Supporting Information

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SI Materials and Methods

To determine the half-life of RA101295 in circulation, one dose (10 mg/kg) was injected subcutaneously. Blood samples were collected on lepirudin anticoagulant before (T0), then at 1, 2, 4, 6, 8, and 24 h postinjection and the amount of RA101295 in plasma was determined by LC-MS.

To determine the duration of RA101295 inhibitory activity, two doses of RA101295 (10 mg/kg) were injected subcutaneously at T+5 min and T+8 h. Blood samples were collected on lepirudin anticoagulant before (T0), then at 1, 2, 4, 6, 8, and 24 h post-injection. The plasma was incubated in vitro with 25 μg/mL PGN, a potent complement activator (9), for 30 min at 37 °C. The formation of sC5b-9 was quantified using ELISA as described in the main text. sC5b-9 formation in sample collected at T0 and treated with PGN was considered as 100%. On the basis of the sC5b-9 formation, the treatment time of RA101295 was decided in LD100 Escherichia coli sepsis model. The time course of bioavailability of C5 inhibitor was also examined by using the classic pathway-mediated hemolysis assay. Plasma was diluted (1:10) with GVB++ buffer (Complement Technology). Next, 50 μL of diluted plasma was mixed with 50 μL of antibody-sensitized sheep erythrocytes (Complement Technology) and incubated for 30 min at 37 °C. Reactions were stopped by adding 150 μL ice-cold GVB++/5 mM EDTA. The plate was centrifuged and supernatants were transferred to a new plate. The plate was read at 412 nm. The value of the T0 plasma sample was considered 100% and percent hemolysis was calculated (49).

Fig. S1. Effect of delayed treatment with RA101295 on E. coli induced complement activation in whole-blood in vitro model. Lepirudin-anticoagulated whole baboon blood was incubated with 5 × 10⁷ E. coli/mL. Treatment with 1 μM RA101295 was done 15 min before (pretreatment) or 5, 15, 30, 60 min after E. coli challenge. Samples where E. coli was replaced by saline served as control. The total incubation time was 2 h. Data are presented as mean ± SEM (n = 3). Data are compared between E. coli no treatment and E-coli+RA101295 using two-tailed Student t test: ****P < 0.0001.
Fig. S2. Time-course of RA101295 exposure and efficacy. (A) A noninfected baboon was given a subcutaneous dose of 10 mg/kg RA101295 at $t=0$ h and plasma levels were determined using LC-MS. (B) A 10 mg/kg dose of RA101295 was subcutaneously injected at T0 and at T+8 h (arrows). Blood samples were collected at indicated time-points and PGN-induced complement activation was measured as sC5b-9 generation. (C) Complement levels in the blood samples collected in B were tested using a classic pathway-induced hemolysis assay.
Fig. S3. C3b binding on circulating aggregated platelets of *E. coli* challenged baboons. Blood collected at T+2 h was smeared on slides and triple-labeled with antibodies against P-selectin as platelet marker (A), lipid A as LPS/bacteria marker (B), and C3b (C). (D) Merged image show colocalization of C3b with aggregated platelets and bacteria. (Scale bar, 10 μm.)
Fig. S4. Changes in vital signs during *E. coli* sepsis in baboons in the presence/absence of RA101295. (A) Heart rate, (B) mean systemic arterial pressure (MSAP), (C) respiration rate, (D) oxygen saturation, (E) body temperature. Data are presented as mean ± SEM. Same time-point data are compared between LD100 and LD100+RA101295 using two-tailed Student *t* test: *P* < 0.05, **P** < 0.01.

Fig. S5. Effect of RA101295 treatment on plasma markers of organ function. (A) Lactate, (B) creatinine, (C) BUN, (D) alanine aminotransferase (ALT). Data are presented as mean ± SEM. Same time-point data are compared between LD100 and LD100+RA101295 using two-tailed Student *t* test: *P* < 0.05, **P** < 0.01.