Role of serum amyloid P component in bacterial infection: Protection of the host or protection of the pathogen

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Serum amyloid P component (SAP) binds to *Streptococcus pyogenes*, and we show here that it also binds to *Neisseria meningitidis*, including a lipopolysaccharide (LPS)-negative mutant, and to rough variants of *Escherichia coli*. Surprisingly, this binding had a powerful antiopsonic effect both *in vitro* and *in vivo*, reducing phagocytosis and killing of bacteria. Furthermore, SAP knockout mice survived lethal infection with *S. pyogenes* and rough *E. coli* J5, organisms to which SAP binds. The susceptibility of $SAP^{-/-}$ mice was fully restored by injection of isolated human SAP. However, $SAP^{-/-}$ mice were more susceptible than wild-type animals to lethal infection with *E. coli* O111:B4, a smooth strain to which SAP does not bind, suggesting that SAP also has some host defense function. Although SAP binds to LPS *in vitro*, $SAP^{-/-}$ mice were only marginally more susceptible to lethal LPS challenge, and injection of large amounts of human SAP into wild-type mice did not affect sensitivity to LPS, indicating that SAP is not a significant modulator of LPS toxicity *in vivo*. In contrast, the binding of SAP to pathogenic bacteria enabled them to evade neutrophil phagocytosis and display enhanced virulence. Abrogation of this molecular camouflage is thus potentially a novel therapeutic approach, and we show here that administration to wild-type mice of (*R*)-1-[6-(*R*)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid, a drug that inhibits SAP binding, significantly prolonged survival during lethal infection with *E. coli* J5.

The pentraxin plasma proteins, C-reactive protein (CRP), the classical acute phase protein, and serum amyloid P component (SAP) (1), belong to the highly conserved lectin fold superfamily (2) with functions related to specific calcium-dependent ligand binding (3, 4). SAP binds to DNA in chromatin (5) exposed to or released in the extracellular environment by apoptosis or necrosis (6, 7), stabilizes it, and protects it from degradation (8, 9). SAP-deficient mice, created by gene targeting (10), degrade chromatin more rapidly than normal, have enhanced antibody responses to exogenous chromatin, and spontaneously develop marked anti-chromatin autoimmunity (9). A major normal function of SAP is thus to modulate handling of chromatin autoantigens and prevent loss of tolerance. On the other hand, SAP also binds to all types of amyloid fibrils, the abnormal protein aggregates responsible for the amyloidosis diseases (1). Binding of SAP stabilizes amyloid fibrils *in vitro* (11) and contributes to pathogenesis of amyloidosis *in vivo*, as shown by delayed and reduced amyloid deposition in SAP-deficient mice (10). However, in addition to these normal and pathological endogenous ligands, SAP also binds to some bacteria, including the important human pathogen *Streptococcus pyogenes*, suggesting a possible role in host defense (12). Here we report studies of bacterial infection in SAP knockout mice and the surprising finding that although SAP does contribute to survival during some infections, it also inhibits phagocytosis of those bacteria to which it binds and thus enhances their virulence.

Materials and Methods

***In Vivo* Infection Experiments.** Pure line 129SV mice with targeted deletion of the gene for SAP and total deficiency of SAP ($SAP^{-/-}$) were created as previously reported (10). The SAP gene deletion was also introduced into pure line C57BL/6 mice by back-crossing for at least 10 generations before the animals were used in the present experiments. Groups of 15–24 $SAP^{-/-}$ and wild-type 129SV mice and of 10–13 $SAP^{-/-}$ and wild-type C57BL/6 mice, all 10–14 weeks old, weighing 20–25 g, and age weight and sex matched within each experiment, were infected on day 0; morbidity and mortality were then recorded until no more deaths occurred. Bacteria were grown in broth as described below and harvested in log phase, and the concentrations used for infection were checked by colony counting. Pure human SAP was isolated as described (13).

Treatment of Infection by Inhibition of SAP Binding. A potent, nontoxic inhibitor of SAP binding, (*R*)-1-[6-(*R*)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (designated here as compound R), which inhibits and reverses binding of SAP to amyloid deposits *in vivo* (14), has been developed as a possible therapeutic agent for amyloidosis (refs. 11 and 14; patent EP-A-915088). This drug in solution in water or neutral pH buffer was administered by repeated i.p. injection to groups of 10 age-, weight-, and sex-matched adult wild-type C57BL/6 mice receiving an LD_{90} dose of *Escherichia coli* J5, and the response to infection was observed as above. Controls received the solvent alone.

Bacteria. *E. coli* O111:B4 (from B. J. Applemelk, Vrije Universiteit, Amsterdam, The Netherlands), *E. coli* J5, and *E. coli* F515 (from U Mamat, Forschungzentrum, Borstel, Germany) were grown overnight to stationary phase in Luria broth (Oxoid, Basingstoke, UK) and then reinoculated and grown to log phase with an optical density of 1.0, corresponding to about 10^9 cells per ml, and checked by colony counting. *S. pyogenes* H305, a scarlet fever serotype M1T1 clinical isolate from a positive blood culture of a patient with invasive streptococcal infection (rederived from strain H250 provided by the Public Health Laboratory Service, Colindale, England), used extensively in murine models of infection (15), was grown overnight to stationary phase in Todd Hewitt broth containing 0.2% yeast extract (Oxoid) and then reinoculated and grown to log phase with an optical density of 1.0, and the number of cells per milliliter was checked by colony counting. In some experiments

Abbreviations: CRP, C-reactive protein; SAP, serum amyloid P component; compound R, (*R*)-1-[6-(*R*)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid; TCB buffer, 10 mM Tris-buffered 140 mM NaCl containing 2 mM $CaCl_2$ and 4% wt/vol BSA at pH 8.0; LPS, lipopolysaccharide.

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Table 1. Binding of human SAP to bacteria *in vitro*

Bacterial species	SAP binding (molecules of SAP bound per bacterial cell)
E. coli O111:B4 (smooth)	$<5 \times 10^2$
E. coli J5 (Rc)	$2.1–6.0 \times 10^4$
E. coli F515 (Re)	2.8×10^4
S. pyogenes H305	$2.8–6.0 \times 10^3$
N. meningitidis clinical isolates, types A, B, and C	4.3×10^3 to 1.7×10^4
N. meningitidis H44/76 (LPS-sufficient wild type)	4.9×10^4
N. meningitidis H44/76[pHBK30] (LPS-deficient mutant)	$2.8–4.0 \times 10^4$

These representative values may vary by up to 2-fold between different cultures of the same organism.

organisms were killed by heating for 30 min at 80°C before testing, but this had no effect on SAP binding. *Neisseria meningitidis* strains (from P. Brandtzaeg, Ullevål University Hospital, Oslo, Norway) were isolated from typical cases of meningococcal disease, apart from the lipolpolysaccharide (LPS)-negative strain provided by P. van der Ley (Bilthoven, The Netherlands) (16). All were grown overnight in brain heart infusion broth containing 5% FCS, then centrifuged, heat killed (80°C for 30 min), and resuspended to standard optical density corresponding to 2×10^8 cells per milliliter. Calcium-dependent binding of SAP from 1-ml aliquots of pooled whole normal human serum, containing ≈30 mg/liter SAP, to aliquots of 10^9 to 10^{10} bacterial cells, was tested precisely as described in ref. 12 by using both immunoassay for SAP eluted with EDTA and direct counting of bound ^{125}I-SAP spiked into the serum. Identical results were obtained with the two methods, and the values shown are representative of reproducible replicate experiments with each organism. The number of SAP molecules bound was calculated from the number of bacteria used and the molecular mass, 127,310 Da, of the physiological pentameric form of human SAP (17, 18).

Phagocytosis by Neutrophils. Smooth *E. coli* O111:B4 and rough (Rc) mutant *E. coli* J5 were transformed, by using plasmid pFPV25.1 containing the *gfp-mut 3a* gene under the constitutive S13 ribosomal protein promoter (from D. Holden, Imperial College School of Medicine, London), to express green fluorescent protein (19), and were grown as described above. After washing in sterile PBS, pellets of 10^9 organisms were resuspended and incubated for 1 h at 4°C in 1-ml volumes of the following reagents: 10 mM Tris-buffered 140 mM NaCl containing 2 mM $CaCl_2$ and 4% wt/vol BSA at pH 8.0 (TCB buffer) [this concentration of albumin is essential for preventing the autoaggregation of isolated human SAP (18)]; TCB buffer containing isolated pure human SAP (13) at 30 mg/liter; whole heat-inactivated (56°C, 30 min) pooled normal human serum containing 30 mg/liter SAP; the same serum from which all SAP had been removed by absorption with sheep anti-SAP antibodies immobilized on CNBr-activated Sepharose (20); whole serum, heat inactivated as above, absorbed with immobilized sheep anti-C-reactive protein antibodies instead of anti-SAP (21); and SAP-depleted heat-inactivated serum reconstituted with 30 mg/liter of isolated SAP. Leukocytes from healthy normal individual donors were isolated by centrifugation and washing after lysis of erythrocytes with ammonium chloride, resuspended in Hanks' balanced salt solution, and incubated for 30 min at 37°C with 0.1 μg/ml dihydroethidine. Volumes of 10 μl of bacteria suspended in the various reagents and 100 μl of leukocyte suspension in Hanks' were then mixed to provide 10 bacteria per polymorph and were rotated at 37°C for 15 min to allow phagocytosis to proceed before fixing in 1% paraformaldehyde in PBS. Ingestion of bacteria containing green fluorescent protein (absorption 480 nm, emission 540 nm; ref. 22) and triggering of the respiratory burst, indicated by oxidation of dihydroethidine to red fluorescent ethidium bromide (absorption 480 nm, emission 593 nm; ref. 23), were quantified by flow cytometry (Coulter Epics XL-MCL with System II software) with the forward scatter threshold set to 140 to exclude free bacteria from the analysis. At least 10,000 neutrophils, identified by well-established light scatter and autofluorescence parameters, were analyzed in each sample. Within each experiment, the relative phagocytosis of bacteria in the different reagents was quantified by comparison of mean cellular green fluorescence in cells that showed red fluorescence, demonstrating that they had mounted an oxidative burst response; this quantification excluded neutrophils that had not actively phagocytosed bacteria. For investigation of neutrophil phagocytosis of *N. meningitidis*, the serogroup B strain H44/76 was grown in broth and then heat killed, as described above, before labeling by incubation at 10^9 organisms per milliliter with 50 μg/ml fluorescein isothiocyanate in 0.1 M $NaHCO_3$ at pH 9.6. In two separate sets of experiments, labeled bacteria were mixed with either undiluted whole serum immunodepleted of SAP, as described above, or control serum treated with immobilized normal sheep IgG or with 10% vol/vol SAP-depleted whole serum in TCB buffer, containing 1% wt/vol BSA, to which was added a range of concentrations of isolated pure SAP. After preincubation for 1 h at 37°C the mixtures were added to leukocyte preparations, and the phagocytosis assay and flow cytometry were performed as described above for *E. coli*. Three different individual leukocyte donors were used in each experiment with each bacterium. For display purposes, results within each experiment have been normalized to a value of 100% assigned to the mean of the maximal phagocytosis in that experiment.

Bacterial Killing *in Vivo*. An acute phase response was induced in both wild-type $SAP^{+/+}$ and $SAP^{-/-}$ C57BL/6 mice by s.c. injection of 0.5 ml of aqueous 2% wt/vol $AgNO_3$, and pooled serum collected 24 h later contained 210 mg/liter SAP from the wild-type mice and 0 mg/liter SAP from the $SAP^{-/-}$ animals. Isolated pure mouse SAP (24) was added, to 100 mg/liter, to some of the $SAP^{-/-}$ serum, and all of the reagents were then heat inactivated at 56°C for 30 min. *E. coli* J5 were washed in PBS, and separate pellets containing 10^8 organisms were resuspended and then rotated for 1 h at 4°C with 100-μl volumes of each of the serum reagents described above. The bacterial suspensions were then diluted to 10^7 organisms per milliliter in Hanks' balanced salt solution, and 1-ml volumes were injected intraperitoneally into individual adult female C57BL/6 mice weighing 20–25 g each. Exactly 15 min later the mice were killed by CO_2 narcosis, the peritoneal cavity of each was washed out with 10 ml of PBS, and the number of surviving bacteria was determined by colony counting. The results shown are pooled from two identical experiments, each containing five mice per group.

Lipopolysaccharide Toxicity *in Vivo*. Groups of 6–12 adult BALB/c mice, age, weight, and sex matched in each experiment, received i.p. injections of purified human SAP (100 mg/kg) or equimolar amounts of purified human serum albumin or human CRP (25), all in solution in Tris-buffered physiological saline (pH 8.0).

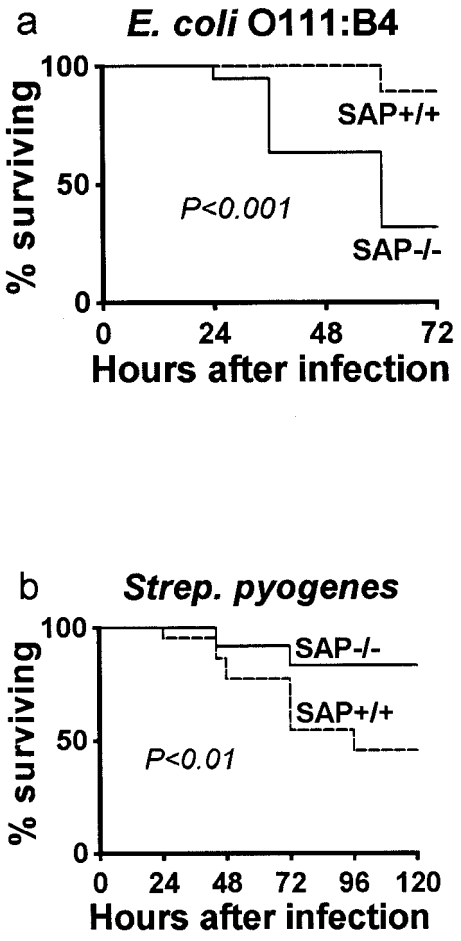


Fig. 1. Effect of SAP deficiency on survival of 129SV mice during bacterial infection. (*a*) *E. coli* O111:B4, 4.4×10^8 organisms injected i.v. (*b*) *S. pyogenes* H350, 2.1×10^5 organisms injected i.p. *P* values (log rank test) represent significant differences in survival between $SAP^{-/-}$ and $SAP^{+/+}$ mice. Each result shown is typical of two to four identical experiments with each organism.

Twenty minutes later all animals received 200 μg per mouse of *Salmonella typhimurium* LPS by i.p. injection at time 0. In some experiments, a second injection of each protein was given after 4 h. The toxicity of *E. coli* O111:B4 LPS was also compared in groups of 10 age-, weight-, and sex-matched $SAP^{-/-}$ and wild-type $SAP^{+/+}$ C57BL/6 mice, after i.p. injection of 10 mg/kg or 1 mg/kg of LPS. In all experiments, morbidity and mortality were observed until no more deaths occurred.

Results

Binding of SAP to Bacteria *in Vitro* and the Outcome of Bacterial Infection in SAP-Deficient Mice. Pure line 129SV $SAP^{-/-}$ mice infected with *E. coli* O111:B4, a mouse pathogenic smooth strain to which SAP shows no appreciable binding *in vitro* (ref. 12; Table 1), had significantly greater morbidity and mortality than wild-type $SAP^{+/+}$ controls (Fig. 1*a*). SAP evidently contributes to survival during infection with this organism. However, surprisingly, and in marked contrast, infection with *S. pyogenes*, a pathogen to which SAP binds in substantial amounts (ref. 12; Table 1), produced significantly lower mortality in the SAP knockouts than in wild-type controls (Fig. 1*b*).

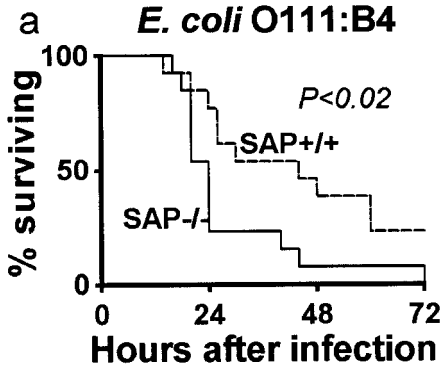
SAP specifically recognizes cyclic pyruvate acetals (1, 12, 26), but the ligand for SAP expressed by *S. pyogenes* is not known. However, binding of SAP to Gram-negative bacterial LPS has recently been reported (27), and we therefore investigated the interaction of SAP with smooth and rough *E. coli* strains expressing different forms of LPS. No binding to the smooth strain was detected, but substantial amounts of SAP bound to rough variants on which the central and core structures of LPS were exposed (Table 1). Nevertheless LPS is not the only SAP ligand on Gram-negative bacteria, as there was marked binding of SAP to an LPS-deficient mutant of *N. meningitidis* (Table 1).

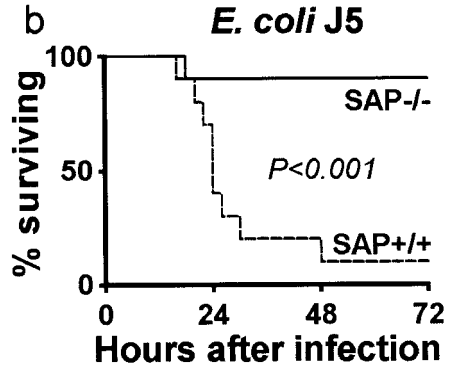
The difference between binding of SAP to rough and smooth *E. coli in vitro* was reflected in a dramatic difference in survival between $SAP^{-/-}$ and $SAP^{+/+}$ mice after lethal infection *in vivo*. Thus whereas pure line C57BL/6 $SAP^{-/-}$ mice were again more susceptible than wild-type animals to infection with *E. coli* O111:B4 (Fig. 2*a*), SAP-deficient animals survived remarkably better than wild-type mice when infected with the rough *E. coli* J5 strain (Fig. 2*b*). The critical specific role of SAP itself was confirmed by the administration of isolated pure human SAP to $SAP^{-/-}$ mice during infection with *E. coli* J5. This administration of human SAP completely restored the greater mortality typical of wild-type $SAP^{+/+}$ animals (Fig. 2*c*).

Treatment of Bacterial Infection by Inhibition of SAP Binding. The binding of SAP to amyloid fibrils makes a significant contribution to the pathogenesis of amyloidosis (10, 11). We have therefore lately developed compounds that inhibit ligand binding by SAP *in vitro* and *in vivo*, for use as drugs to treat amyloidosis (14). The addition of one of these inhibitors, compound R, to human serum at 50 μM completely inhibited binding of SAP to *E. coli* J5. For example, in a typical experiment, 4×10^4 molecules of SAP bound to each bacterial cell in the presence of Tris-buffered saline containing calcium, whereas no SAP binding was detectable in the presence of 10 mM EDTA or any concentration of compound R between 5 mM and 50 μM. Lower concentrations of compound R were not tested. To investigate the effect of compound R on the course of infection *in vivo*, animals received the drug i.p. at 10 mg per mouse immediately before and 4 h after infection with *E. coli* J5. They showed significantly prolonged survival compared with mice receiving solvent only (Fig. 3*a*). In a second experiment the survival of treated animals was similarly prolonged significantly by doses of 5 mg per mouse immediately before infection followed by 2 mg per mouse at hourly intervals up to 12 h (Fig. 3*b*).

SAP Reduces Killing of Bacteria *in Vivo*. When *E. coli* J5 organisms were incubated with acute phase serum, either from $SAP^{-/-}$ or $SAP^{+/+}$ mice, or with $SAP^{-/-}$ serum reconstituted with isolated pure mouse SAP, and then introduced into the peritoneal cavity of $SAP^{-/-}$ animals, there was significantly more immediate killing of the bacteria in the absence of SAP (Fig. 4). Binding of SAP evidently enables the organism to evade the host's essential early defenses.

SAP Binding Inhibits Phagocytosis of Bacteria by Neutrophils *in Vitro*. Comparison of *in vitro* phagocytosis of *E. coli* J5 by human neutrophils in the presence of whole heat-inactivated serum, in SAP-depleted serum, or in SAP-depleted serum replenished with isolated pure human SAP, showed robustly reproducible inhibition by SAP (Table 2). Indeed, the presence of isolated human SAP alone notably reduced phagocytosis. The presence or absence of SAP had no effect on the phagocytosis of *E. coli* O111:B4, to which SAP does not bind appreciably (Table 2). These *in vitro* observations with human cells, serum, and SAP indicate that SAP binding is likely to promote bacterial virulence in humans as it does in the mouse. However, rough *E. coli* strains are not human pathogens, and *E. coli* J5 was used only as a convenient model system. In contrast, the binding of SAP to *N. meningitidis* (Table 1), the pathogen responsible for the serious human infections meningococcal meningitis and septicemia, is clearly of considerable clinical interest. *N. meningitidis* does not





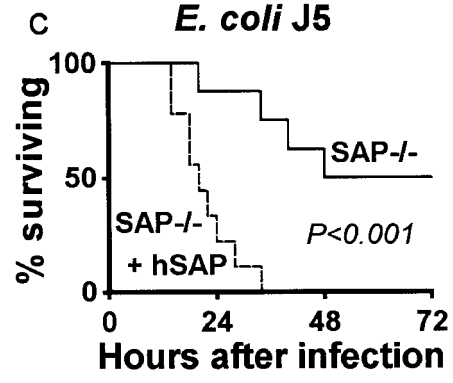
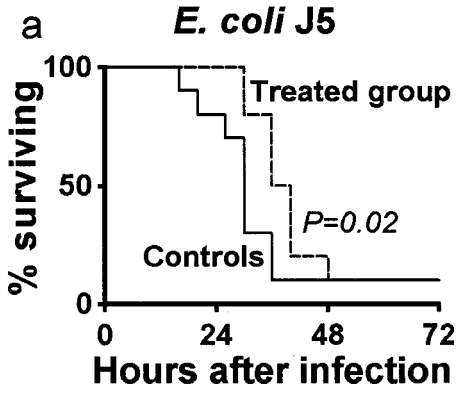


Fig. 2. Effect of SAP deficiency on the survival of C57BL/6 mice during bacterial infection. (*a*) *E. coli* O111:B4, 10^8 organisms injected i.v. (*b*) *E. coli* J5, 10^9 organisms injected i.v. Each result shown in *a* and *b* is typical of at least two identical experiments with each organism. (*c*) *E. coli* J5, 10^9 organisms injected i.v. at time 0; isolated human SAP, 10 mg per mouse, was injected i.p. at −2 h and 16 h. *P* values (log rank test) represent significant differences in survival between $SAP^{-/-}$ and $SAP^{+/+}$ or SAP-reconstituted $SAP^{-/-}$ mice.



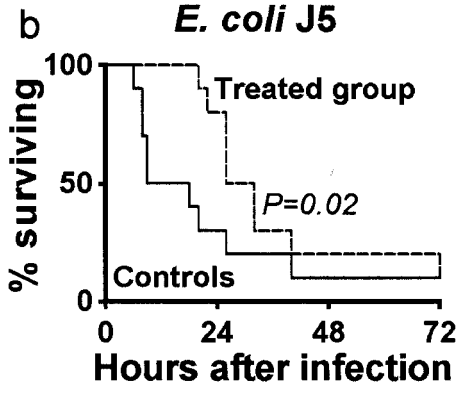


Fig. 3. Effect of inhibition of SAP binding on the survival of C57BL/6 mice during bacterial infection. All mice received an LD_{90} of *E. coli* J5, 10^9 organisms injected i.v. (*a*) The treated group received compound R at 10 mg per mouse by i.p. injection immediately before infection and at 4 h. (*b*) The treated group received compound R in doses of 5 mg per mouse by i.p. injection immediately before infection and 2 mg per mouse at hourly intervals up to 12 h. In both experiments the control group received injections of solvent alone at the same times. Note that the repeated manipulation and injections involved in *b* were associated with accelerated morbidity and mortality, but the treated group still showed significantly prolonged survival. *P* values (Wilcoxon test based on Kaplan–Meier analysis) represent significant differences in survival between treated and control groups.

infect mice, and virulence studies of the type used here for *E. coli* and *S. pyogenes* are therefore not possible. However, binding of SAP markedly inhibited neutrophil phagocytosis of *N. meningitidis in vitro* (Table 2). The effect was dose dependent and demonstrable below and within the physiological concentration range of circulating human SAP, 15–40 mg/liter (28).

Effect of SAP on Lipopolysaccharide Toxicity *in Vivo*. Administration to wild-type mice of even a very large amount of isolated human SAP (100 mg/kg) shortly before challenge with a lethal dose of 10 mg/kg of LPS did not confer any protection at all, with the same observed mortality rate as that observed after administration of human serum albumin as a control. In contrast, an equimolar quantity of human CRP significantly reduced morbidity and mortality (Fig. 5*a*), as previously reported in mice transgenic for rabbit CRP.[¶] In further experiments in which a second equal dose of human SAP or CRP was given 4 h after injection of LPS, exactly the

[¶]Xia, D., Lin, C., Yun, J., Wagner, T., Magnuson, T. & Samols, D. (1991) *FASEB J.* **5,** 1628 (abstr.).

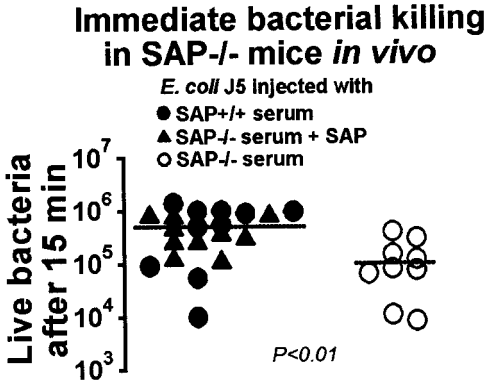
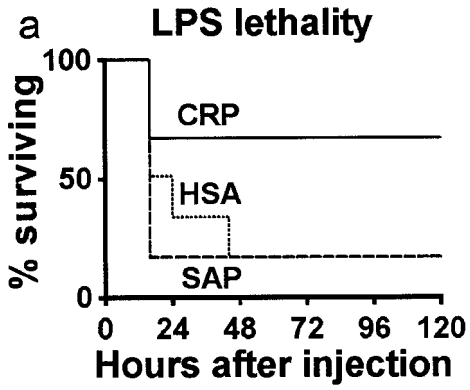


Fig. 4. Effect of SAP on immediate killing of *E. coli* J5 in $SAP^{-/-}$ mice *in vivo*. *E. coli* J5 was preincubated with whole acute phase serum from wild-type $SAP^{+/+}$ mice, or from $SAP^{-/-}$ animals, or with $SAP^{-/-}$ acute phase serum reconstituted by the addition of isolated mouse SAP, and 10^7 organisms were then injected i.p. into individual C57BL/6 $SAP^{-/-}$ mice. After 15 min the peritoneal cavity was lavaged, and the numbers of bacteria surviving in each animal were determined. Horizontal lines indicate medians, and the *P* value (Wilcoxon rank sum test) indicates the significant difference between bacterial killing in the presence or absence of SAP.

same result was obtained, with a final percentage of survivors in one experiment of 62.5% with CRP treatment, 25.0% with SAP, and 28.6% with albumin, and in a second, separate experiment, 25% with CRP and 0% with SAP. Furthermore, although $SAP^{-/-}$ mice showed accelerated morbidity after a lethal challenge with 10 mg/kg of LPS, the eventual mortality was the same as in $SAP^{+/+}$ wild-type animals (Fig. 5*b*). Importantly, challenge with 1 mg/kg of LPS, which is not clinically toxic for wild-type C57BL/6 mice, similarly caused no morbidity or mortality in $SAP^{-/-}$ mice. Thus, in contrast to the *in vitro* observations of de Haas *et al.* (27, 29), there is no evidence that SAP plays a significant role in protection from, or modulation of, LPS toxicity *in vivo*.

Table 2. Effect of human SAP binding on phagocytosis of bacteria by human neutrophils

Bacterium	Medium	Relative GFP or FITC fluorescence of ethidium positive neutrophils Mean (SD), $n = 3$
E. coli J5	TCB buffer	100.0 (10.3)
	SAP in TCB buffer	22.5 (4.6)
E. coli J5	Whole serum	32.9 (4.2)
	CRP-depleted serum	33.1 (0.9)
	SAP-depleted serum	100.0 (9.4)
	SAP-repleted serum	44.5 (7.1)
E. coli O111:B4	TCB buffer	100.0 (4.6)
	SAP in TCB buffer	97.7 (3.3)
E. coli O111:B4	Whole serum	94.4 (8.6)
	CRP-depleted serum	98.4 (18.0)
	SAP-depleted serum	100.0 (16.2)
	SAP-repleted serum	105.2 (17.2)
N. meningitidis H44/76	Whole serum	40.3 (7.7)
	SAP-depleted serum	100.0 (6.0)
N. meningitidis H44/76	SAP-depleted serum	100.0 (10.4)
	+ SAP 0.1 mg/l	91.9 (19.0)
	+ SAP 1.0 mg/l	89.3 (16.0)
	+ SAP 10.0 mg/l	62.6 (12.2)
	+ SAP 100 mg/l	47.3 (6.5)
	+ SAP 1000 mg/l	41.7 (7.4)



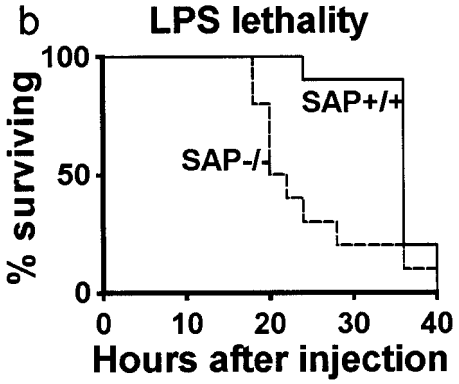


Fig. 5. Effect of SAP deficiency on survival after LPS challenge. (*a*) BALB/c mice received i.p. injections of 100 mg/kg of isolated pure human SAP, or equimolar amounts of isolated pure human CRP or serum albumin, followed after 20 min by 200 μg per mouse of *S. typhimurium* LPS, injected i.p. at time 0. (*b*) $SAP^{-/-}$ or wild-type $SAP^{+/+}$ C57BL/6 mice received 10 mg/kg of whole LPS from *E. coli* O111:B4, about 200 μg per mouse, injected i.p. at time 0.

Discussion

The antiopsonic property of SAP is presumably critical for its physiological function of retarding degradation of autologous chromatin in the extracellular environment (9). However, bacteria that express ligands to which SAP binds evidently use this mechanism to their advantage, and this is an example of a normal host protein acting like an antiopsonin, promoting bacterial survival, pathogenicity, and virulence.

In mice the only cellular site of significant *in vivo* catabolism of SAP is the hepatocyte (30), and the present findings confirm the apparent absence of receptors for SAP on human neutrophils that presumably underlies the antiopsonic effect of bound SAP. In addition to the direct, complement-independent effect of SAP that we show here using isolated SAP in serum-free medium or heat-inactivated sera, ligand binding by SAP may also interfere with opsonization by complement. Artificially aggregated SAP activates complement (8, 31), as do some of the macromolecular and particulate ligands to which SAP binds, including DNA and certain bacteria. However, the complex of SAP with chromatin, its physiological ligand, does not activate complement (8). This absence of activation is presumably important for the normal role of SAP in retarding and modulating chromatin clearance (9), but it has been subverted by bacteria that have evolved ligands for SAP, and, for

example, binding of SAP to the rough *S. typhimurium* Re strain inhibits complement activation by these organisms (32).

As well as binding to bacteria, SAP also binds to influenza virus (33), and we have lately demonstrated that, in comparison with wild-type mice, $SAP^{-/-}$ mice show markedly enhanced survival during lethal influenza virus infection and mount accelerated and increased anti-viral antibody responses (M. Kopf, B. Ecabert, G. Köhler, M.B.P., and M.F. Bachmann, unpublished observations). Thus in addition to its antiopsonic effects, binding of SAP can suppress the immunogenicity of microbial antigens as well as chromatin autoantigens (9), although the duration of the present bacterial infection models was too brief for this mechanism to have been significantly involved.

Regardless of the underlying mechanisms, the enhancement of virulence conferred by bound SAP suggests a possible new therapeutic target in infections caused by SAP-binding pathogens, and we have shown here, in an experimental model system, that *in vivo* administration of a drug that inhibits SAP binding prolongs survival during a lethal bacterial infection. Compound R is a dicarboxylic acid that must be given by injection, and it is very rapidly excreted. When given by continuous infusion from an osmotic minipump, doses as low as 30 μg per mouse per day are sufficient to completely inhibit and reverse all SAP binding *in vivo* (M.B.P., unpublished observations). However, implantation of such pumps was not compatible with the established infection models used here. Although the very short course of drug R administered by repeated injection in these experiments did not affect the final number of survivors, the present results (Fig. 3) and the reconstitution of $SAP^{-/-}$ mice with pure SAP (Fig. 2*c*) clearly confirm the role of SAP in enhancing bacterial virulence. The results with *S. pyogenes* infection and our *in vitro* observations with *N. meningitidis*, human SAP, and human neutrophils (Tables 1 and 2) suggest that this mechanism may well be operative in clinically significant human infections.

In contrast to the effects of SAP binding that are harmful to the host, SAP evidently also contributes to survival during infections with organisms to which it does not bind. We show this contribution here for *E. coli* O111:B4 and have previously found it with smooth *S. typhimurium* 12023, an intracellular Gram-negative bacterium to which little or no SAP binds (ref. 14; M. Botto, J. Warren, M. J. Walport, and M.B.P., unpublished observations). We have also observed increased parasitemia in $SAP^{-/-}$ animals compared with wild-type mice after infection with the malarial parasite *Plasmodium chabaudii* (M. Botto, P. Taylor, M. J. Walport, and M.B.P., unpublished observations). Several mechanisms may underlie the role of SAP in host resistance in these different infections, although our present results do not support the idea that binding to LPS is important. It is not yet clear whether SAP has any specific anti-infective property or whether it may also be beneficial during other inflammatory or tissue-damaging processes. The toxicity and proinflammatory properties of materials released by cell death of both autologous and microbial cells contribute to morbidity and mortality during infection. The critical role of SAP in appropriate handling of chromatin, and perhaps other ligands, exposed or released by apoptosis and necrosis (9), may thus be important. For example, prokaryotic DNA is toxic in mice (34), and the major DNA-binding property of SAP (5) may be protective.

The contrasting roles of SAP, which we report here, in host resistance to infections are a novel example of the interplay between evolution in the host and in the pathogen. The protean and important protective functions of the evolutionarily ancient SAP molecule, with respect to antinuclear autoimmunity and perhaps some pathogens, probably accounts for its high degree of conservation. In contrast, the evolution by some bacteria of ligands to which SAP binds permits evasion of host defenses, causing enhanced virulence. Inhibition of this binding may offer a new approach to therapy.

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