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Selective Inhibition of the Lectin Pathway of Complement with Phage Display Selected Peptides against Mannose-Binding Lectin-Associated Serine Protease (MASP)-1 and -2: Significant Contribution of MASP-1 to Lectin Pathway Activation

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The complement system, an essential part of the innate immune system, can be activated through three distinct routes: the classical, the alternative, and the lectin pathways. The contribution of individual activation pathways to different biological processes can be assessed by using pathway-selective inhibitors. In this paper, we report lectin pathway-specific short peptide inhibitors developed by phage display against mannose-binding lectin-associated serine proteases (MASPs), MASP-1 and MASP-2. On the basis of the selected peptide sequences, two 14-mer peptides, designated as sunflower MASP inhibitor (SFMI)-1 and SFMI-2, were produced and characterized. SFMI-1 inhibits both MASP-1 and MASP-2 with a K_I of 65 and 1030 nM, respectively, whereas SFMI-2 inhibits only MASP-2 with a K_I of 180 nM. Both peptides block the lectin pathway activation completely while leaving the classical and the alternative routes intact and fully functional, demonstrating that of all complement proteases only MASP-1 and/or MASP-2 are inhibited by these peptides. In a C4 deposition inhibitor assay using preactivated MASP-2, SFMI-2 is 10-fold more effective than SFMI-1 in accordance with the fact that SFMI-2 is a more potent inhibitor of MASP-2. Surprisingly, however, out of the two peptides, SFMI-1 is much more effective in preventing C3 and C4 deposition when normal human serum containing zymogen MASPs is used. This suggests that MASP-1 has a crucial role in the initiation steps of lectin pathway activation most probably by activating MASP-2. Because the lectin pathway has been implicated in several life-threatening pathological states, these inhibitors should be considered as lead compounds toward developing lectin pathway blocking therapeutics. ***The Journal of Immunology*****, 2010, 185: 4169–4178.**

The complement system is an important component of the innate immunity. It consists of ~30 proteins (circulating in the serum or membrane bound) forming a sophisticated molecular network capable of recognizing, tagging, and eliminating invading pathogen microorganisms and altered host cells (e.g., apoptotic and necrotic cells) via Ab-independent mechanisms (1). Thus, the complement system provides the first line of defense before the adaptive immune response builds up. Moreover, the complement system bridges the innate and adaptive immunity, because the activated complement components facilitate the phagocytosis of pathogens by the host's leukocytes and initiate inflammatory reactions by recruiting and stimulating the cellular elements of the immune system.

Key components of the complement system are serine proteases (SPs) that are present in the circulation as zymogens (2). During complement activation, these proteases activate each other in a cascade-like manner. There are three ways through which the complement system can be activated: the classical, the lectin, and the alternative pathway. In the case of the classical and the lectin pathways, collagen-containing pattern recognition molecules bind to Ag–Ab complexes and carbohydrate residues, respectively, on the surface of the pathogenic cells. Upon this binding, the recognition molecule-associated SPs become activated and initiate the proteolytic cascade (3). The activation of the alternative pathway is initiated by slow and spontaneous deposition of C3 onto foreign surfaces, although recently it has been shown that properdin can act as a recognition component in this pathway (4). Because all three pathways result in the activation of C3, the alternative pathway functions as an amplification loop for the other two pathways.

C1q is the only known recognition molecule of the classical pathway. It consists of six globular heads binding to the IgG or IgM portion of immune complexes and six collagen-like arms, which bind the SP components (two C1r and two C1s zymogens) forming the C1 complex. C1r is capable of autoactivating and consequently

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Abbreviations used in this paper: APTT, activated partial thromboplastin time; IR, ischemia-reperfusion; MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; PT, prothrombin time; SFMI, sunflower mannose-binding lectin-associated serine protease inhibitor; SFTI, sunflower trypsin inhibitor; SGCI, *Schistocerca gregaria* chymotrypsin inhibitor; SP, serine protease; TT, thrombin time.

activating C1s via limited proteolysis. Activated C1s cleaves C4 and C2, the components of the C3-convertase complex (C4b2a).

In the case of the lectin pathway, the system is much more complex, and there are many open and debated issues concerning the composition and stoichiometry of the initiation complexes and the roles of the associated SPs. To date, four pattern recognition molecules have been identified: mannose-binding lectin (MBL) (5) and three ficolins (M-, L-, and H-ficolin, also called ficolin-1, -2, and -3, respectively) (6). These molecules bind to arrays of carbohydrates or acetylated compounds on the cell surface. There are three proteases (mannose-binding lectin-associated SP [MASP]-1, -2, and -3) (7) and two noncatalytic molecules (MBL-associated protein 19 and MBL-associated protein 44) (8), which associate with the recognition molecules to form the initiation complexes of the lectin pathway. In vitro experiments demonstrated that a complex consisting of an MBL or ficolin molecule and a MASP-2 homodimer is able to initiate the complement cascade (9). MASP-2 combines the enzymatic properties of C1r and C1s: it can autoactivate and cleave C4 and C2.

The physiological role of MASP-1 is not so well established, although several potential substrates have been suggested, including C2 (10), C3 (11), fibrinogen, factor XIII (12, 13), and protease-activated receptor 4 (14). Experiments with MASP-1 knockout mice and with depleted sera showed that MASP-1 enhances the efficiency of the lectin pathway activation, probably through facilitating the autoactivation of MASP-2 (15) and/or cooperating with MASP-2 to generate C3-convertase (C4b2a) through cleavage of C2 (16).

The role of MASP-3, MBL-associated protein 19, and MBL-associated protein 44 is even more enigmatic, although it seems likely that these proteins do not activate the lectin pathway, rather they downregulate the activity of MASP-2 and probably that of MASP-1 (17, 18).

The complement system is essential for fighting against infection and maintaining the immune homeostasis. Furthermore, it is now becoming evident that the complement system has important roles in diverse physiological processes that are distinct from the classical immunological functions (19). A striking example is that the classical pathway plays a crucial role in the elimination of inappropriate synapses in the postnatal brain and retina (20).

Erroneous or uncontrolled activation of the complement system contributes to the development of numerous diseases (21). A prominent example is the ischemia-reperfusion (IR) injury, which is responsible for tissue damage in a number of clinical conditions including myocardial infarction, stoke, and transplant rejection (22). Therefore, inhibition of pathological complement activation seems to be a promising therapeutic strategy (23). Inhibition of the SPs is an obvious choice to effectively block the complement cascade (24). Several attempts have been made to develop small-molecule inhibitors against complement proteases (25). Although these inhibitors (e.g., nafamostat also called FUT-175) have anticomplement and anti-inflammatory activity (26), the lack of specificity has hindered successful drug development.

The purpose of our work was to develop highly specific low m.w. inhibitors against complement proteases by in vitro evolution technology: phage display. Our target proteases were the MASP-1 and MASP-2, the initiator proteases of the lectin pathway. As a starting point for inhibitor selection, we used the sunflower trypsin inhibitor (SFTI), which has the shortest scaffold (only 14 aa) among the known natural SP inhibitors (27–30). The selected peptides inhibit exclusively the lectin pathway among the three activation pathways of complement. Specific inhibitors against MASP-1 and MASP-2, besides being lead molecules for drug development, are unique tools for studying the physiologic and pathophysiologic roles of the lectin pathway. Using such inhibitors, one could assess the contribution of the lectin pathway to different biological processes without compromising the activity of the other two pathways. Moreover, one could study the individual roles of MASP-1 and MASP-2 in the lectin pathway activation.

Materials and Methods

Reagents

All common laboratory reagents were from Sigma-Aldrich (St. Louis, MO) and Merck (Whitehouse, NJ). Maxisorp plates were from Nunc (Roskilde, Denmark). Restriction endonucleases and DNA-modifying enzymes were from New England Biolabs (Beverly, MA) and Fermentas (St. Leon-Rot, Germany). The bacterial strains XL1Blue and CJ236 were from Stratagene (La Jolla, CA) and New England Biolabs, respectively, whereas SS320 was a gift from Genentech (South San Francisco, CA). The M13-KO7 helper phage was from New England Biolabs. PCR products were isolated by the GenElute PCR Clean Up kit from Sigma-Aldrich.

The sunflower MBL-associated SP inhibitor library

The sunflower MBL-associated SP inhibitor (SFMI) library displaying phagemid vector pSFMI-prolib was constructed from pBluescript II KS(−) (Stratagene), pMal-p2× (New England Biolabs), and our previous *Schistocerca gregaria* chymotrypsin inhibitor (SGCI) displaying vector (31) by the combination of various DNA manipulation techniques such as PCR, digestion by restriction enzymes, ligation, and synthetic adapters. The sequence of the final vector pSFMI-prolib is deposited in the GenBank under the accession number HM356027 (www.ncbi.nlm.nih.gov/genbank). The vector contains a FLAG epitope cassette, two serine-glycine linkers, an SGCI module to ensure monovalent display of the SFMI library, and the bacteriophage p8 coat protein coding sequence (Fig. 1*A*). We proved that SGCI does not interact with the MASP enzymes (data not shown). The FLAG-tag allows for testing the display level of the SFMI peptides independently of their inhibitory properties.

The SFMI library was introduced into pSFMI-prolib as a PCR fragment, and the codon randomization is indicated using International Union of Pure and Applied Chemistry nomenclature. The SFMI_lib primer contains six hard randomized (NNK) codons, whereas the binary ARA randomization allows for only Arg or Lys at the P1 position. The sequence of the SFMI_lib primer is 5′-ccgccgcctcggcgctagca ggt nnk tgt nnk ara nnk nnk cct ccg nnk tgt nnk ccg gat ggcgggtcgggtggatccggtgg-3′. The SFMI coding segment is underlined. The other PCR primer, named p8_3′ (sequence: 5′-gctagttattgctcagcggtggcttgctttcgaggtgaatttc-3′), anneals to the p8 coat protein coding region. The above primer pair and the pSFMI-prolib vector as template were used in a PCR generating the SFMI library containing PCR product. This product was purified, digested with NheI and Acc65I enzymes and ligated into the NheI/Acc65I-treated pSFMI-prolib vector. The sequence of the library-carrying plasmid, named pSFMI-lib, was also deposited in the GenBank under the accession number HM356026 (www.ncbi.nlm.nih.gov/genbank). SS320 cells were transformed with pSFMI-lib by electroporation yielding a library of 2×10^9 SFMI-phage clones. Cells were infected with M13 KO7 helper phages and grew overnight. Phage particles were isolated as described previously (32).

Preparation of MASP-1 and MASP-2 catalytic fragments

The catalytic fragments containing the complement control protein 1-complement control protein 2-SP domains of MASP-1 and MASP-2 were used as targets. These fragments are unable to dimerize and bind to MBL but carry all the proteolytic activity and specificity of their respective enzymes. The catalytic fragments were produced as recombinant proteins and purified as already described elsewhere (10).

Selection of the SFMI library

Three independent selections on three different targets were performed. Nunc MaxiSorp ELISA plates were coated with MASP-1, MASP-2, or anti–Flag-tag Ab, respectively. Selection on the anti–Flag-tag Ab was done to assess display bias. The protein concentration was 20 μg/ml for the MASPs and 2 μg/ml for the Ab. Three selection rounds were carried out on each target separately as described previously (32). The eluted phage population was amplified in XL1 Blue cells superinfected with helper phage. Binding properties of individual SFMI-phage clones were tested in a phage-ELISA format as described elsewhere (32). MASP-selected SFMI-phage clones were tested both on their original target as well as on the other MASP enzyme to detect cross-reactivity.

Sequence analysis

The genes of individual SFMI-phage clones producing an ELISA signal on their target 3-fold above background (measured on BSA containing wells) were sequenced by the Big Dye Terminator version 3.1 cycle Sequencing Kit (Applied Biosystems). To eliminate the effects of display bias, the amino acid frequencies determined for the MASP-1 and MASP-2 binding SFMI-phage populations were normalized by data from the anti–Flag-tag selected population. For a logo representation of the normalized results, an input sequence data set containing 100 sequences was generated. This data set represented the normalized amino acid frequencies at each randomized positions. The sequence logos were generated by the online application WebLogo (33) and are available at the following Web site: http://weblogo.berkeley.edu/logo.cgi.

Peptide synthesis

Solid-phase peptide synthesis was performed using the standard Fmoc [*N*-(9 fluorenyl)methoxycarbonyl] chemistry (34). The lyophilized product was dissolved in water at 0.1 mg/ml concentration, and the formation of the single native disulfide bond was facilitated via air oxidization by stirring the solution at pH 8–9 (adjusted with adding *N*,*N*-diisopropyl-ethylamine). The final product was purified by reversed-phase HPLC.

SFTI is produced in nature as a cyclic peptide lacking N or C termini (30). For the SFMI-1 variant, this cyclic version was synthetically produced by introducing a peptide bond between the N and C termini. In this case, synthesis of the peptide was carried out on 2-chlorotritil resin, and then, the product was cleaved from the resin by 1% trifluoroacetic acid in dichloromethane. Under these conditions, the side chain protecting groups remain intact. After a reversed-phase HPLC purification, the peptide was dissolved in dimethylformamide to a final concentration of 0.1 mM. Then, 1.1 equivalence of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triasolo[4,5-b] pyridinium-3-oxide hexafluorophosphate and 3.0 equivalence of diisopropylethylamine were added, and the solution was stirred at room temperature. The process of cyclization was followed on reversed-phase HPLC.

All synthetic peptides were analyzed by mass spectrometry performed on a HP1100 series HPLC-electrospray ionization-mass spectrometry system using flow injection method with a 10 mM ammonium formiate buffer (pH 3.5). The total ion current chromatogram was obtained in positive ion mode by scanning in the 300–2000 mass/charge range. The mass information was evaluated with the Agilent ChemStation software. The theoretical and the measured mass values of all peptides were in perfect agreement.

WiELISA

The WiELISA kit (COMPL300; Wieslab, Lund, Sweden) was originally developed for testing the capacity of human serum to be activated separately through the three complement activation pathways (35). We applied this kit to test the pathway-selective inhibitory properties of our MASP inhibitor peptides. Following the manufacturer's guidelines, blood samples were incubated at room temperature for 1 h, and aliquots of normal human serum were prepared and kept frozen at −80°C. Serum was diluted with the provided buffers according to the manual and incubated at room temperature for 20 min. Then, serial dilution of the inhibitors was added to the serum, and the samples were further incubated for another 20 min and transferred to the WiELISA plate. The same serum sample was used for all measurements, and two parallels were measured for each data point. In all three complement activation assays (the classical, the alternative, and the lectin route), the level of serum activity in the presence of the SFMI peptide was expressed as a percentage of the corresponding activity measured in the absence of the inhibitor.

Inhibitory constant measurements

The equilibrium K_Is of the SFMI peptides were determined on four different enzymes: MASP-1, MASP-2, trypsin, and thrombin. A method developed for determining the equilibrium K_I values of tight binding inhibitors was applied (36). A thioesther substrate, Z-L-LysSBzl hydrochloride (C3647; Sigma-Aldrich), was used in the photometric assay. MASP-1, MASP-2, or trypsin was incubated with serial dilutions of the inhibitors for 1 h at room temperature. The reactions were conducted in 1 ml final volume at room temperature in 20 mM HEPES, 145 mM NaCl, 5 mM $CaCl_2$, and 0.05% Triton X-100 (pH 7.6). The sulfhydryl of the cleaved substrate reacted with the cosubstrate dithiopyridine (143057; Sigma-Aldrich) producing the product detected at 324 nm. Enzyme concentration of the stock protease solutions was determined by active site titration. In the case of trypsin and thrombin 4-methylumbelliferyl-4-guanidinobenzoate (51010; Sigma-Aldrich), whereas in the case of MASP-1 and MASP-2, a C1 inhibitor was used for the titration. The concentration of the free enzyme in the inhibitory assay was determined by the ratio of the residual enzyme activity (in the presence of inhibitor) and the total enzyme activity (in the absence of inhibitor). The K_I values were determined by nonlinear regression analysis using the following equation: $[E]/[E]_0 = 1 - ([E]_0 + [I]_0 + K_I - \mathrm{sqr}[([E]_0 + [I]_0 + K_I)^2 - 4 \times [E]_0 \times [I]_0)]/(2 \times [E]_0)$, where $[E]$, $[E]_0$, and $[I]_0$ represent the molar concentration of the free enzyme, the total enzyme, and the total inhibitor, respectively. Data were calculated from two parallel measurements. In the case of thrombin, the same experimental logic was applied, but Z-Gly-Arg-pNA was used as a substrate, and the cleavage product was detected at 405 nm.

Blood coagulation assays

Dose-dependent inhibitory capacities of the SFMI peptides to slow down the coagulation process were tested by measuring their effects in three standard coagulation assays, the thrombin time (TT), prothrombin time (PT), and the activated partial thromboplastin time (APTT). Blood was collected from a healthy individual by vein punction. The blood was treated with sodium citrate (3.8% w/v) and centrifuged. The inhibitory effects on the extrinsic pathway of blood coagulation were tested through (PT) measurements on the automated instrument Sysmex CA-500 (Sysmex, Kobe, Japan) with Innovin reagent (Dale Behring, Marburg, Germany).

The inhibition of the intrinsic pathway of blood coagulation was tested through (APTT) measurements, whereas the direct effects of the peptides on thrombin were determined through (TT) measurements. APTT and TT were assessed on an automated Coag-A-Mate MAX (BioMerieux, France) system with TriniClot reagent (Trinity Biotech, Wichlow, Ireland) and Reanal reagent (Reanal Finechemical, Budapest, Hungary).

The C3 deposition assay from serum

C3 deposition was assessed by ELISA as previously described (16) with some modifications. Greiner high-binding microtiter plates were coated with 10 μg/ml mannan in 15 mM Na_2CO_3 and 35 mM $NaHCO_3$ (pH 9.6) (coating buffer) overnight at 4°C. Wells were blocked with 0.5% BSA in TBS buffer for 2 h at 37°C, then washed with TBS containing 0.05% Tween 20 and 5 mM $CaCl_2$ (wash buffer). Serum was diluted 100-fold in barbital buffer and was preincubated in Eppendorf tubes with serial dilutions of the SFMI peptides for 30 min at 37°C. Samples were applied to the microtiter plate and further incubated for 30 min at 37°C. After washing, 2000-fold diluted anti-human C3c (catalog number A0062; DakoCytomation, Glostrup, Denmark) in TBS, 0.05% Tween 20, 5 mM $CaCl_2$, and 0.5% BSA (Ab dilution buffer) was added to the wells and incubated for 1 h at 37°C. The plate was washed, and 40,000-fold–diluted HRP-conjugated anti-rabbit Ab in Ab dilution buffer was added to the wells. After 30 min of incubation at 37°C and washing, the plate was developed as follows. First, 0.1 ml of 1 mg/ml *o*-phenylenediamine substrate in 50 mM citrate buffer (pH 5) containing 0.1% H_2O_2 was added, and after 5–7 min of incubation, the reaction was stopped with 50 μl/well 1 M H_2SO_4. The absorbance was read at 490 nm. The signal developed in the absence of SFMI inhibitor was considered as 100% activity.

Deposition of C4 by preactivated MASP-2

Measurements were carried out as described previously (37). A mannan-coated plate was incubated for 1 h at 37°C with serum diluted 1/1 (v/v) with 20 mM HEPES, 140 mM NaCl, and 10 mM $CaCl_2$ (pH 7.4) buffer. Wells were washed with high-salt buffer [20 mM HEPES, 1 M NaCl, 5 mM $CaCl_2$, and 0.1% Tween 20 (pH 7.4)]. To detect MASP-2 activity, 0.1 μg/well purified C4 and serial dilution of SFMI peptides were introduced to the wells in the same time and incubated for 1 h. Anti-human C4 Ab from Santa Cruz Biotechnology (Santa Cruz, CA; catalog number C4HYB162) was used in 250-fold dilution. Wells were developed using *o*-phenylenediamine substrate as described for the C3 deposition assay. The signal developed in the absence of SFMI inhibitor was considered as 100% activity.

Deposition of C4 from serum

The assay was performed as in the case of C3 deposition. Serum was diluted 60-fold with barbital buffer. Deposition was measured using anti-human C4c Ab (catalog number Q0369; DakoCytomation) in 1000-fold dilution.

Results

Design and construction of the inhibitory peptide-phage library

As we aimed to evolve selective MASP inhibitors, the peptide-phage library was designed based on our previous structural studies of the MASP-1 and MASP-2 enzymes (38–40). The MASPs

are trypsin-like enzymes; therefore, the library was designed based on a trypsin-inhibitor scaffold. The crystal structure of the free MASP-2 enzyme (40) showed that in the absence of bound substrate or inhibitor, the substrate binding groove on the enzyme is largely obstructed by long surface loops. Lacking structural information about the positions of these loops in a complex, we decided to design the library based on the smallest known natural trypsin inhibitor scaffold of the SFTI (27–30).

SFTI is a 14-aa peptide containing one disulfide bridge. It is produced in nature in a cyclic form inhibiting trypsin with subnanomolar equilibrium constant. Previous studies showed that an opened version of SFTI retains near wild-type affinity as long as the disulfide is intact (27, 41, 42). On the basis of its structure, SFTI mimics the protease interacting loop of the much larger canonical inhibitor scaffold of Bowman-Birk inhibitors (28).

The opened-up form of SFTI enabled its display on phage, whereas the comparative analysis of Bowman-Birk inhibitor sequences guided the library design (Fig. 1*C*). There are six conserved residues on the inhibitory loop of this family. Using the nomenclature of Schechter and Berger (43), the conserved residues are two cysteines at the P3 and P6′ positions, two prolines at P3′ and P4′, a serine at P1′, and a threonine at the P2 position (44–49). The cysteines and the prolines have only structural roles, but the P2 Thr has two functions. Its methyl group contacts the protease, whereas its hydroxyl group together with the hydroxyl of the P1′ Ser participates in an intramolecular H-bonding network stabilizing the interacting loop (46, 47). Because P2 Thr is a protease-contacting residue and because the P1′ Ser was shown not to be essential for the integrity of the protease-binding loop, we randomized both, despite being naturally conserved.

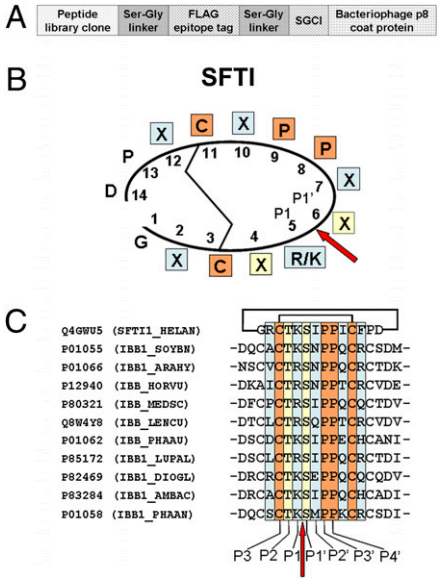

FIGURE 1. Construction of the phage-peptide library. *A*, Peptide library clones were fused to the p8 coat protein of the bacteriophage via linkers. The FLAG epitope tag is used to control display bias. SGCI ensures the monovalent appearance of peptides on each clone. The peptide library is based on the SFTI. *B*, Concept of the library design. Wild-type SFTI has a cyclic conformation with an internal disulfide bridge. In our peptide library, the ring was opened between Gly and Asp according to previous studies. The nonconserved randomized positions are shaded blue, the conserved randomized positions are shaded yellow, and the highly conserved positions are orange. *C*, Alignment of the SFTI sequence with homologous segments from Bowman-Birk inhibitors illustrates characteristic amino acid conservations. Coloring is the same as in *B*. UniProt numbers are indicated. Red arrow points to the protease cleavage site.

Thus, we kept the structurally important cysteines and prolines as well as the presumably nonprotease-contacting SFTI residues as wild-type and fully randomized the P4, P2, P1′, P2′, P5′, and P7′ positions. Because MASPs are trypsin-like enzymes, we allowed only Lys and Arg at the P1 (Fig. 1*B*).

For developing high-affinity binders, it is important to apply a monovalent display format; otherwise, multiple binding ("avidity") promotes the selection of inherently weak binders. In a previous study, we demonstrated that the display of a chymotrypsin inhibitor peptide, SGCI, on the p8 coat protein is monovalent (31). Therefore, we fused the SFTI library N-terminal to the SGCI–p8 construct, in which—in terms of MASP inhibition—SGCI is an indifferent module, ensuring monovalency (Fig. 1*A*). The construct also contained a FLAG epitope tag allowing for display level and display bias detection. In the randomization scheme, we introduced 32 different codons at six positions and two different ones at one position; thus, the theoretical diversity was $32^6 \times 2 = 2.15 \times 10^9$. The SFMI phage library contained $\sim 2 \times 10^9$ individual clones approaching the theoretical library size.

Three types of selections were performed independently on anti-epitope tag with the sequence DYKDDDDK (FLAG-tag) Ab, MASP-1, and MASP-2. Individual SFMI-phage clones were tested in a phage-ELISA format for binding to their original target. MASP-1–selected clones were also tested for binding to MASP-2 and vice verse to test for cross-specificity.

Analysis of the selected sequences reveals common and distinct sequence features and two functionally distinct populations

SFMI clones selected on MASP-2 fell into two functionally distinct groups. Clones belonging to the first group recognized only MASP-2, whereas those from the second group recognized both MASP enzymes. In contrast, MASP-1–selected SFMI clones fell into a single functional category, as all recognized MASP-2, as well. Thus, we found essentially two inhibitor populations: a MASP-2–selective one and a nonselective one recognizing both MASP enzymes; therefore, no MASP-1–selective clone was identified.

From the MASP-2 selection, 63 clones were sequenced yielding 21 unique sequences. Of these 12 were MASP-2 selective and 9 nonselective. From the MASP-1 selection, 20 clones were sequenced, and only 9 unique clones were found. Overall we identified 12 unique MASP-2–selective and 18 unique nonselective clones. The characteristic display bias normalized MASP-2–selective and nonselective sequence populations are illustrated as WebLogo diagrams (Fig. 2) (33).

As it was expected, at the P1 position, Arg was more prevalent than Lys, in good accordance with the known Arg preference of both MASP enzymes. At the P1′ position, the wild-type Ser was selected back. Quite unexpectedly, at the P2 position, a Ser was exclusively selected instead of the naturally conserved Thr. At the other randomized positions, the two populations showed distinct sequence features. A characteristic feature of the MASP-2–selective clones is the dominance of aromatic amino acids at P4 and P2′ and the preference for a Val at P5′. In the case of the nonselective population, P4 is dominantly Ile, there is no clear amino acid preference at P2′, and Ile is the most frequent residue at P5′.

Kinetic analysis of synthetic peptides designed based on the selected sequence patterns

First, two peptides were designed representing the consensus sequences of the two functionally distinct populations. SFMI-1 with the sequence GICSRSLPPICIPD represents the nonselective whereas SFMI-2 with the sequence GYCSRSYPPYCIPD the MASP-2–selective population. The three differences between the two sequences are underlined. Several variants of SFMI-1 and

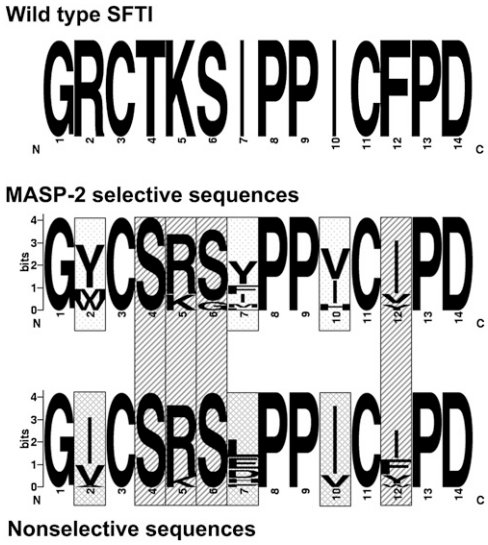

FIGURE 2. WebLogo diagram of phage-selected sequences. Framed letters indicate randomized positions. Position heights represent the degree of conservation. Letter heights indicate normalized amino acid frequencies. Sequences of phage-selected clones were grouped in two subsets according to different degree of MASP selectivity of the corresponding clones. Dotted areas indicate positions with amino acid distributions characteristic to MASP-2–selective clones. Checkered areas highlight amino acid distributions characteristic to the nonselective subset. Striped areas highlight positions with amino acid distributions shared by the two functionally distinct subsets.

SFMI-2 were also produced. These are three point mutants of SFMI-2 representing alternative versions with the second or third most prevalent amino acid types, a cyclic version of SFMI-1 representing the natural SFTI format and a double mutant of SFMI-1. The K_Is of these peptides and of the wild-type SFTI were measured on MASP-1, MASP-2, and trypsin and for SFTI, SFMI-1, and SFMI-2 on thrombin as well. The results are summarized in Table I.

Both for the selective MASP-2 binder population as well as for the nonselective MASP binder population the peptide having the consensus sequence was found to be the optimal variant. Therefore, only SFMI-1 and SFMI-2 were investigated further.

The inhibitory peptides SFMI-1 and SFMI-2 selectively block the lectin pathway

We applied the WiELISA kit (Wieslab) assay to test the inhibitory properties of SFMI-1 and SFMI-2 on the three complement activation pathways separately, because this assay selectively measures the activation of the three different routes (35).

As it is shown in Fig. 3, both SFMI-1 and SFMI-2 inhibit exclusively the lectin pathway activation and have no measurable effect on the classical and alternative routes. The WiELISA assay detects the formation of the common late complement component "activated" C9. Thus, the observed lectin pathway selectivity means that neither the classical and alternative pathway-selective proteases nor the common route proteases are inhibited by these peptides. It is noticeable that SFMI-1, which inhibits both MASP-1 and MASP-2, is 3-fold more effective than SFMI-2, which is a selective MASP-2 inhibitor. Compared with SFMI-2, SFMI-1 is a six times weaker inhibitor of MASP-2. Moreover, SFMI-1 is 15-fold more effective on MASP-1 than on MASP-2. Altogether these indicate that although MASP-2 inhibition in itself is able to block the activation of the lectin pathway, parallel inhibition of both MASP-1 and MASP-2 is more efficient in this respect.

The effects of SFMI-1 and SFMI-2 on C3 and C4 deposition

The fact that the less selective, and in terms of MASP-2 inhibition less potent, SFMI-1 inhibits lectin pathway activation more efficiently than SFMI-2 suggested that MASP-1 is a significant contributor of the lectin pathway activation. To clarify how MASP-1 can contribute to the activation of lectin pathway, C3 and C4 deposition assays were carried out.

The results of the C3 deposition assay (Fig. 4*E*, 4*F*) show the same trend already observed in the WiELISA assay: the nonselective, and in terms of MASP-2 inhibition less potent, SFMI-1 peptide inhibits the process five times more efficiently than the selective and more potent MASP-2 inhibitor SFMI-2. This confirms that MASP-1 contributes to the production of C3b. The IC_{50} values are lower than those in the WiELISA assay, which is most likely just a matter of higher sensitivity achieved in the C3 deposition assay.

In case of the C4 deposition assay, we conducted two types of experiments. First, we measured the inhibition of activated MASP-2 by adding purified C4 to the immobilized, mannan-activated MBL–MASP complexes. In this assay format, the trend of the IC_{50} values mirror those of the K_I data: the MASP-2–selective SFMI-2 is 10-fold more effective in this assay than SFMI-1, which compared with SFMI-2, inhibits MASP-2 six times weaker (Fig. 4*A*, 4*B*).

In the other type of the C4 deposition assay, instead of activated MBL–MASP complexes, we used serum in which the MBL–MASP complexes are not yet activated. In this sense, this format is analogous to the one used in the C3 deposition assay. Thus, in this C4 deposition assay, MASP-2 has to become activated and needs to remain active to cleave C4. As it is shown in Fig. 4*C* and 4*D*, the nonselective SFMI-1 peptide was an ~10 times more efficient inhibitor compared with the MASP-2–selective SFMI-2 in this assay. Although the IC_{50} values of SFMI-2 were the same in the two different C4 assays (~2.7 μM), SFMI-1 showed 100-fold

Table I. K_Is *of the synthetic peptide inhibitors on different proteases*

Inhibitor	Sequence	K_I (nM)			
		Trypsin	Thrombin	MASP-1	MASP-2
Wild-type SFTI-1 (open)	GRCTKSIPPICFPD	0.1	140,000	ND[a]	ND
SFMI-1	GICSRSLPPICIPD	260	10,000	65	1,030
SFMI-1 cyclic	[GICSRSLPPICIPD]	350	–	275	750
SFMI-1 (I2V, I12W)	GVCSRSLPPICWPD	170	–	140	5,000
SFMI-2	GYCSRSYPPVCIPD	1,000	550,000	ND	180
SFMI-2 (Y2M)	GMCSRSYPPVCIPD	4,000	–	4,000	1,500
SFMI-2 (Y2W)	GWCSRSYPPVCIPD	1,700	–	ND	580
SFMI-2 (Y7I)	GYCSRSIPPVCIPD	160	–	ND	7,000

[a]No detectable inhibition under the given experimental conditions (see *Materials and Methods*).
–, data not measured.

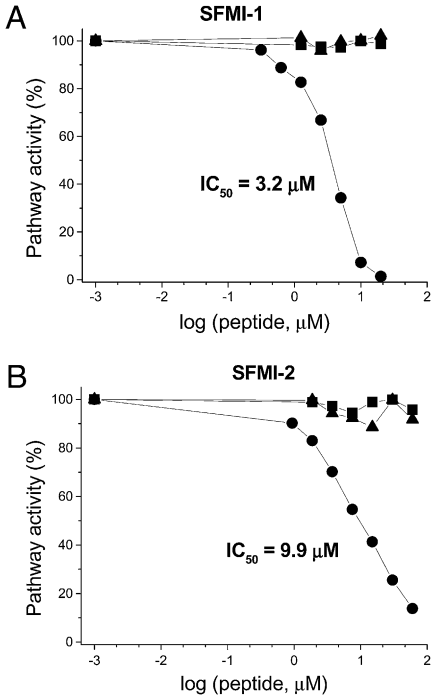

FIGURE 3. Inhibitory effect of SFMI peptides on the complement pathways. WiELISA assay was applied to test the effect of SFMI-1* (*A*) and SFMI-2* (*B*) on the classical, lectin, and alternative routes of complement separately. Serial dilutions of peptides were added to freshly collected serum, and the activities of pathways were measured. Signal without SFMI inhibitors was considered as 100% pathway activity. SFMI-1 and SFMI-2 peptides inhibit the lectin (●) pathway with an IC_{50} of 3.2 and 9.9 μM, respectively. However, none of them shows an effect on the classical (■) and alternative (▲) routes. Asterisk, the sequences of the SFMI-1 and SFMI-2 peptides are shown in Table I.

stronger inhibition in serum, which contains zymogen MASPs, than in the assay using activated MASP-2 (0.23 versus 25 μM).

Effects of SFMI-1 and SFMI-2 on blood coagulation

Besides the complement, the serum contains several other protease cascade-based systems. The most complex of these involving the largest variety of proteases is the blood coagulation system. Cross-reactivity of the SFMI peptides with blood coagulation proteases was tested in the three standard blood coagulation tests, TT, PT, and APTT. Similarly to the WiELISA assay, these tests were developed to separately measure the functionality of different cascades, but these assays can also be used for testing the SFMI inhibitors.

In case of the TT assay, activated thrombin is added to the plasma; therefore, we can test whether the SFMI peptides can inhibit thrombin in the presence of all plasma components. In the PT test, the extrinsic pathway of the blood coagulation system is activated; therefore, we can test whether the SFMI peptides can inhibit any of the proteases (VIIa, Xa, and thrombin) involved in this activation route. Finally, in the APTT assay, the intrinsic activation pathway is tested; therefore, we can test whether the peptides can inhibit XIIa, XIa, IXa, Xa, or thrombin. The results in Fig. 5 show that in all assays SFMI-1 has significant inhibitory effect, whereas SFMI-2 inhibits these processes only at extremely high concentration, and the level of inhibition is negligible.

Discussion

The high degree of parallelism in complement activation hinders a better understanding of the individual roles and relative importance of the three activation pathways both in physiological as well as in pathological processes. Specific inhibitors are extremely useful tools for basic research and therapeutic purposes. Previously, there were attempts to develop pathway-selective inhibitors by preventing the binding of the recognition molecules (C1q and MBL) to their targets (50, 51). Each activation pathway is associated with unique proteases, which could be appropriate targets for such inhibitors. Although SPs are among the most druggable targets of the complement system, early drug development efforts failed to yield specific complement inhibitors (21).

There are several X-ray structures of complement initiator proteases, but none of these present the protease in complex with an interacting peptide substrate or inhibitor (38–40, 52, 53). Without such a binding partner, the functional binding site of the protease cannot be mapped accurately. In most cases, the substrate binding groove is obstructed by long surface loops complicating the in silico design of specific inhibitors.

Of the three activation routes, the lectin pathway is the latest discovered, and perhaps not surprisingly, relatively little is known about the specific steps of its activation. The lectin pathway is activated when its pattern recognition molecules, the MBL or the ficolins, recognize characteristic molecular patterns on bacterial surfaces or on altered host cells. This triggers the activation of the MBL-associated protease zymogens, MASP-1 and MASP-2 (7). There are many open questions about the specific roles of these two proteases. MASP-1 is capable to autoactivate, and the activated form can cleave C2 with moderate efficiency. However, in vitro experiments demonstrated that MASP-1 cannot cleave C4 (10, 54). Thus, MASP-1 alone appears to be unable to activate the lectin pathway. Moreover, MASP-1 has some similarities to thrombin, because it can also specifically cleave fibrinogen and some other thrombin-specific substrates (12–14, 55). This suggests that MASP-1 could represent an evolutionary link between the two plasma cascades: the complement and the blood coagulation system (2).

In contrast to MASP-1, MASP-2 has all the activities required for lectin pathway activation. It can autoactivate and cleave both C4 and C2. The concentration of MASP-1 in the plasma exceeds that of MASP-2. This observation and the fact that MASP-1 and MASP-2 have partially overlapping activities suggest that MASP-1 might be an auxiliary component of the lectin pathway.

We decided to develop lectin pathway-selective inhibitors by targeting MASP-1 and MASP-2, because selective MASP inhibitors should help us in deciphering the particular roles that these enzymes play in the lectin pathway activation. As both MASP enzymes have a partially blocked substrate binding groove, rational inhibitor design did not seem to be a promising approach. Therefore, we used a high-throughput, in vitro, directed evolution method: phage display (56, 57). Because of the blocked substrate binding sites, we started the directed evolution with the smallest natural trypsin inhibitor, the 14-aa-long SFTI peptide (30), and used it as a scaffold displayed on the surface of bacteriophage M13. By randomizing seven potential protease binding positions, we built an SFTI-phage library comprising >2 billion variants.

The library was separately selected on MASP-1 and on MASP-2, and large numbers of individual binding clones were sequenced. Selection on MASP-2 resulted in two characteristically different sequence families. Two inhibitors, SFMI-1 and SFMI-2, were designed representing the two consensus sequences. Clones resembling SFMI-1 recognized both MASP enzymes, whereas those with SFMI-2 characteristics recognized only MASP-2. From the MASP-1 selection, only one sequence pattern emerged, and quite interestingly, it was practically identical to the SFMI-1 type. Accordingly, all the MASP-1–selected clones recognized both MASP enzymes.

As it was anticipated, SFMI-1 inhibited both MASP enzymes, whereas SFMI-2 was selective for MASP-2. It has already been

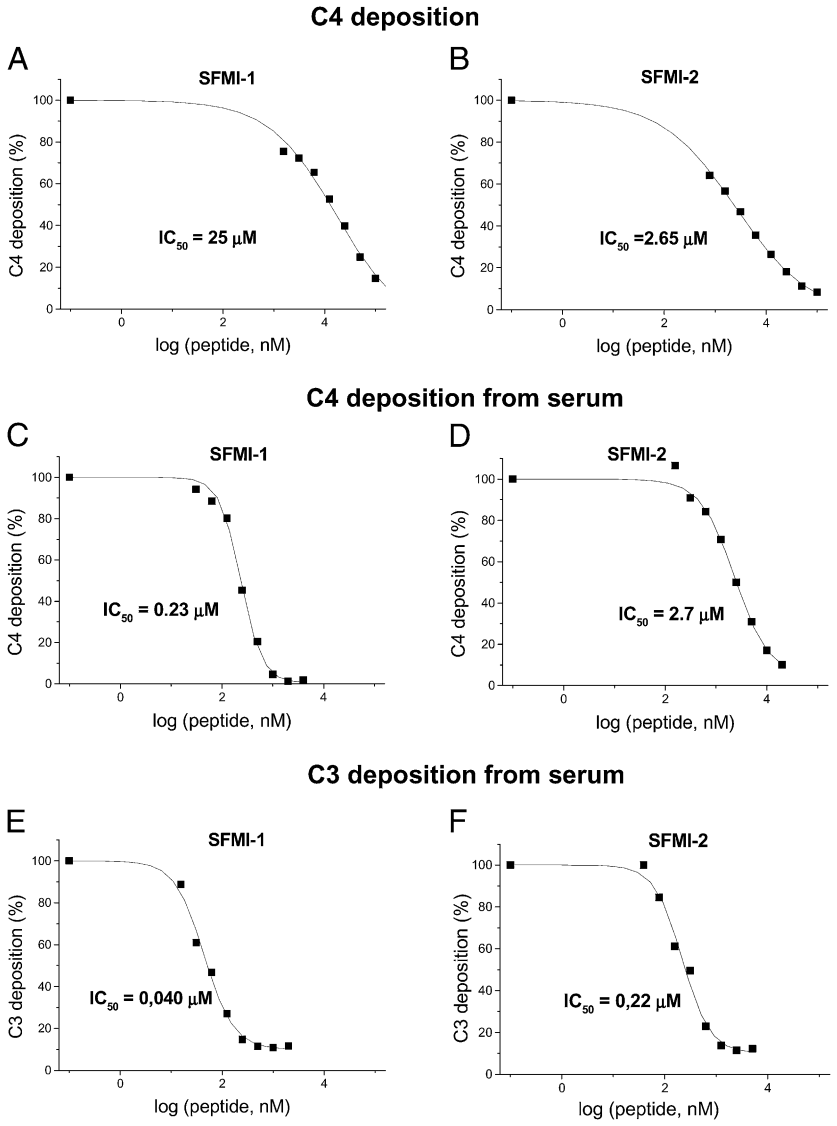

FIGURE 4. C3 and C4 deposition assays. MBL–MASP complexes from serum were captured on mannan-coated plates. In the case of C4 deposition (*A*, *B*), purified C4 was added, and C4b deposition was measured with polyclonal Ab. C3 deposition (*E*, *F*) and C4 deposition from serum (*C*, *D*) were carried out the same way: MBL–MASP complexes from serum were captured on mannan-coated plates, and the C3 and C4 cleavage occurred in situ. C3c and C4c deposition were measured with specific Abs, respectively. In the presence of serial dilution of SFMI peptides, decreased deposition was measured, whereas signal without inhibitor was considered as 100% deposition.

suggested that from the two MASP enzymes MASP-1 is the more ancient one (2). It appears that through the SFMI-1 type clones, directed evolution explored a set of binding sites on MASP-2 homologous to those existing on the more ancient MASP-1. In contrast, directed evolution also produced a distinct set of SFMI-2–type inhibitors against the evolutionary more modern MASP-2, which appear to bind to additional or alternative binding sites not present in MASP-1.

The more ancient MASP-1 can specifically cleave the typical thrombin substrates fibrinogen, factor XIII, and protease activated receptor PAR4, suggesting that the evolutionary past of MASP-1 might date back to a time when the complement and blood-clotting functions were not yet entirely separated (12–14). To this end, it is noticeable that SFMI-1, which inhibits both MASP-2 and MASP-1, is a 55-fold stronger thrombin inhibitor than the MASP-2–selective SFMI-2, which is almost ineffective on thrombin.

This difference also showed up in all the three standard blood-clotting assays where SFMI-2 was almost ineffective, suggesting that it does not inhibit any of the blood coagulation proteases even with moderate affinity. SFMI-1, in contrast, appeared to be a relatively potent inhibitor in all three assays. It is yet to be clarified whether SFMI-1 inhibits any of the blood coagulation enzymes preceding thrombin in the cascades, because the thrombin-inhibiting activity of SFMI-1 can mask such additional inhibitory potentials.

Furthermore, a recent in vivo study by Takahashi et al. (58) demonstrated that MASP-1/3 knockout mice have significantly prolonged bleeding time following tail tip excision. This result strengthens our previous observation that MASP-1 mediates thrombin-like activity. Taking these into consideration, we cannot rule out the possibility that the observed blood clotting inhibitory effects of SFMI-1 are partially due to MASP-1 inhibition.

A couple of sequence features were shared by all the selected clones. As we already mentioned at the P1 position, an Arg was preferred in both selections. This is in good accordance with the known absolute Arg preference of the two MASP enzymes (10), both having an Asp in their S1 substrate-binding pocket. Perhaps the most striking common feature is the absolute conservation of a Ser at the P2 position, where natural evolution strongly conserved a Thr in case of the Bowman-Birk family. The role of this Thr is thoroughly established. With its hydroxyl group, it participates in a hydrogen bond network that stabilizes the canonical protease interacting loop, whereas its methyl group fits into the S2 binding site of the inhibited protease (46, 47). The strict selection for a Ser by directed evolution suggests that this Ser maintains the same loop stabilizing role through its hydroxyl. The elimination of the methyl group in contrast suggests that, at least when presented in this particular scaffold, it would clash with both MASP enzymes. At the P1′ position also a serine was selected, but in this

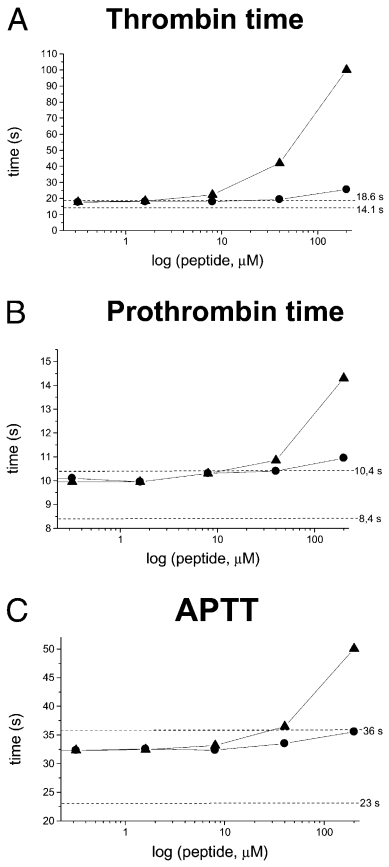

FIGURE 5. Effects of SFMI-1 and SFMI-2 on blood coagulation. Three characteristic data of blood coagulation: TT (*A*), PT (*B*), and activated partial thromboplastin time (APTT) (*C*) were measured by using standard clinical tests. Serial dilution of SFMI-1 (●) and SFMI-2 (▲) was added to fresh serum, and blood coagulation time was detected. The range of normal blood coagulation time is indicated between the dashed lines. Data points represent the average of two parallel experiments.

case, it is the wild-type SFTI residue, which is also highly conserved in the Bowman-Birk family. The side chain of this Ser participates in the same important loop stabilizing H-bonding network that was already mentioned for the P2 position. Its absolute reselection is unexpected only in the light of a previous study showing that a P1′ Ser is not essential for the integrity of the protease binding loop of Bowman-Birk inhibitors (45). Finally, a further common feature is the dominance of an Ile at the P7′ position, although Val or aromatic residues are also allowed here.

The differences between the SFMI-1 and SFMI-2 types of clones mapped to only three positions, P4, P2′, and P5′. None of these positions carries strictly conserved residues in the Bowman-Birk family, although at P2′, Ile was found to be optimal in peptides mimicking a Bowman-Birk interacting loop (59). Without high-resolution structures of the corresponding inhibitor–protease complexes, we cannot interpret the structural relevance of the selected sequence features. Nevertheless, it is important to note that at least for the P4 and P5′ positions a very low level of sequence variation was observed. This suggests that on the SFTI scaffold only a few solutions exist for efficient MASP inhibition. Moreover, both for SFMI-1 and SFMI-2, even small alterations of the consensus sequence led to a deterioration of the inhibitory properties. It seems that only a very narrow sequence space is available for a stable interaction with these proteases.

The most significant finding of this study is that both SFMI peptides inhibit the lectin pathway of the complement selectively without compromising either the classical or the alternative pathway. As far as we know, these are the first highly selective inhibitors against complement SPs, promising a breakthrough on the field of complement research.

The kinetic measurements using purified MASPs and small synthetic substrates showed that compared with SFMI-1, SFMI-2 is a 6-fold more potent inhibitor of MASP-2. Thus, if MASP-2 inhibition alone would be responsible for the observed lectin pathway inhibition, we would expect SFMI-1 to be 6-fold weaker than SFMI-2. However, an important finding of this study is that SFMI-1 is actually a >3-fold more potent inhibitor of lectin pathway activation than SFMI-2. The same trend is observed in the case of the C3 deposition assay where SFMI-1 is five times more potent inhibitor than SFMI-2. This 15- to 20-fold difference between the ratios of the expected and observed inhibitory potencies has to be due to the MASP-1 inhibitory activity of SFMI-1. Thus, these results clearly show that MASP-1 plays an important role in the activation of the lectin pathway.

However, the most striking difference shows up between the C4 deposition inhibitory effect of SFMI-1 in serum (IC_{50} = 0.23 μM) versus in the assay using activated MASP-2 (IC_{50} = 25 μM). The 100-fold difference in the IC_{50} values convincingly demonstrates that MASP-1 has a significant contribution to the activation of MASP-2. Although MASP-2 alone can autoactivate and initiate complement activation (9, 39), the presence of MASP-1 appears to facilitate this process. Our results are in agreement with that of Takahahsi et al. (15), who observed a depressed C4 and C3 deposition activity on mannan-agarose in MASP-1/3–deficient mouse serum compared with the normal one.

Our results unambiguously demonstrate that MASP-1 contributes to MASP-2 activation, but further studies are required to clarify the underlying molecular mechanism. It is also known that MASP-1 has moderate C2 cleaving activity (10), which to some degree could contribute to lectin pathway activity. This latter activity could explain why every C4b deposited by MBL–MASPs complex can form C4b2a convertase, whereas only one of four C4b deposited by the classical pathway C1 complex can do the same (60). Both types of MASP-1 contributions require close proximity of MASP-1 and MASP-2. This can only happen if both proteases are present on the same MBL or ficolin or if the complexes containing different MASPs deposit in close proximity on the activator surface.

Previous experiments with MASP-depleted human serum (16) and serum derived from MASP-1/3 knockout mice (15) also indicated a synergic effect of MASP-1 and MASP-2 in the lectin pathway activation. Because we used normal human serum, our experimental conditions are physiologically more relevant than those using manipulated sera. In addition to that, the low m.w. peptide inhibitors can inhibit the protease activity without dis-

Table II. *The P4-P3′ sequences of SMFI peptides and natural MASP substrates*

	P4	P3	P2	P1	P1′	P2′	P3′
SFMI-2	**Y**	**C**	**S**	**R**	**S**	**Y**	**P**
MASP-2	T	G	G	R	I	Y	G
C2	S	L	G	R	K	I	Q
C4	G	K	Q	R	A	L	E
C1 inhibitor	S	V	A	R	T	L	L
SFMI-1	**I**	**C**	**S**	**R**	**S**	**L**	**P**
MASP-1	L	M	A	R	I	F	N
C3(H_2O)	G	L	A	R	S	N	L
Fibrinogen β-chain	F	S	A	R	G	H	R
Factor XIII	V	V	P	R	G	V	N
Antithrombin	I	A	G	R	S	L	N
Protease-activated receptor 4	P	A	P	R	G	Y	P

sociating the MBL–MASPs complexes. The peptide inhibitors can freely penetrate into the activating complexes without any steric constraint that could arise in the case of protein inhibitors or inhibitory Abs.

Only a few other peptide inhibitors have hitherto been developed against various components of the complement cascade by phage display. A C1q-binding peptide (designated 2J) (51) binds to the globular head of the C1q and efficiently prevents classical pathway activation (IC_{50} = 2–6 μM). Compstatin is a 13-residue cyclic peptide that binds to primate C3 and precludes its cleavage by the convertases (61, 62). It inhibits both the classical (IC_{50} = 63 μM) and the alternative pathway (IC_{50} = 12 μM) with different efficiency. Since the time of the discovery of compstatin, numerous analogs have been developed with higher efficiency. The best analog with substitution of Val4 for Trp(Me)4 has an IC_{50} value of 0.205 μM (63). The very recently published complin (64), an 11-residue cyclic peptide, was developed against factor B; however, it inhibits all three pathways with comparable good efficiencies: IC_{50} values for the classical, the alternative, and the lectin pathway being 6.1, 31.5, and 3.1 μM, respectively (Wieslab assay). Our inhibitors have similar IC_{50} values for the lectin pathway (3.2 μM for SFMI-1 and 9.9 μM for SFMI-2), but most importantly, they do not inhibit the other two pathways at all. Another major difference between our peptides and all the above mentioned ones is that all the other peptides were evolved from naive peptide libraries, whereas we started the in vitro evolution procedure from a natural canonical SP inhibitor scaffold (SFTI). Consequently, while inhibitors evolved from a naive peptide library can bind to any suitable site on the surface of the target protein, our newly developed inhibitors target specifically the active center of the SP.

The high pathway selectivity of the SFMI inhibitors is somewhat unexpected considering that these are substrate-like inhibitors, and the key enzymes of the classical pathway and the lectin pathways, C1s and MASP-2, respectively, have common specific substrates, C2 and C4. Moreover, C1s and MASP-2 (as well as C1r, MASP-1, plasma kallikrein, fXIa and fXIIa) are inhibited by the substrate-like irreversible serpin type C1 inhibitor. Nevertheless, structural studies already pinpointed that despite their overlapping substrate specificities, MASP-2 and C1s have quite different protease binding sites suggesting alternative binding modes upon substrate recognition (40). Apparently, these differences in the substrate binding apparatus allow for the observed selectivity of the two SFMI peptides.

Because the SFMI peptides are substrate-like inhibitors, one might think that their protease-interacting loops should mimic the sequence of the selective substrates of their target proteases. Table II lists natural substrate sequences containing MASP-1 and MASP-2 cleavage sites, and we compared these to the sequences of the SFMI-1 and SFMI-2 protease interacting loops. Comparing six known natural MASP-1 cleavage sites from P4 to P3′, it appears that all have Arg at the P1, and most of them carry small residues at the P4, P3, P2, and the P1′ positions, whereas there is a greater chemical variability at the P2′ and P3′ positions. MASP-1 is inhibited by the SFMI-1 peptide, which also has small residues at P4 (Ile/Val), at P3 (a Cys in disulfide), at P2 (a Ser), and at P1′ (also a Ser).

In the case of MASP-2, only four natural substrates are known, which is too few to interpret trends confidently. Nevertheless, P1 is also strictly an Arg, P4, P3, P2, and P1′ tend to be small, P2′ is usually aliphatic, but a Tyr is also accepted in this study, P3′ is variable, whereas P4′ is small aliphatic. This pattern is quite similar to the one shown for MASP-1 substrates; therefore, it also fits the sequence of SFMI-1. SFMI-2 in contrast has a large Tyr at P4, where small residues are present in the substrates.

Two important conclusions can be drawn from these comparisons: 1) most SFMI sequence features do not contradict the characteristics of natural substrates, but 2) the rather fuzzy sequence trends of natural substrates would not have helped much in designing these specific inhibitors.

Activation of the complement system and particularly the lectin pathway is involved in a number of pathological conditions. A prominent example is the IR injury, a severe condition resulted in by deprivation and subsequent restoration of the tissues' blood supply. After reperfusion, the complement system attacks the effected tissue (65). IR injury can occur in a number of clinical conditions including acute myocardial infarction, stroke, or organ transplantation and may also be a complication of coronary artery bypass graft surgery. Although the lectin pathway is implicated to be a major player in IR (66), further experiments are needed to assess the exact contribution of this pathway and particularly to define the roles of MASP-1 and MASP-2 to develop effective, specific, and safe treatment. From now, this problem can be tackled with our MASP-specific inhibitors in well established disease models.

In conclusion, we have produced, to our knowledge, the first lectin pathway-specific SP inhibitors. These selective MASP inhibitors are valuable tools for basic research aiming to explore the detailed mechanism of complement activation in different physiological situations as well as they can serve as lead molecules for subsequent drug development process.

Acknowledgments

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Disclosures

P.G., G.P., A.K., and P.Z. have a pending patent regarding the SFMI peptides.

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