced sepsis in human and porcine blood (Barratt-Due et al. 2011). Orthopox viruses, on the other hand, produce several RCA mimics, such as the vaccinia virus complement control protein (VCP) and the smallpox inhibitor of complement enzymes (SPICE) (Ahmad et al. 2007); VCP has been explored in several disease studies (Ghebremariam et al. 2010; Kulkarni et al. 2011). Finally, *S. aureus* appears to be an especially rich source of complement inhibitors and immune modulators that act on a variety of targets and show a fascinating diversity of inhibitory mechanisms (Laarman et al. 2010; Chavakis et al. 2007), including allosteric modulation of C3b (Chen et al. 2010).

1.4 Aim Before You Shoot: Targeting of Complement Drugs

In the vast majority of complement-related disorders, the detrimental activation and amplification process occurs directly on surfaces that trigger the defensive actions of complement, including microbial particles, foreign biomaterials (e.g., implants or drug delivery systems), transplanted cells or organs, or diseased cells and tissues. Systemic complement inhibitors have frequently proven effective in suppressing such surface-induced inflammatory effects (Ekdahl et al. 2011; Nilsson et al. 2010), as, for example, in the case of hemodialysis filter membranes (Kourtzelis et al. 2010) or implantable devices (Sokolov et al. 2011). Yet rather than saturating circulation with soluble inhibitors, the selective blockage of complement activation directly on the triggering surface appears to be a more elegant and focused approach that may better preserve systemic complement activity and reduce drug doses and cost. Fortunately, recent years have produced a panel of creative and diverse strategies of targeted surface protection from complement attack (Fig. 1.6). Similar to systemic inhibition, the task is a delicate one and has to be tailored to the clinical situation. For example, biomaterials can be coated with inhibitors during production, and transplants may be perfused with inhibitor solutions *ex vivo*, thereby allowing the use of targeting entities with broader specificity. In contrast, the targeted inhibition on selected cells types such as erythrocytes during PNH requires a much more selective strategy.

In the case of biomaterials, coating with heparin has been explored and demonstrated to largely prevent activation of both complement and coagulation; however, the demand on production and cost of such surfaces as well as the broad binding specificity of heparin impose potential limitations. PEG coatings, on the other hand, effectively reduce protein binding and certain initiation processes but have shown ambiguous success in preventing complement activation. The use of small molecular entities to recruit physiological regulators such as C4BP or FH to surfaces by means of mimicking self-protection

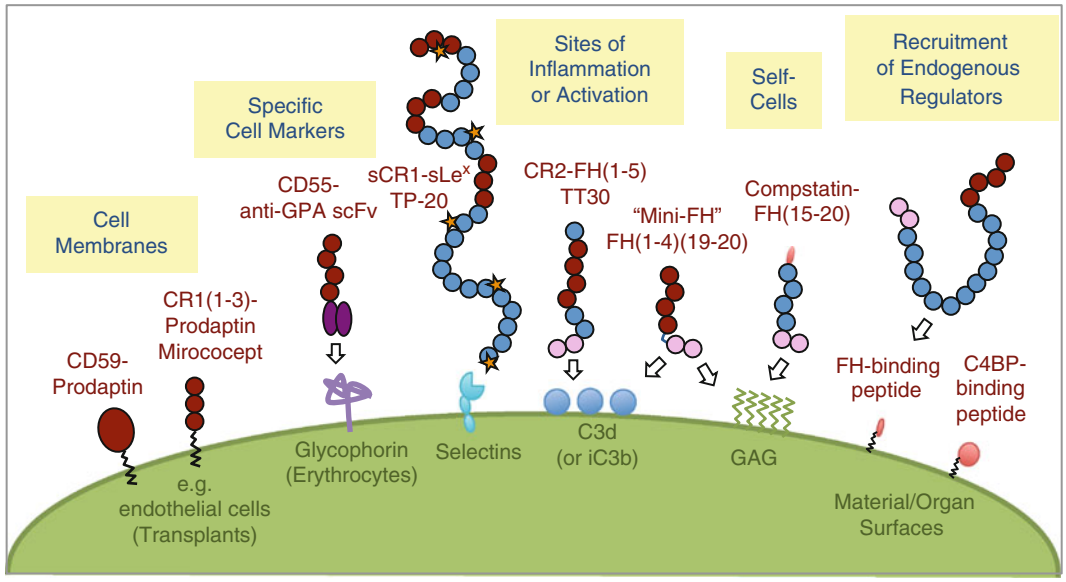

Fig. 1.6 Approaches to target complement inhibitors to various surfaces either in circulation or after *ex vivo* coating/perfusion. Regulatory/inhibitory and recognition domains are depicted in *dark red* and *pale pink*, respectively. Antibody fragments are shown in *purple*, sialyl Lewis x moieties are represented as *orange stars*, and inhibitory or recruiting peptides in *bright red*

of human cells or complement evasion strategies by certain pathogens has recently resulted in promising applications (Ekdahl et al. 2011). For example, coating of model biomaterials with small peptides that bind to the nonregulatory part of FH and thereby attracting this regulator in a functional way were shown to significantly reduce complement activation (Wu et al. 2011).

Perfusion of organ transplants with membrane-targeted inhibitors after removal from the donor has emerged as another highly promising application. Mirococept (APT070) is a particularly interesting and well-studied candidate in this context. This combination of a truncated CR1 fragment (CCP1-3) with a lipopeptide anchor that attaches to cell membranes was initially described several years ago (Smith and Smith 2001; Smith 2002) and had been shown to ameliorate I/R-injury effects in rat intestine and models of myocardial infarction and renal isograft (Banz et al. 2007; Souza et al. 2005; Patel et al. 2006). Though the compound was not clinically developed for several years, Mirococept has recently regained attention after its clinical evaluation in kidney transplantations indicated highly promising effects based on significantly increased life spans for transplants that were perfused with this targeted inhibitor (Sample 2010). A similarly targeted variant of CD59 (APT542) has also been described (Fraser et al. 2003) and was recently tested in a model of CNV (Bora et al. 2010).

Whereas targeting of a systemically administered complement inhibitor to a specific cell type imposes considerable challenges, this problem can be partially circumvented by instead targeting sites of ongoing activation/amplification. Surfaces under attack by the complement system accumulate opsonins such as C3b that are subsequently transformed into cleavage fragments (e.g., iC3b, C3d). By coupling regulatory RCA modules to moieties that bind to such opsonization fragments, one can achieve complement inhibition that is focused on activation sites. A well-described inhibitor that follows this promising strategy is TT30 (Alexion), a chimeric protein that combines regulatory domains from human FH (CCP1-5) with C3d-binding domains from CR2 (CCP1-4) and potently inhibits AP activation (Fridkis-Hareli et al. 2011). TT30 and its rodent-specific analogs have shown encouraging results in a series of AP-mediated disease models ranging from CNV and I/R injury to arthritis and PNH (Fridkis-Hareli et al. 2011; Rohrer et al. 2012; Rohrer et al. 2010; Khan et al. 2011; Banda et al. 2009;

Huang et al. 2008). Alternative approaches for *in vivo* targeting of cell surfaces include the coating of sCR1 with sialyl Lewis x to address selectins at sites of inflammation (Schmid et al. 2001), the combination of RCAs with antibodies against cellular markers (Spitzer et al. 2004, 2005), or the coupling of compstatin to the C-terminus of FH to achieve protection of (attacked) self-surfaces (Zipfel et al. 2011) (Fig. 1.6). Very recently, the new structural information concerning the N- and C-terminus of FH (Wu et al. 2009; Morgan et al. 2011; Kajander et al. 2011) was utilized to design a truncated regulator construct with full regulatory (via CCP1-4) and versatile targeting activities (to both sites of activation and self-surfaces via CCP19-20) that showed promising results in PNH models (Schmidt et al. 2012). The modularity of RCA and CR proteins, the potent activity of RCAs on the convertase level, and the increased knowledge about the molecular involvement of individual CCP domains based on mutational analysis will likely uphold regulator design and targeting as a powerful source of complement-directed therapeutics.

1.5 Alternative Concepts

Whereas the majority of inhibitory strategies are focused on the selective blockage of individual complement components, alternative approaches based on depletion, reconstitution, or gene therapy have also been pursued. Cobra venom factor (CVF) is the most prominent example of the first category; this snake protein isolated from certain cobra species shares high structural and sequence similarity with C3 and is able to form highly stable convertase complexes that rapidly activate and consume C3 and C5 in circulation (Vogel and Fritzinger 2010). CVF and a recombinant humanized form created by replacing small stretches of the C3 sequence with the stabilizing parts of CVF (HC3-1496, InCode BioPharmaceutics) have been evaluated in various disease models including myocardial I/R injury, CNV, arthritis, PNH, or transplant accommodation (Vogel and Fritzinger 2010; Fritzinger et al. 2010; Chen Song et al. 2011).

Even though administration of purified proteins is preferred for treating diseases caused by deficient, dysfunctional, or mutated complement components, such an option is not available or economically feasible in all cases. Reconstitution with functional complement components can be achieved in such cases by infusion with fresh frozen plasma. This type of therapeutic approach has been utilized for C3 dysfunction (Nilsson et al. 1994) and aHUS (Licht et al. 2005).

In most cases, and as illustrated above, the therapeutic use of complement regulators involves the administration of RCA constructs, their recruitment to surfaces, or reconstitution from plasma. However, in the case of cancer, complement regulators may undesirably protect tumor cells from complement attack, thereby warranting their therapeutic modulation. For example, CD59 inhibitors have been shown to enhance the anticancer activity of cancer-directed antibodies in certain cases (Ge et al. 2011). Similarly, genetic knock-down of membrane-associated RCAs (CD46, CD55, CD59) using small interfering RNA (siRNA) has been beneficial as an adjuvant strategy to antibody-based cancer immunotherapy (Geis et al. 2010). In view of the dual role that complement may have in different types of cancer (Markiewski and Lambris 2009), the potential of using complement inhibitors and "enhancers" has to be more closely explored.

Finally, the complex intertwinement and collaboration of complement with other defense and inflammatory pathways suggests that certain diseases may benefit from combinatorial treatment strategies. Such an approach based on simultaneous inhibition of toll-like receptor and complement pathways (via anti-CD14 mAb and compstatin, respectively) has shown promising and synergistic effects in models of bacteria-induced sepsis (Christiansen et al. 2012). Also, simultaneous inhibition of complement and coagulation targets promises advantages for preventing inflammatory responses induced by biomaterials or transplants (Ekdahl et al. 2011; Fujiwara et al. 1997). Clearly, such combinatorial therapies are just starting to be explored and may be highly potent for the treatment of diseases with complex etiologies.

1.6 Pinpointing Activation Sources: Diagnostic Strategies

The early involvement of complement in many disease processes and its upstream position in the inflammatory system potentially render it an interesting option for biomarker analysis in clinical diagnostics. In this context, approaches have been exploited that measure either the plasma levels of intact complement components, the presence of complement activation products, or the opsonization of tissue with fragments of C3 or C4.

Examples of using total complement component concentrations as markers are the measurement of C3 and C4 levels in SLE, antiphospholipid syndrome, atopic asthma, or cardiovascular disease (Mosca et al. 2011; Engstrom et al. 2007; Palikhe et al. 2007; Ramos-Casals et al. 2004). On the other hand, many activation products, such as C3a, C5a, or FB fragments, are increased in a variety of inflammatory and immune-related diseases and have been used as predictive markers for disorders like pregnancy complications and preeclampsia, heart failure, or adult respiratory distress syndrome (Gombos et al. 2012; Lynch et al. 2008, 2011; Zilow et al. 1990). Recently, several studies also investigated the systemic concentrations of complement proteins and activation products of the alternative pathway in the case of AMD and revealed significant correlations for several markers (Hecker et al. 2010; Scholl et al. 2008; Reynolds et al. 2009). Whereas biomarker analysis at the protein level has several advantages over genetic studies, including the potential assessment of posttranslational modifications and activation fragments, protein-based diagnostic approaches are often dependent on the method and difficult to multiplex, thereby complicating biomarker discovery and analysis. However, recent developments in advanced screening methods are increasingly facilitating such endeavors. For example, assay platforms based on SOMAmers (i.e., chemically optimized aptamers that form tight complexes with protein targets; SOMAscan, SomaLogic) offer multiplexing capabilities and diagnostic versatility in low sample quantities (Kraemer et al. 2011; Gold et al. 2010). Several other diagnostic platforms are also available based on mass spectrometry or antibody arrays (Sanchez-Carbayo 2011; Blonder et al. 2011).

In many cases, though, the most profound accumulation of complement activation products occurs directly on the surface of diseased organs, as in the case of various kidney diseases (Berger 1974; Brown et al. 2007) and organ/cell transplantations (Asgari et al. 2010; Kato et al. 2003), as well as in AMD where such fragments are found both in drusen and on the subretinal tissue (Anderson et al. 2010). Opsonizing complement fragments such as C3b or C4d have long been used for diagnostic purposes, but their deposition has mainly been determined through tissue biopsies and immunohistochemistry applications. More recently, noninvasive technologies have been developed that utilize the same principles of addressing sites of ongoing complement activation as described above for inhibitor targeting. For example, iron oxide nanoparticles have been coated with the C3d-binding domains of CR2 to perform magnetic resonance imaging of inflammatory kidney disease in a mouse model of lupus nephritides with promising results (Serkova et al. 2010). Similar approaches with targeted contrast agents or other diagnostic entities may therefore be developed and could allow a more dynamic and noninvasive route to monitor complement activation *in vivo*; they also might be useful in the decision-making process to determine which patients may benefit from complement-targeted treatments.

1.7 Conclusions and Outlook

With its strong involvement in many inflammatory, I/R-injury-related, immune and degenerative diseases, its 50 potential targets, and its cascade organization that allows various points of intervention, complement presents itself as an increasingly attractive target network for pharmaceutical intervention. Currently, the approved therapeutics only cover orphan diseases (PNH, aHUS, HAE),

thereby influencing both cost and recognition in the field. However, additional and much bigger markets ranging from transplantation medicine to prevalent diseases like AMD are already looming on the horizon. It will be interesting to follow the changes and new opportunities in the field of complement-directed therapeutics once new drugs that target different levels of the cascade hit the market. Similarly, much may be learned when those new therapeutics, along with existing drugs such as Eculizumab and rC1-INH, have their applications gradually extended through off-label use or newly approved indications. The shifting dogmas and increased knowledge about complement in health and disease, and the high level of diversity and creativity in complement-targeted drug discovery, surely paint a bright picture and promise an exciting future for treating complement-mediated disorders.

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Disclosure J.D.L. is the inventor of the C3 inhibitor compstatin and holds several patents about the development and clinical application of compstatin analogs. He has previously served as a member on the Scientific Advisory Board of Potentia Pharmaceuticals and is the founder of Amyndas Biotherapeutics, which perform clinical development of compstatin analogs for various indications.

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