Supporting Information

Héja et al. 10.1073/pnas.1202588109

SI Materials and Methods

Reagents. All common laboratory reagents were from Sigma-Aldrich and Merck. High-binding microtiter plates were from Greiner Bio-One. Acetylated BSA was prepared by incubation of 10 mg/mL BSA in 0.5 M Hepes pH 7.4 with acetic anhydride in threefold molar excess to amino groups.

Serum. Blood was drawn from 10 healthy donors after informed consent. Blood samples were incubated for 1 h at room temperature and after coagulation the serum was collected, pooled, and stored at −80 °C until use.

Wieslab Assay. The Wieslab kit (COMPL 300; Wieslab) measures activation of the classical, alternative, and lectin pathways separately (1). We applied the kit to test pathway-selective inhibitory properties of the SGMI variants. 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 and the samples were incubated for another 20 min and transferred to the Wieslab plate. The same serum sample was used for all measurements and two parallels were measured. The level of serum activity in the presence of the SGMI variant was expressed as percentage of activity measured in the absence of the inhibitor.

C3 Deposition Assay. C3 deposition from human serum was measured as described previously (2). In brief, Greiner high-binding microtiter plates were coated with 10-μg/mL mannan or 50-μg/mL acetylated BSA (3) overnight at 4 °C. Wells were blocked with 0.5% BSA in Tris-buffered saline (TBS) for 2 h at 37 °C and then washed three times. Human serum was diluted 100-fold in barbital puffer and was preincubated with serial dilutions of the inhibitors for 30 min at room temperature. Samples were applied to the microtiter plate and further incubated for 30 min at 37 °C. After washing, deposited C3 was detected by antihuman C3c (A0062; DakoCytomation) primary- and HRP-conjugated secondary antibodies (A1949; SIGMA).

C4 Deposition Form Intact Serum. The C4 deposition from intact serum was measured in a similar way to the C3 deposition. Deposited C4 was detected by antihuman C4c (Q0369; DakoCytomation) antibody and HRP-conjugated secondary antibody (A1949; SIGMA).

C4 Deposition by Preactivated MASP-2. The ELISA for measuring C4 deposition by preactivated MASP-2 was performed as described by Petersen et al. (4) with minor modifications. Mannan-coated or acetylated-BSA-coated wells were incubated with 100 μL of pooled human serum diluted 1:1 in 40 mM Hepes, 2 M NaCl, and 10 mM $CaCl_2$, pH 7.4 (high salt serum dilution buffer) for 1 h at 4 °C. The wells were then washed three times with 300 μL of high salt wash buffer (20 mM Hepes, 1 M NaCl, 5 mM $CaCl_2$, and 0.1% Tween 20, pH 7.4), and three times with 300 μL of normal wash buffer (20 mM Hepes, 5 mM $CaCl_2$, and 0.1% Tween 20, pH 7.4) at 37 °C. Purified C4 (0.4 μg/well) in 100-μL wash buffer was added to the wells and incubated for 1 h at 37 °C. Following three further washes with wash buffer, deposited C4 was detected by antihuman C4c (Q0369; DakoCytomation) antibody and HRP-conjugated secondary antibody (A1949; SIGMA).

Pathway-Specific Complement Activation Assays at Twofold Serum Dilution. The assays were carried out as described previously (3, 5). Greiner high-binding microtiter plates were coated with 10 μg/mL IgG (classical pathway), 10 μg/mL LPS (alternative pathway), or 5 μg/mL mannan (lectin pathway). The wells were then blocked with TBS (50 mM Tris, and 150 mM NaCl, pH 7.4) containing 1% BSA (BSA) and 0.1% Tween 20.

Selective measurement of the lectin pathway was achieved by using SPS at a final concentration of 100 μg/mL (6). Human serum was diluted to twofold with barbital buffer (4 mM barbiturate, 145 mM NaCl, 0.5 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1% Tween 20, pH 7.4) containing the SPS and the SGMI inhibitors. The samples were applied to the mannan-coated plates and complement activation was allowed for 30 min at 37 °C. After washing, the deposition of C4 was detected by using antihuman C4c antibody 1:20,000 (DakoCytomation; Q0369). The activation of the classical pathway was measured as above, but on IgG-coated surface and the SPS was omitted from the buffers.

In the case of the alternative pathway measurements, the SGMI inhibitors were dissolved in Mg^{2+}–EGTA–barbital buffer (4 mM barbiturate, 145 mM NaCl, 4 mM $MgCl_2$, 10 mM EGTA, and 0.1% Tween 20, pH 7.4) and the deposition of C3 was detected by antihuman C3c antibody 1:30,000 (DakoCytomation; A0062) on the LPS-coated plates.

The final development of the microtiter plate was carried out as described in Kocsis et al. (2). Signal amplitudes were converted to percentages, where signal obtained in the absence of inhibitors was defined as 100%.

Recombinant MASPs. Recombinant active MASP-1 catalytic fragment (CCP1–CCP2–SP) was prepared as described (7), but with the absence of benzamidine. Recombinant active MASP-2 catalytic fragment (CCP1–CCP2–SP) was produced according to Ambrus et al. (8). A variant form of recombinant MASP-2 (CCP-1–CCP2–SP), in which the catalytic serine was replaced by alanine (S633A) using mutagenesis, was prepared as described for the wild-type form (8), however in this case the procedure yields proenzymic (one chain) MASP-2. The zymogen (one chain) form of recombinant wild-type MASP-2 catalytic fragment (CCP1–CCP2–SP) was prepared as described (9). MASP-3 CCP–SP (V^{365}–R^{728}) containing an ASMT peptide at the N terminus was expressed and refolded as described for the analogous fragment of MASP-2 (8). After purification by cation-exchange chromatography and gel filtration, MASP-3 CCP–SP was concentrated to about 3 mg/mL in 150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH7.5, where it became activated in 2 d at room temperature. Precursor numbering was used throughout.

Kinetics of the Cleavage of MASP-2 S633A by Wild-Type MASP-2 and MASP-1. Recombinant MASP-2 (CCP1–CCP2–SP) S633A at a concentration of 3 μM was incubated with 500 nM recombinant active MASP-2 (CCP1–CCP2–SP), or 50 nM recombinant active MASP-1 (CCP1–CCP2–SP) in 140 mM NaCl, 50 mM Hepes, and 0.1 mM EDTA, pH7.4. Samples were removed periodically and heated in the presence of reducing SDS/PAGE sample buffer. The samples were run on reducing SDS-PA gels, which was followed by densitometry using a GEL DOC 1000 instrument and the Molecular Analyst software (both from Bio-Rad). The disappearance of the intact (uncleaved) MASP-2 (CCP1–CCP2–SP) S633A band was quantified. The catalytic efficiency (k_{cat}/K_M) was determined by nonlinear regression using the $I = I_B + I_o \times \exp(-k_{cat}/K_M \times [E]_T \times t)$ equation, where I is the intensity, I_o is the intensity at the zero time point, I_B is the background, and $[E]_T$ is the total active enzyme concentration.

Inhibition of MASP-3 by SGMI-1 and -2. Recombinant active MASP-3 (CCP2–SP) was assayed using the Z–Gly–Arg–S–Bzl (MP Biomedicals) substrate (10) at 20 μM final concentration and 4,4′-dithiodipyridine (Sigma) cosubstrate at 40 μM final concentration in 50 mM Hepes, 140 mM NaCl, 0.1 mM EDTA, pH 7.4, and 0.1% PEG-3350 buffer. The activity of 10 nM MASP-3 was measured alone or in the presence of 2–10-μM SGMI-1, or SGMI-2. The inhibitory constant (K_i) was calculated for SGMI-2 using $K_i = [I] \times V/(V_o - V)$, where $[I]$ is the inhibitor concentration, V and V_o are the measured activities in the presence, or absence of inhibitor, respectively. SGMI-1 showed no inhibition up to 10-μM concentration.

Inhibition of Autoactivation of Isolated MASP-2 Catalytic Fragment. To follow the autoactivation process, the recombinant zymogen MASP-2 was incubated at a concentration of 0.3 μM in 20 mM Hepes and 145 mM NaCl, pH 7.4 at 37 °C with or without the inhibitors (1-μM SGMI-1 or SGMI-2) and samples were taken at various time points. The samples were visualized on reducing SDS/PAGE and analyzed by densitometry using GEL DOC 1000 instrument and Molecular Analyst software (Bio-Rad). The autoactivation was followed by the appearance of the cleaved catalytic domain of MASP-2.

Blood Coagulation Assays. Inhibitory capacities of the two SGMI variants to slow down the coagulation process were tested by measuring their effects in three standard coagulation assays, the thrombin time, prothrombin time, and the activated partial thromboplastin time. Blood was collected from a healthy individual by vein puncture after informed consent. The blood was treated with sodium–citrate (3.8% wt/vol) 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) with Innovin reagent (Dale Behring, Marburg, Germany).

The inhibition of the intrinsic pathway of blood coagulation was tested through APTT measurements, and 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) system with TriniClot reagent (Trinity Biotech) and Reanal reagent (Reanal Fine Chemicals).

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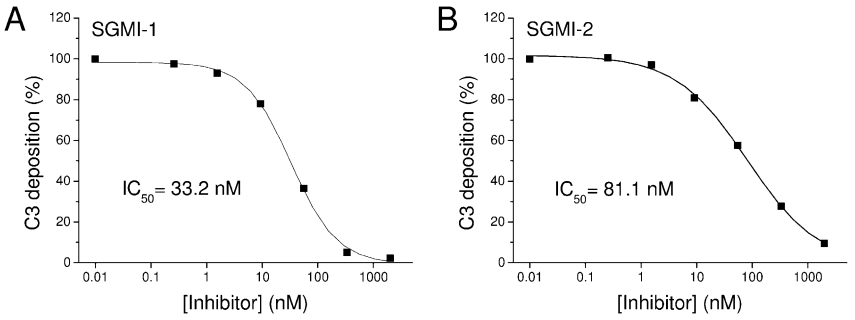

C

Identification number of serum donors	IC_{50} values (nM)	
	SGMI-1	SGMI-2
1.	12.1	12.0
2.	7.7	46.2
3.	33.2	81.1

Fig. S1. SGMI-1 and SGMI-2 effectively inhibit the lectin-pathway activation in individual serum samples. Dose-dependent inhibitory effect of SGMI-1 (*A*) and SGMI-2 (*B*) on C3 deposition was measured in a conventional assay system using 100-fold diluted normal human serum. Sera from three healthy donors were tested separately. Representative plots of a typical experiment (*A*) and (*B*) and the IC_{50} values (*C*) from three individual serum samples are shown. The IC_{50} values vary from case to case most probably due to the individual differences in the concentration of the lectin-pathway components.

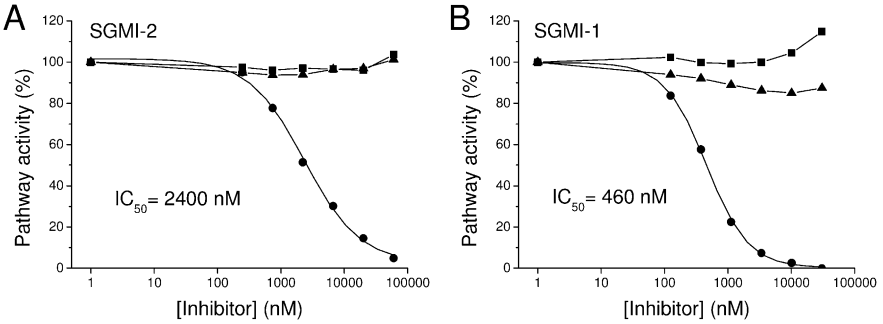

Fig. S2. Inhibitory effects of SGMI-1 and SGMI-2 on the three pathways of complement activation in twofold diluted human serum. Normal human serum was diluted 1:1 with barbital buffer containing different concentrations of SGMI-2 (*A*) or SGMI-1 (*B*). In the case of the lectin-pathway-specific assay, the buffer also contained 100 μg/mL SPS. Complement activation was triggered by incubation the sera on pathway-selective activation surfaces. Activity of the classical (■) and lectin (●) pathways was measured through C4 deposition, and for alternative pathway (▲) C3 deposition was determined.

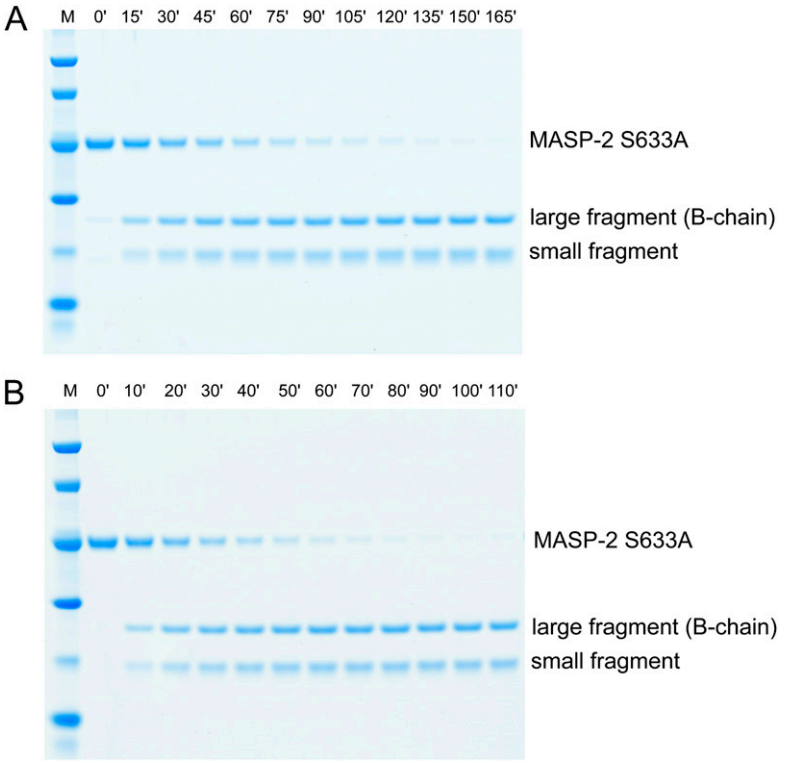

Fig. S3. Kinetics of the cleavage of recombinant zymogen MASP-2 S633A by active MASP-2 and MASP-1. Recombinant zymogen MASP-2 (CCP1–CCP2–SP) S633A at a concentration of 3 μM was incubated with (*A*) 500 nM recombinant active MASP-2 (CCP1–CCP2–SP), or (*B*) 50 nM recombinant active MASP-1 (CCP1-CCP2-SP) and analyzed by SDS/PAGE as described in *Materials and Methods*. Incubation times are indicated in minutes. For the MASP-2/MASP-2 S633A cleavage $k_{cat}/K_M = 6.0 \pm 0.3 \times 10^2$ M^{-1} s^{-1}, and for the MASP-1/MASP-2 S633A cleavage $k_{cat}/K_M = 1.2 \pm 0.1 \times 10^4$ M^{-1} s^{-1} values were determined after densitometry and nonlinear regression. For size comparison a marker (M) composed of 97, 66, 45, 30, 20.1, and 14.4 kDa proteins (GE Healthcare) was used.