20. Compstatin: A Complement Inhibitor on its Way to Clinical Application

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Abstract. Therapeutic modulation of the human complement system is considered a promising approach for treating a number of pathological conditions. Owing to its central position in the cascade, component C3 is a particularly attractive target for complement-specific drugs. Compstatin, a cyclic tridecapeptide, which was originally discovered from phage-display libraries, is a highly potent and selective C3 inhibitor that demonstrated clinical potential in a series of experimental models. A combination of chemical, biophysical, and computational approaches allowed a remarkable optimization of its binding affinity towards C3 and its inhibitory potency. With the recent announcement of clinical trials with a compstatin analog for the treatment of age-related macular degeneration, another important milestone has been reached on its way to a drug. Furthermore, the release of a co-crystal structure of compstatin with C3c allows a detailed insight into the binding mode and paves the way to the rational design of peptides and mimetics with improved activity. Considering the new incentives and the promising pre-clinical results, compstatin seems to be well equipped for the challenges on its way to a clinical therapeutic.

1 Tackling Complement at its Core

Therapeutic intervention in the human complement system has long been recognized as a promising strategy for the treatment of a series of ischemic, inflammatory and autoimmune diseases (Lambris and Holers 2000; Ricklin and Lambris 2007a). In principle, the large network of soluble and cell-surface-bound proteins, which builds the base of the complement cascade, offers a variety of potential drug targets. However, the quest for complement-specific therapeutics proved to be much more challenging than initially anticipated. With the therapeutic antibody eculizumab (Soliris®, Alexion Pharmaceuticals, Inc.) against paroxysmal nocturnal hemoglobinuria, the first drug with proven complement connectivity has been marketed only recently (Ricklin and Lambris 2007a; Rother et al. 2007). A second complement-associated compound, purified C1 esterase inhibitor (C1-INH), is available as a therapeutic option for the treatment of hereditary angioedema in several countries. However, its mechanism of action may be closer related to the bradykinin-kallikrein than the complement cascade (Davis 2006).

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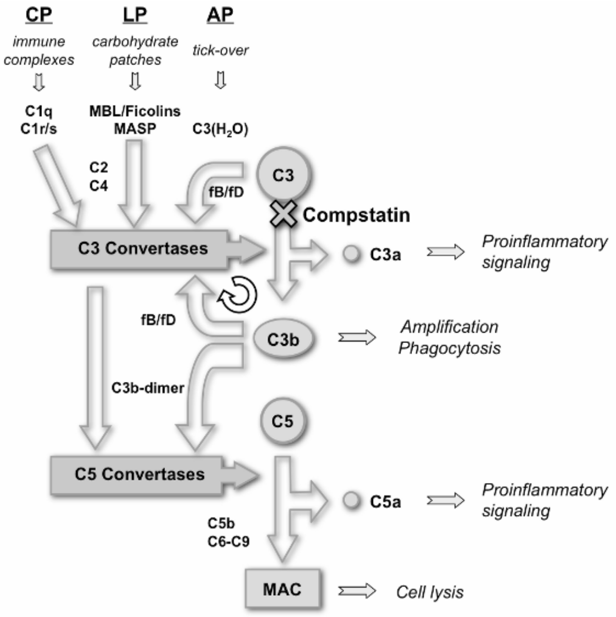


Fig. 1 Compstatin inhibits the cleavage of native C3 to its active fragments C3a and C3b. As a consequence, the deposition of C3b, the amplification of the alternative pathway and all downstream complement actions are prevented

Strikingly, both drugs cover relatively rare diseases and have been developed with the aid of orphan drug regulations. Yet, for many of the more common inflammatory or autoimmune conditions there are no complement drugs on the market. Any extension of the current complement-specific therapeutic arsenal is therefore highly desired.

Part of the problem in complement-directed drug discovery is the selection of the right target (Ricklin and Lambris 2007a). A controlled, localized modulation at the core of complement activation is considered to be the most promising approach in many cases. On a molecular level, inhibition at the level of C3, including the C3 convertases, is of particular interest since both the amplification of all initiation pathways and the generation of anaphylatoxins (C3a, C5a) and the membrane attack complex (MAC) are affected (Fig. 1). In this respect, C3 can be regarded as a central hub that mediates and controls the upstream activation and downstream effector functions of complement (Degn et al. 2007). Initial attempts in developing small molecule drugs for inhibiting the conversion of C3 focused on the various serine proteases that are involved in the convertase formation and activity. However, lack of potency and specificity as well as short half-lives so far limited clinical success of these compounds. Similarly, soluble forms of physiological complement regulators (e.g. complement receptor 1) have mostly been

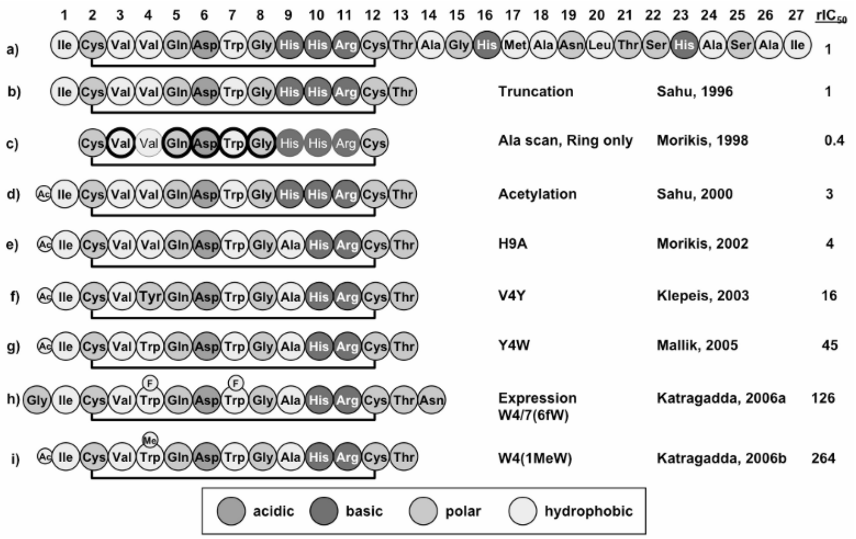


Fig. 2 Important achievements in the optimization of the compstatin peptide sequence as expressed by their relative activities (rIC$_{50}$) for inhibiting complement activation. The initial C3b-binding peptide from the phage display library (**a**) could be truncated to the *N*-terminal 13 residues without losing activity (**b**). While an alanine scan identified positions 3, 5, 6, 7, and 8 as essential for its functions (*bold rings*), the removal of the flanking amino acids caused a slight loss of activity (**c**). Acetylation of the N-terminus (**d**) and the exchange of His-9 by alanine (**e**) led to minor and the introduction of aromatic side chains at position 4 to large activity improvements (**f, g**). Beneficial effects due to modifications on the indole ring of tryptophan were observed for both compstatin expressed in *E. coli* (**h**) and the synthetic peptide (**i**), leading to a dramatic increase in the inhibitory activity

discontinued or put on hold (Ricklin and Lambris 2007a). Compared to these previous examples, which indirectly target activation of C3 via the convertases or C3b, the peptidic inhibitor compstatin exerts its function via direct binding to native C3 (Fig. 1). This unique mechanism, its comparatively small size, and promising pre-clinical results make compstatin an interesting candidate for further clinical development. In the following sections, we illustrate both the structural and functional, as well as the clinical properties of this versatile molecule.

2 Discovery and Initial Characterization

Compstatin has been discovered more than 10 years ago by screening phage-display libraries in the search for C3b-binding peptides (Sahu et al. 1996). In comparison with small drug molecules, peptides face some specific challenges (e.g. stability or short plasma half-lives) but also feature several benefits such as high selectivity and low toxicity (Sato et al. 2006). In addition, peptidic drugs are more successful in inhibiting large protein-protein interactions, which are common in the complement cascade. It is not surprising, therefore, that complement therapeutics in the pipelines almost exclusively encompass biopharmaceuticals like peptides, antibodies, and other soluble proteins (Ricklin and Lambris 2007a).

Phage-display technology represents an ideal technology for screening large peptide libraries of high structural diversity in an efficient manner (Kay et al. 2001; Ladner et al. 2004; Sarrias et al. 1999; Yu and Smith 1996). The method has proven especially important for drug discovery in the academic sector, since their access to small molecule libraries is usually largely restricted. In case of compstatin, a random 27-mer peptide library containing 2×10^8 unique clones had been screened for binding to C3b (Sahu et al. 1996). One selected clone showed strong affinity for C3, C3b, and C3c but not for the C3d fragment. Even more, the isolated peptide (Peptide I; Fig. 2a) was able to inhibit both the classical and alternative pathway of complement activation with IC_{50} values of 65 and 19 μM, respectively. No significant loss in activity was observed when the C-terminal half of the peptide was removed, leaving a cyclic 13-mer (ICVVQDWGHHRCT; Fig. 2b). Reduction and alkylation of the intramolecular disulfide bond between cysteins 2 and 12 resulted in a completely impaired functionality indicating the importance of the cyclic structure (Sahu et al. 1996). The cyclic peptide, which was later named compstatin, bound to C3 in a reversible manner and inhibited the convertase-mediated cleavage of C3 to C3a and C3b. A series of surface plasmon resonance (SPR) studies confirmed and quantified the selectivity and binding mode of compstatin (Ricklin and Lambris 2007b). While C3 and its hydrolyzed form $C3(H_2O)$ featured similar affinities (K_D = 60–130 nM) for the immobilized peptide, the binding of C3b and C3c was found to be reduced by a factor of 20 and 70, respectively. Again, no binding to C3d could be detected (Sahu et al. 2000). This finding was rather surprising since the vast majority of physiological C3 ligands interact with its active form C3b, while only few ligands are known to bind native C3 (Sahu et al. 1998). In situ formation of the C3 convertase on a SPR sensor chip further revealed that compstatin indeed inhibited the formation and deposition of active C3b when it was co-injected with C3 (Nilsson et al. 1998). The activity of compstatin on the classical and alternative pathway has later been confirmed and further investigated by independent groups from both academic research and pharmaceutical industry (Furlong et al. 2000; Klegeris et al. 2002).

Since compstatin did only block enzymatic removal of the C3a/ANA domain by the C3 convertase but not by trypsin, a steric inhibition of the C3a cleavage site (Fig. 3b) seemed to be unlikely as functional explanation. Furthermore, the peptide did not destabilize the C3 convertase by interfering with the interaction between C3b and factor B, nor did it inhibit the binding to the convertase-stabilizing protein properdin (Sahu et al. 1996). As a consequence, the induction of a conformational change (Fig. 3c) or the steric hindrance of substrate binding (Fig. 3d) remained as

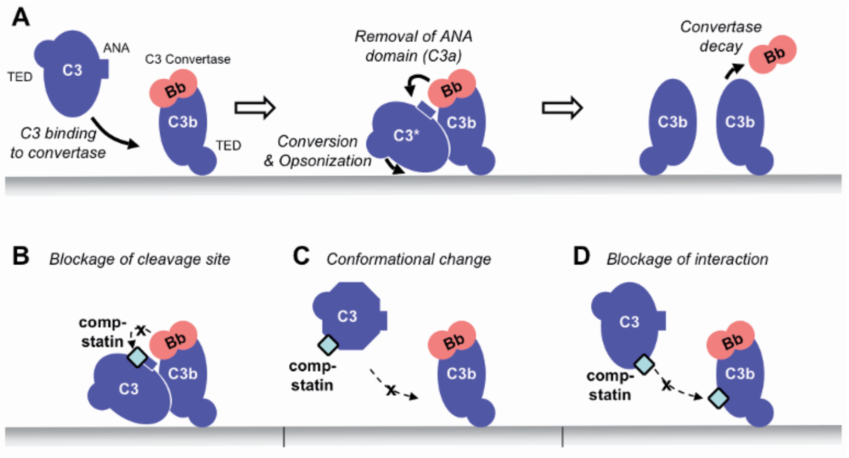


Fig. 3 In a current model of C3 activation, native C3 interacts with the surface-bound C3 convertase (C3bBb in case of the alternative pathway) and brings the anaphylatoxin domain (ANA) in close proximity to the enzymatic site on Bb. After enzymatic removal of ANA (i.e. C3a), the thioester domain (TED) undergoes a large relocation and exposes the active thioester, which leads the deposition of the newly generated C3b on the surface (**a**). In principle, compstatin may inhibit this process in several ways: (**b**) by sterically hinder the access of the convertase to the scissile bond; (**c**) by inducing a conformational change in C3 that prevents binding and/or cleavage by the convertase; or (**d**) by blocking the initial interaction between native C3 and the convertase-associated C3b

more plausible explanations. However, neither hypothesis could be undermined substantially without structural information (see Chap. 4).

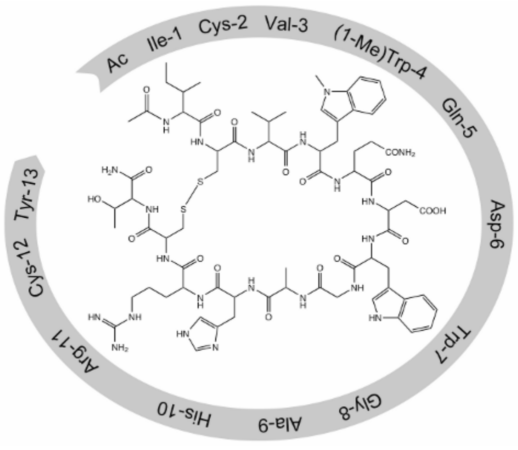
Surprisingly, compstatin features a high selectivity for human and primate C3. While binding to C3 from baboons and other primates showed comparable interaction profiles as its human counterpart, no binding was detected for C3 from lower mammalian species such as mice, rats, guinea pigs, rabbits, or pigs (Sahu et al. 1996, 2003). While this selectivity has direct consequences for the clinical development of the peptide (see Chap. 6), the recent description of the molecular foundation for this selectivity may allow circumventing this issue (see Chap. 4).

Owing to its strong inhibition at the C3 level of complement, compstatin nowadays is not only a promising candidate for the development of complement-targeting drugs (see Chaps. 6 and 7) but also an indispensable tool for studying the role of complement in a variety of biological systems (examples in (Gronroos et al. 2005; Nielsen et al. 2007; Pedersen et al. 2007; Ritis et al. 2006)).

3 Tuning the Structure

While the original compstatin peptide represented a highly selective and potent lead structure itself, the combination of rational, combinatorial, and computational optimization methods was able to drastically improve its activity (Fig. 2). Early approaches in this direction have been reviewed previously (Holland et al. 2004; Morikis and Lambris 2002; Morikis et al. 2004). An initial alanine scan experiment identified Val-3 and the stretch between positions 5 and 8 as essential for complement inhibition (Fig. 2c). The study also showed that the flanking residues (Ile-1 and Thr-13) contribute to the overall activity since their removal led to a threefold decrease in activity (Morikis et al. 1998). The NMR-derived structure of compstatin (PDB code 1A1P; (Morikis et al. 1998)) offered first information about the conformation of the peptide in solution. The disulfide bond was found to form a hydrophobic cluster around the termini (Fig. 6a) and to restrain the conformational flexibility. As a consequence, residues at positions 5–8 (Gln-Asp-Trp-Gly) were arranged in a type I β-turn (Fig. 6a), which is highly prevalent in naturally occurring proteins and peptides and is often involved in molecular interactions (Rotondi and Gierasch 2006). Maintenance of this turn structure seemed to be essential for the functionality of compstatin, since both the linearization as well as the replacement of residues 5–8 by alanine led to a dramatic loss in activity (Morikis et al. 1998). Similarly, the introduction of D-amino acids into the compstatin ring in an attempt to reinforce the β-turn conformation led to a complete loss of activity in most cases (Furlong et al. 2000). Finally, a cycle size of 11 residues was shown to be mandatory since none of the shorter deletion analogs maintained activity (Sahu et al. 2000).

In contrast to the restriction concerning the ring structure, compstatin was found to be more tolerant to the replacement or modification of the terminal residues (Ile-1, Thr-13), which may be beneficial for improving the stability towards exopeptidases (Furlong et al. 2000; Sahu et al. 2000). Surprisingly, acetylation of the N-terminus did not only improve the degradation profile (see Chap. 7) but also increased complement inhibition potency by a factor of 3 (Fig. 2d) (Sahu et al. 2000). This effect has later been attributed to electrostatic effects, i.e. the reduction of charges at the N-terminus (Soulika et al. 2003). Extensive studies on the contributions of the turn-cluster arrangement revealed a more potent lead structure (Fig. 2e), in which His-9 had been replaced by alanine in order to reduce bulkiness and provide more conformational flexibility (Morikis et al. 2002). Using a two-stage computational protein design approach, the replacement of Val-4 by tyrosine was predicted to increase the fold stability and therefore improve peptide activity (Klepeis et al. 2004). Indeed, the synthesized peptide showed a sevenfold increase in potency (Fig. 2f). An impressive demonstration of rational design at residue 4 was presented, when this position was screened for aromatic amino acids and yielded a tryptophan analog (Fig. 2g) with 45-fold increased potency (Mallik et al. 2005).



4(1MeW)7W compstatin

MW = 1628 g/mol
CLogP = -3.85

IC_{50} = 0.205 μM
rIC_{50} = 264

K_D (C3) = 15 nM

Fig. 4 Optimized lead structure of compstatin (Ac-ICV(1MeW)QDWGAHRCT-NH_2) with key biophysical properties. The tridecapeptide chain is synthesized using solid phase peptide synthesis and cyclized via a disulfide bond between the cysteine residues at positions 2 and 12. The IC_{50} values represent inhibition of the classical pathway of complement activation both as absolute value and relative to the original compstatin

By introducing hydrophobic, non-natural amino acids at the same position (e.g. 2-naphthylalanine), this effect could even be increased to a nearly 100-fold activity (Mallik et al. 2005). Further thermodynamic analyses indicated that the C3-compstatin interaction is largely driven by enthalpic contributions. In this context, the beneficial effects of a valine-to-tryptophan substitution at position 4 (and also histidine-to-alanine at position 9) could be linked to an increased enthalpy and binding affinity to C3 (Katragadda et al. 2004).

In an attempt to make the production of compstatin more economical, a bacterial expression system was successfully established in *Escherichia coli,* which generated high yields of compstatin analogs with an N-terminal glycine instead of the acetyl group (Katragadda and Lambris 2006). Incorporation of substituted tryptophan derivatives resulted in a highly active analog that carried two 6-fluorotryptophan residues at positions 4 and 7 (Fig. 2h). Encouraged by these improvements, the role of hydrophobic contributions and hydrogen bonding at position 4 and 7 was further elucidated using various tryptophan derivatives (and naphthylalanine) in combination with ELISA and calorimetric analysis (Katragadda et al. 2006). While increasing the hydrophobicity at position 4 was found to be generally beneficial, the introduction of 1-methyl tryptophan had the most prominent effect. With a C3 binding affinity of 15 nM and a 267-fold increased complement inhibition potency compared to the original peptide, 4(1MeW)7W compstatin is the most active analog published so far (Figs. 2i and 4).

The arsenal of active and inactive analogs in combination with the known solution structures led to the development of sophisticated in silico models based on QSAR, molecular dynamics, or conformational space annealing (Mallik et al. 2003; Mallik and Morikis 2005; Mulakala et al. 2007; Song et al. 2005; Tamamis et al. 2007). However, the most important breakthrough was reached with the release of the first co-crystal structure between a compstatin analog and C3c (see Chap. 4). These developments will augment our understanding about the binding mode and function of compstatin and pave the way to the development of analogs with even higher potency.

4 Exploring the Binding Site and Mode

The initial studies with compstatin already provided important hints for the localization of its binding site on C3, since both the active phage clone and the isolated peptide only bound to C3 fragments that contained the C3c portion of the protein (Sahu et al. 1996). Biophysical data confirmed a single binding site for compstatin on C3, while deviation from a simple kinetic 1:1 model in SPR indicated a conformational rearrangement of the peptide and/or protein upon binding (Katragadda et al. 2004; Sahu et al. 2000). Later, the binding region could be narrowed down to the C-terminal 40-kDa part of C3, which includes several macroglobulin domains (MG 3–6^{β}) as well as the linker (LNK) domain (Soulika et al. 2006). But even with this information, a clear description of the binding mode has proven difficult. The release of several key crystal structures such as C3, C3b, and C3c (Janssen et al. 2006; Janssen and Gros 2007; Wiesmann et al. 2006) offered an unprecedented insight into the complement activation process. After removal of the ANA domain (C3a) by the C3 convertase, the C3 structure undergoes a large structural rearrangement, in which the thioester-containing domain (TED) is completely relocated (Fig. 5). Interestingly, the core ring formed by the MG3-MG6 domains, which also included the proposed compstatin binding site, remained conformationally stable throughout these transformations. Hence, one of the most important breakthroughs in compstatin development was reached with the recent publication of a co-crystal structure of the compstatin analog 4W9A (Fig. 2g) and the C3c protein (PDB code 2QKI; Janssen et al. 2007). Alongside with indispensable information about this peptide-protein interaction, the structure also revealed a number of surprises.

First of all, the single binding site of compstatin could be localized to a shallow groove between the MG4 and MG5 domains in the stable β-ring of C3c. While this finding confirmed previous predictions of the binding area and a 1:1 interaction mode, it also had consequences for the peptide's potential mechanism of action. When assuming the same site in native C3, compstatin is too far distant from the ANA domain to allow a direct steric inhibition of C3a cleavage by the C3 convertase (Figs. 3b and 5). Similarly, an interference with the translocation of the TED domain is unlikely based on the location of the binding site. In addition, the

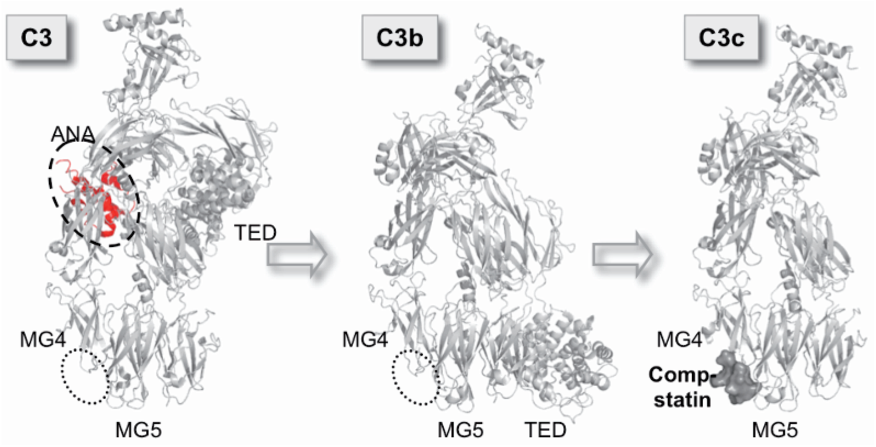
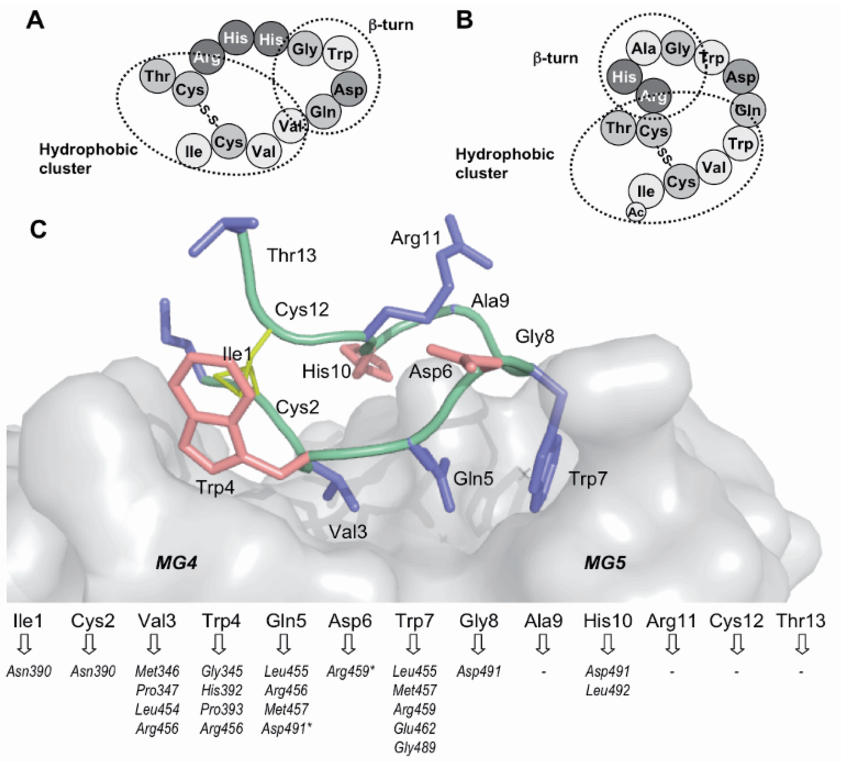


Fig. 5 The co-crystal structure of compstatin with C3c (right; PDB code 2QKI) shows that the peptidic inhibitor binds to a shallow site between macroglobulin domains 4 and 5 (MG4/5) in the core ring of C3c. Interestingly, the corresponding binding area (dotted circle) in native C3 (left; PDB code 2A73) is clearly distant from the C3a domain (ANA) that is cleaved during the activation of C3 to C3b. Furthermore, the site is nearly unaffected by the massive conformational changes during the conversion to C3b (middle; PBD code 2I07) that lead to a repositioning of the TED domain

discovery of a unique binding pocket away from other known C3 binding sites also confirms earlier observations that compstatin does not interfere with complement regulation proteins or formation of the convertase (Sahu et al. 1996).

Secondly, no large domain rearrangement could be detected between bound and unbound C3c (Janssen et al. 2007). While only a co-crystal of compstatin with native C3 may disqualify a conformational change of C3 as the functional mechanism (Fig. 3c), even the current C3c-based structure makes this option largely implausible. This leaves the steric hindrance of the C3 substrate binding to convertase-based C3b as the most likely explanation (Fig. 3d). Further support for this hypothesis came from the crystal of C3b, in which a C3b-C3b crystallographic symmetry-related contact area that includes the compstatin binding site was observed (Janssen et al. 2006, 2007). The authors therefore suggested, that this area may be involved in C3b-dimerization or C3-C3b binding, and that compstatin may interfere with these interactions (Janssen et al. 2007). A similar mechanism has been proposed for another complement inhibitor, the physiological receptor CRIg, that binds C3b at the same face of the β-chain (although via domains MG3/MG6) and selectively inhibits the alternative pathway convertases (Katschke et al. 2007; Wiesmann et al. 2006).



Ile1	Cys2	Val3	Trp4	Gln5	Asp6	Trp7	Gly8	Ala9	His10	Arg11	Cys12	Thr13
⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩	⇩
Asn390	*Asn390*	*Met346* *Pro347* *Leu454* *Arg456*	*Gly345* *His392* *Pro393* *Arg456*	*Leu455* *Arg456* *Met457* *Asp491**	*Arg459**	*Leu455* *Met457* *Arg459* *Glu462* *Gly489*	*Asp491*	-	*Asp491* *Leu492*	-	-	-

Fig. 6 The special arrangement and conformation of compstatin in solution (**a**) and in the bound form (**b**) are clearly distinct, which indicates an induced fit upon binding. During this process, the β-turn shifts from residues 5–8 to 8–11. In the co-crystal structure of 4W9A-compstatin and C3c (**c**; PDB code 2QKI), most of the residues in compstatin (main chain as ribbon, side chains as sticks) make contacts with C3c residues that form a shallow groove between domains MG4 and MG5 (surface representation), while the charged/polar amino acids Asp-6, Arg-11, and Thr-13 point out into the solvent. *Gln-5/Asp-491 and Asp-6/Arg-459 were mediated by water and bromine in the co-crystal, respectively

Although compstatin did not change the domain arrangement of C3c, there were still some structural adaptations within the binding site. Strikingly, the side chain arrangement did more closely resemble the local structure of native C3 than that of unbound C3c, which may also explain the higher relative affinity of compstatin to C3 (Janssen et al. 2007). Even more surprising, however, were the findings on the structure of bound compstatin, which was clearly distinct from the published solution structure (Janssen et al. 2007; Morikis et al. 1998). While bound compstatin still contains a β-turn element, it now encompasses residues 8–11 (instead of amino acids 5–8 as in the unbound peptide). As a consequence, the overall shape of the peptide changed significantly. The MG4/5 binding groove encloses 40% of the molecular surface of compstatin with Val-3, Trp-4, Gln-5, Trp-7, Gly-8, and His-10 pointing towards the C3c surface and making hydrogen bonds and hydrophobic contacts through both main and side chain atoms. On the other hand, the polar or charged amino acids Asp-6, Asp-11, and Thr-13 are pointing away from C3c towards the solvent (Fig. 6c). Additional interactions, i.e. Gln-5/Asp-491 and Asp-6/Arg-459, may be mediated through water or bromine molecules, respectively. However, these interactions are contradicting between the structures in the asymmetric unit or may be an artifact of the crystallization conditions (Janssen et al. 2007).

Analysis of the binding site also explains the species specificity of compstatin. C3c residues Gly-345, His-392, Pro-393, Leu-454, and Arg-459 all show direct interaction with compstatin and are highly conserved in primates but not in other species (Janssen et al. 2007). Unfortunately, these large variations in the binding sites of different animals makes the development of a ‘universal’, less species-specific compstatin analog that could be used in a wide panel of disease models rather unlikely. On the other hand, the creation of transgenic animals (e.g. mice) with ‘humanized’ compstatin binding sites may develop into a potential approach to circumvent this problem and could provide a compstatin-sensitive animal model.

Finally, our knowledge of the binding pocket and the contribution of individual compstatin residues to the interactions greatly facilitate further development and rational design approaches towards its human target. Despite the major differences between the bound and solution structure of compstatin, many predictions from previous structure-function-studies were in good agreement with the co-crystal (Janssen et al. 2007) and these models may further support development efforts.

5 First Steps Towards Therapeutic Applications

The potential to block the central step of complement activation makes compstatin an attractive candidate for a therapeutic use in a variety of clinical setups (Holland et al. 2004; Ricklin and Lambris 2007a). First steps in this direction were undertaken by investigating its effect on complement activation during extracorporeal blood circulation. Many artificial surfaces (e.g. plastics) and biomaterials are known to activate complement and induce inflammatory reactions (Nilsson et al. 2007). During cardiopulmonary bypass (CBP) surgery or haemodialysis, where whole blood is circulated extracorporeally, complement activation is a major complication and can influence the clinical outcome. When compstatin was added to blood circulating in PVC tubes, all readouts (C3a and MAC generation, C3b deposition, expression of CD11b on polymorphonuclear leukocytes) were clearly reduced when compared to the linear control peptide (Nilsson et al. 1998). By selective inhibition of the classical and alternative pathways using Mg-EGTA and compstatin, respectively, the major contribution of biomaterial-induced complement activation could be attributed to the alternative pathway (Andersson et al. 2005). Extended studies using the PVC tube model showed that expression of CD11b on both granulocytes and monocytes and the formation of platelet-granulocyte conjugates was attenuated and that induction of leukotriene B4 was reduced by acetyl-compstatin, while the formation of monocyte-platelet conjugates and the release of myeloperoxidase, lactoferrin, thrombospondin, thromboxane B4, and prostaglandin E2 was not affected (Lappegard et al. 2004, 2005). Very recently, the anti-inflammatory effect of compstatin in this model was impressively confirmed by monitoring a panel of 27 inflammation mediators during biomaterial-activation of complement. Compstatin efficiently reduced the formation of 12 of the 14 mediators (e.g. IL-8, FGF, VEGF) that were induced by PVC tubes (Lappegard et al. 2007). During CBP surgery, complement activation is not only triggered by blood contact with artificial surfaces and reperfusion of the arrested heart, but also by the postoperative administration of protamine in order to neutralize heparin and restore blood coagulation (Cavarocchi et al. 1985). In a primate model of protamine/heparin-induced complement activation, a combined injection and infusion of compstatin effectively inhibited the generation of C3 activation products without influencing heart rate, blood pressure or hematological parameters (Soulika et al. 2000). In this context, the study demonstrated for the first time both the efficacy and safety of compstatin as a complement inhibitory drug in an in vivo animal model. The use of this compound to prevent biomaterial-induced complement activation was further undermined by a study showing a largely reduced activation of neutrophils (as assessed by expression of the Mac-1 receptor) when the peptide was added to polymer-exposed blood (Schmidt et al. 2003).

Beneficial effects have also been described for an ex vivo model of xenotransplantation. Complement-mediated inflammation plays an essential role in the pathophysiology of hyperacute rejection. In the study, the survival time of pig kidneys that were perfused with human blood was remarkably increased in the presence of compstatin compared to the control (380 and 90 min, respectively). Furthermore, the study also confirmed that compstatin indeed acts at the level of C3 in vivo, since only the concentrations of C3 fragments and MAC but not C1 and C4 were decreased in the compstatin group (Fiane et al. 1999a,b). In another transplantation model, compstatin helped to elucidate the role of complement in delayed xenograft rejection. For this purpose, the expression of E-selectin on porcine aortic endothelial cells that were stimulated with human serum was monitored in the presence of compstatin, C1-inhibitor as well as C5, C7, and fD-specific antibodies. While attenuation of C1, C5, and C7 inhibited E-selectin expression completely, compstatin led to a markedly decreased expression level. No effect was observed for the anti-fD antibody. Together, these data suggested that complement activation via the classical pathway is predominantly involved in the pathophysiology and that MAC mediates endothelial cell activation (Solvik et al. 2001).

In a whole-blood model of *E. coli*-induced inflammation, compstatin not only reduced the formation of various complement-activation products, but also drastically inhibited oxidative burst in granulocytes and monocytes as well as formation of interleukin (IL)-8 (but not IL-6 and IL-10). The authors concluded that complement is a primary inducer of inflammation and that its inhibition could serve as a promising approach for treating sepsis or inflammatory diseases (Mollnes et al. 2002). Cytokine production (IL-12p40) was also influenced by compstatin-mediated complement inhibition in mononuclear cells that were stimulated by immune complexes (Tejde et al. 2004). In this respect, compstatin may be beneficial for treating immune complex-associated diseases such as rheumatoid arthritis or systemic lupus erythematosus.

6 From Bench to Bedside: Clinical Development

Regardless convincing concepts, potent lead compounds, and promising preclinical data, the road from the experimental entity to the marketed drug is usually long and bumpy. Several complement-targeting therapeutics have already taken this road and many of them have been discontinued (Ricklin and Lambris 2007a). Besides a high efficacy, several parameters such as stability, safety, toxicity, or pharmacokinetics have to be considered in the development process. Even though there are no clinical data available for compstatin so far, there are nevertheless many indicators from in vitro, ex vivo, and in vivo studies that constitute a high degree of drug-likeliness to the peptide.

Degradation, metabolism, and rapid (renal) excretion are some of the recurrent challenges in the development of peptidic drugs, which often lead to short plasma half-lives (Sato et al. 2006). In case of compstatin, the cyclic structure is largely responsible for a rather low degree of biotransformation in serum compared to previously investigated peptides for complement inhibition (Sahu et al. 2000). This high stability has been confirmed both in human and baboon plasma (Sahu et al. 2000; Soulika et al. 2000). Acetylation of the N-terminus even improved the stability in human plasma and resulted in a very low inactivation rate of 0.03%/min (Sahu et al. 2000). So far, no pharmacokinetic data regarding distribution and excretion are available for compstatin. However, since the peptide is likely to be used in acute phase situations as well as local or extracorporeal applications, a short plasma half-live may not be as limiting as in the case of chronic, systemic administration. On the other hand, unfavorable pharmacokinetic properties could potentially be modulated by chemical modifications such as PEG-ylation (Sato et al. 2006).

The high species specificity of compstatin so far limited the preclinical development in animal models. Complement inhibition has only been achieved in human and primate systems (e.g. baboon or rhesus monkey) but not for rodents (mouse, rat, guinea pig, rabbit) or pigs (Sahu et al. 2003). While residual activity has been observed in dog blood, compstatin activity was clearly reduced by two orders of magnitude (Furlong et al. 2000). The peptide was found to be highly specific for human C3 and did not affect blood clotting or the activity of serine proteases such as thrombin, trypsin, or elastase (Furlong et al. 2000). No adverse effects on heart rate or hematological parameters have been detected during in vivo experiments with baboons (Soulika et al. 2000). Furthermore, compstatin did not show any cytotoxicity towards polymorphonuclear cells (Furlong et al. 2000).

In its first attempt to enter clinical development, compstatin was licensed from the University of Pennsylvania by Potentia Pharmaceuticals Inc. in 1996 (Potentia 2006). Recently, the company announced the initiation of phase I clinical trials for the treatment of both dry and wet forms of age-related macular degeneration (AMD) (Potentia 2007). Since AMD represents the primary cause of blindness in industrialized nations, therapies that are able to prevent, retard, or even reverse this disease are highly desirable. While the formation of lipoprotein-rich deposits (drusen) and penetration of new blood vessels in the area between the choroids and the retinal pigmented epithelium has long been identified as the primary cause of the impaired vision, the contributions of different cellular pathways is still matter of investigation. Vascular endothelial growth factor (VEGF) has been found to be a key element in the neovascularization, and anti-VEGF antibody fragments (ranibizumab; Lucentis®, Genentech Inc.) or aptamers (pegaptanib; Macugen®, Eyetech Inc.) have already been introduced as treatment options for wet AMD (Blick et al. 2007). Although these drugs show high efficacy, they also require frequent injections into the eye and are very expensive. Despite an early intervention would be beneficial for reducing the risk of disease progression, there are currently no therapeutics available to treat the more prevalent dry form of AMD (Petrukhin 2007). The presence of several complement components (e.g. C3, C5, MAC) in the drusen, the correlation with mutations or polymorphisms in several complement genes (e.g. for factor H), and reported promoting effects of anaphylatoxins (C3a, C5a) to the neovascularization all indicate an involvement of the complement system in AMD pathogenesis (Jha et al. 2007; Markiewski and Lambris 2007; Petrukhin 2007). Local inhibition of complement activation is therefore considered a promising approach for treating both forms of AMD, and compstatin is the first complement-specific candidate that enters clinical trials for this indication. While other AMD drugs like Macugen or Lucentis have to be administered by monthly injections into the eye, Potentia is developing a intravitreal delivery system that should release a continuous therapeutic dose of a compstatin analog (POT-4) during a period of at least 12 months. Considering the novel form of administration and the first use of compstatin for human in vivo studies, the results from this clinical trial are keenly awaited.

7 Conclusions and Perspectives

Its target at the core of complement and its favorable properties as a rather small and stable peptide drug uniquely position compstatin in the repertoire of complement-specific therapeutics. The first clinical studies for an application in AMD is considered an important indicator for its potential as a drug and will certainly help collecting pharmacokinetic and toxicological parameters for this compound. Even though the outcome concerning efficacy is difficult to predict in view of the unknown impact of complement in the pathogenesis of AMD, a favorable safety profile may pave the way to the development of alternative clinical applications of compstatin. In this context, the prevention of complement-induced

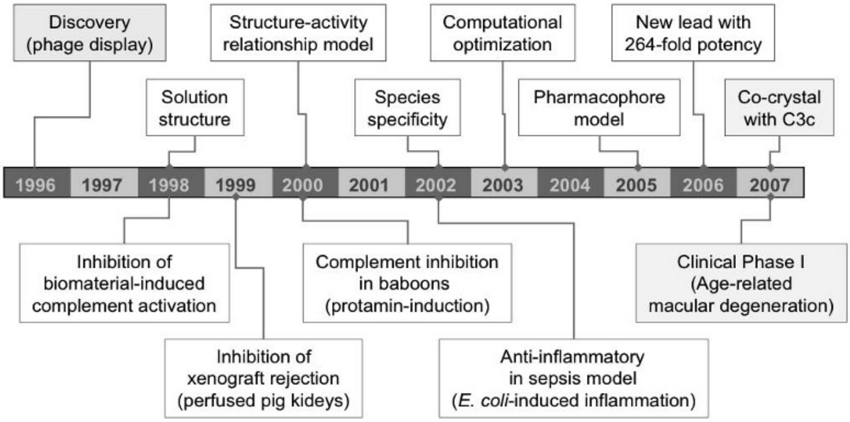


Fig. 7 Milestones in the first decade of compstatin development with an emphasis on structural/analytical (*upper part*) and clinical (*lower part*) improvements and findings

inflammation during haemodialysis or CBP surgery could be promising candidates, even more since compstatin already has proven efficacy in the baboon model and biomaterial-related studies (see above).

Meanwhile, the structural development has gained new impetus with the C3c/compstatin co-crystal. The detailed knowledge of the binding site and contact residues will allow a rational drug design. Alongside with an optimization of the peptide sequence, a transition to smaller, non-peptidic structures may allow to improve the drug-like properties of the compound. For this purpose, β-turn mimetics could provide a starting point: owing to their high prevalence in protein interaction sites, β-turn peptides and mimetics thereof are regarded as promising scaffolds in drug design (Kee and Jois 2003). Considering the proposed mechanism of action by blocking the initial interaction between C3 and the convertase, compstatin could be an interesting addition to the emerging class of low-molecular-weight protein-protein interaction inhibitor drugs (Wells and McClendon 2007). While the current molecular mass (1600 Da) is slightly higher than comparable compounds in this class (500–900 Da), compstatin already has a promising ligand efficiency (0.09 kcal per mole per non-hydrogen atom) and has certainly found a ‘hotspot’ in the C3 activation process.

Compared with other complement therapeutics in late preclinical development or in clinical trial, the way of acting directly on the native C3 protein is rather unique. However, a similar strategy has been described in case of complement evasion by human pathogens (Lambris et al. 2008). In particular, the extracellular fibrinogen-binding protein (Efb) and the related Efb-homologous protein (Ehp) from *Staphylococcus aureus* have been found to efficiently inhibit complement activation by binding to native C3 and all its fragments that contain the thioester domain (Hammel et al. 2007a,b). This binding induces a conformational change in both C3 and C3b and therefore influences the conversion of C3 and C5 by the corresponding convertases (Hammel et al. 2007b; Jongerius et al. 2007). While the bacterial proteins themselves may be too immunogenic for a direct use as therapeutics, they may be used as templates for developing complement-inhibiting drugs.

Finally, the success story of compstatin development illustrates the potential of academia-centered drug discovery efforts. Even without the financial and logistic resources of big pharmaceutical companies, the careful selection of screening methods, the combination of powerful techniques, and a diversified net of collaborations may produce valuable lead compounds for clinical development. Supporting incentives by governmental science foundations, such as the 'Rapid Access to Interventional Development' pilot program (RAID) by the National Institutes of Health (NIH), will clearly further these efforts in the future.

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