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Serum Amyloid P-Component-Induced Enhancement of Macrophage Listericidal Activity

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Purified serum amyloid P component (SAP), the major acute-phase reactant of mice, augmented the in vitro listericidal activity of inflammatory (elicited) macrophages, bone marrow-derived monocytes, and macrophages from a subcutaneous site of inflammation. Monocytes and macrophages from C57BL/B6 mice, which are relatively resistant to *Listeria monocytogenes*, exhibited a significantly greater enhanced killing capacity for listeria than macrophages from listeria-susceptible A/J mice. SAP did not alter the extent of phagocytosis by macrophages of opsonized *L. monocytogenes*, nor was SAP opsonic for listeria. Mannose-derived simple sugars inhibited the binding of SAP to macrophages and consequently prevented the enhanced SAP-dependent listericidal activity. Macrophages from lipopolysaccharide-hyporesponsive mice also had increased microbicidal activity following incubation with SAP. SAP activated macrophages independently of lymphokine. Therefore, SAP may serve as a mediator of the heightened nonspecific host defense response that is associated with the acute phase of the systemic inflammatory response.

Host defense mechanisms to a large extent depend on several nonspecific inflammatory responses. Acute microbial infections initiate events that usually lead to a systemic inflammatory response which is characterized in part by a greatly increased rate of synthesis and secretion of a group of plasma proteins known as acute-phase reactants (APRs) (19, 30). Serum amyloid P component (SAP) is a major APR of mice (21, 31) and is identical to the P component present in all amyloid deposits (3). Mouse SAP is a 230,000-dalton alpha-glycoprotein composed of 10 identical, noncovalently linked subunits arranged as two pentraxins binding face to face (21, 29, 32). SAP is a molecular homolog of C-reactive protein (CRP), the prototypical APR of humans (29). Human SAP possesses a high-affinity Ca^{2+}-dependent binding site for the 4,6-cyclic pyruvate acetal of the galactan in most agarose preparations (12). Endogenous concentrations of SAP among inbred mouse strains vary over a wide range, but still function as a typical APR in all of the strains (25). Recently it was shown that SAP levels in mice are controlled by a major locus, designated *Sap*, that is closely linked to the *Mls* and *Ly-9* loci on the distal portion of chromosome 1 (27). The biological significance of the genetically controlled SAP levels in the inbred mouse strains remains unknown, since no well-defined role for SAP has been established (30).

Natural resistance to infection with the facultative intracellular pathogen *Listeria monocytogenes* in mice is controlled by a single, dominant gene not linked to H-2 (4, 35), and this natural resistance is mediated by monocytes and macrophages (18). C57BL/6 (B_6) and closely related strains are relatively resistant to listeria (L^r), while strains such as A/J, C3H/HeJ, and DBA/2 are more susceptible (L^s) (35). The strain difference in susceptibility to *L. monocytogenes* infection has been attributed to the rapid mobilization of significantly greater number of macrophages (36) or neutrophils (6) to the infected (inflammatory) site in L^r strains than in L^s strains. A defect in the infiltration of phagocytic cells into infected sites in some L^s strains has been associated with C5 deficiency (10). Elicited peritoneal macrophages of mice can be activated for killing *L. monocytogenes* either by sublethal infection of the host (11) or by incubating the macrophages in vitro with lymphokine (LK) preparations (5, 11, 15). CRP, a pentraxin which shares 70% amino acid sequence homology with SAP (30), has been shown to be a potent activator of mouse macrophages for tumoricidal function when it is delivered in vivo encapsulated in liposomes (2, 7). The investigations reported here showed that purified mouse SAP enhanced nonimmune mouse macrophage listericidal activity; however, the response was influenced by the genetic background of the macrophages.

MATERIALS AND METHODS

Mice. Inbred strain A/J, C57BL/6J, C3H/HeJ, and C3H/HeN mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. Strains C57BL/10SN and C57BL/10ScN were obtained from Harlan Sprague-Dawley, Indianapolis, Ind. Mice were housed in a portable containment system (model PCS-80; Hazelton Labs, Bethesda, Md.) with an airflow of 25 ft^3/min (0.7 m^3/min). The housing minimized changes in endogenous levels of APRs (25). Female mice 8 to 16 weeks old were used in all of the experiments. (BXA) F_1 mice were bred in our animal facility.

L. monocytogenes. A virulent strain of *L. monocytogenes* (strain EDG) was kindly supplied by Robert North (Trudeau Institute, Saranac Lake, N.Y.) and was kept as a stock culture at −70°C. A temperature-sensitive mutant of *L. monocytogenes* prepared by Anne Morris-Hooke, Georgetown University, Washington, D.C., was used for the listericidal assays (A. Morris-Hooke, T. A. Tran, F. Gervais, and E. Skamene, unpublished data). This mutant strain does not replicate at 37°C, but is identical to the wild-type parent strain in its susceptibility to phagocytosis and its surface antigenicity (F. Gervais, F. Morris-Hooke, and E. Skamene, unpublished data). The temperature-sensitive mutant was stored at −70°C and was grown in Trypticase soy broth overnight. Logarithmic-phase cultures were washed with Hanks balanced salt solution (HBSS) and incubated with 1.0% fresh normal mouse serum in HBSS at 37°C for 30 min for opsonization. The listeria cells were

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washed and suspended in HBSS at a density of 10^8 cells per ml.

Purification of SAP. Mouse SAP was purified by affinity chromatography as described previously (21). Briefly, acute-phase mouse serum was chromatographed on phospho-ethanolamine-linked agarose beads (Sigma Chemical Co., St. Louis, Mo.) in Tris-saline (0.15 M) buffer (pH 7.5) containing 5 mM Ca^{2+}. After extensive washing, protein was eluted with 0.02 M Tris-buffered (pH 7.5) saline (0.12 M) containing 10 mM EDTA. Fractions containing protein were pooled and further sized by chromatography on a column (1.5 by 60 cm) of ACA-54 Ultrogel (Pharmacia, Inc., Piscataway, N.J.) in the same buffer. The fractions containing SAP eluted at molecular weights of 210,000 to 230,000, and individual fractions were dialyzed against HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered (pH 7.0) 0.15 M saline, filter sterilized, and stored at 4°C. The presence and concentration of SAP were determined by rocket immunoelectrophoresis, using a serum standard of 200 μg/ml; the sensitivity of the assay was 2 μg/ml. The yield of SAP was 60 to 70%. The protein at a concentration of 400 μg/ml migrated as a single band at a molecular weight of 31,000 in a reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis system. This band accounted for 95 to 97% of the protein and corresponds to the subunit size for mouse SAP (21).

Collection of macrophages. Peritoneal exudate cells were harvested 48 h after a single intraperitoneal injection of 3.0 ml of 10.0% proteose peptone (Difco Laboratories, Detroit, Mich.). The proteose peptone contained <0.1 ng of lipopolysaccharide (LPS) per ml, as measured by a chromogenic *Limulus* assay (M. A. Bioproducts, Rockville, Md.). The cells were washed and suspended in Dulbecco modified Eagle medium (DMEM) (M. A. Bioproducts) containing 5.0% fetal bovine serum (FBS) (HyClone, Logan, Utah). Antibiotics were not added to any of the reagents or media used in the listericidal assay. The cell suspension was diluted to a density of 10^6 macrophages per ml, and 1.0 ml per well was added to a 24-well tissue culture plate (Falcon no. 3407). The cells were allowed to adhere for 2 h at 37°C in 5.0% CO_2–air. The wells were washed with warm HBSS five times to remove the nonadherent cells. Samples of the adherent cells were stained with phosphate-buffered Giemsa stain and for cytoplasmic esterase activity (16). The adherent cells were 90 to 95% esterase positive and morphologically phagocytic macrophages.

Lymphokine preparation. Culture supernatant from a spleen (C3H/HeN) cell suspension (4×10^6 cells per ml) incubated for 48 h with 2.0 μg of concanavalin A (3× crystallized; Miles Scientific, Div. Miles Laboratories, Inc., Kankakee, Ill.) per ml and 10 ng of phorbol myristic acetate (Sigma) per ml was used to activate macrophages. A single preparation was used for these studies.

Subcutaneous inflammatory macrophages. Macrophages from subcutaneous inflammation sites were obtained by the method of Fauve et al. (9), with modifications. Briefly, 3.0 ml of 3.5% Bio-Gel P-100 beads (100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) in sterile HBSS was injected subcutaneously into pouches in mice created by the divulsion of dorsal skin, 5.0 mm behind the neck. Four days later the cells were collected by injecting and withdrawing 5.0 ml of cold, sterile HBSS through the same puncture used to inject the beads. This process was repeated three times. The cell suspension was kept at 4°C and filtered through a sterile stainless steel screen (400 mesh) to remove the beads. The cells were washed in cold HBSS, suspended in DMEM containing 5.0% FBS, adjusted to a density of 10^6 cells per ml, and allowed to adhere to plates for the listericidal assay.

BM-derived monocytes. Bone marrow (BM)-derived macrophages were obtained by using the liquid culture method of Stewart (37), with modifications to recover the cells as described by Warren and Vogel (38). BM cells were harvested by flushing a femur with alpha minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with L-glutamine (2.0 mM), gentamicin (50 μg/ml), and 7.5% sodium bicarbonate solution. To culture BM cells, the medium was further supplemented with 10% FBS and 10% L-cell-conditioned medium as a source of macrophage colony-stimulating factor 1 (complete alpha minimal essential medium). BM cells (10^7 cells in 10 ml) in complete alpha minimal essential medium were transferred to a T-75 flask and incubated for 24 h to allow the strongly adherent cells (especially fibroblasts) to attach, leaving the macrophage colony-forming cells in suspension. The nonadherent BM cells at a density of 2×10^5 cells per ml in fresh complete alpha minimal essential medium were transferred to another T-75 flask. After 7 to 10 days the BM monocyte precursors had proliferated and differentiated into monocyte colonies of 20 to 50 cells, which were recovered by using the neutral protease Dispase II (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) (38). Viability of the recovered monocytes was >95%, with a yield of 60 to 80%. The cells were then added to a 24-well plate exactly as described below for the listericidal assay.

Binding of SAP by macrophages. The washed adherent macrophages were incubated at 37°C with SAP in DMEM containing 10.0 mM HEPES and 10.0% FBS. Five sugars [D-(+)-mannose, D-mannose 6-phosphate, alpha-D-(+)-mannose 1-phosphate, methyl-alpha-mannopyranoside, and *N*-acetylgalactosamine (Sigma)] were tested for their ability to inhibit both SAP binding and SAP-enhanced macrophage listericidal activity. Each sugar was added to the adherent macrophages along with purified mouse SAP (5.0 μg/ml) and incubated for 2 h prior to examining the cells for SAP binding and assaying for listericidal activity. The fraction of macrophages which bound SAP was determined by peroxidase staining, using biotinylated SAP followed by avidin horseradish peroxidase. The preparation of the reagents has been described elsewhere (20).

Listericidal assay. For the listericidal assay, 10^6 macrophages in 0.9 ml of 0.01 M HEPES-buffered DMEM were infected with 10^7 opsonized cells of the temperature-sensitive listeria mutant in a 1.0-ml volume. The macrophages were allowed to ingest the opsonized *L. monocytogenes* cells for 20 min at 37°C, after which they were washed with complete DMEM five times to remove the extracellular bacteria (more than 90% of the listeria cells were inside the macrophages). For a zero-time bacterial count, the macrophage layer was lysed with 1.0 ml of water, and the number of CFU of listeria was determined by plating 10-fold dilutions of the lysates on Trypticase soy agar. The bacterial plates were kept at room temperature for 2 days. The results were expressed as percent killing of listeria by comparing the number of CFU at 1 h with the number of CFU at zero time. Macrophages from each of three mice were tested separately. Listericidal activity was also measured on the basis of micrograms of protein (macrophages). There was no difference in the percentage of killing based on amount of macrophage protein and based on macrophage numbers.

Phagocytosis assay. Peritoneal macrophages were tested for phagocytic activity while in suspension by the method of Horwitz and Silverstein (13). Peritoneal exudate cells at a

density of 10^6 cells per ml suspended in 1.0 ml of RPMI 1640 medium containing 10% FBS in a 5-ml polypropylene tube were exposed to 10^7 opsonized listeria cells for 30 min at 37°C. The cells were centrifuged and suspended in medium, and a cytosmear was prepared and then stained with buffered Giemsa stain. The percentage of phagocytic macrophages and the mean number of bacteria per macrophage were determined on slides containing 100 cells, with three to four slides per experimental parameter.

Statistical methods. Data are expressed as the percentage of listeria killed on the basis of the number of listeria at zero time. Data in the form of $\log_{10}$ CFU of listeria were analyzed by a two-tailed Student's t test, and a significant difference was considered to be $P < 0.05$.

RESULTS

Effect of SAP on macrophage listericidal activity. The exposure of elicited peritoneal macrophages from A/J and C57BL/6J mice to various concentrations of purified mouse SAP enhanced their listericidal activity (Fig. 1). The maximum enhancement of the listericidal activity of macrophages from both L^r and L^s strains occurred at SAP concentrations of $\geq$5.0 μg/ml. Macrophages from the L^r strain (C57BL/6J) showed significantly greater listeria killing than macrophages from strain A/J (Fig. 1). When macrophages from the L^r strain were exposed to 5.0 μg of SAP per ml for 0.5 to 16.0 h, we found that only a 2.0-h exposure was necessary for maximum enhancement of the listericidal activity (Fig. 2). Upon exposure to 5.0 μg of SAP per ml, resident macrophages from either strain did not display significantly enhanced listericidal activity (Table 1).

Since the bulk of macrophages that infiltrate a site of infection are recently derived from the BM, we tested monocytes grown in vitro from precursors for their response to SAP. BM-derived macrophages from both C57BL/6J and A/J mice displayed enhanced listericidal activity after exposure to SAP (Table 2). The BM-derived macrophages from C57BL/6J mice exhibited significantly greater killing activity than those from A/J mice.

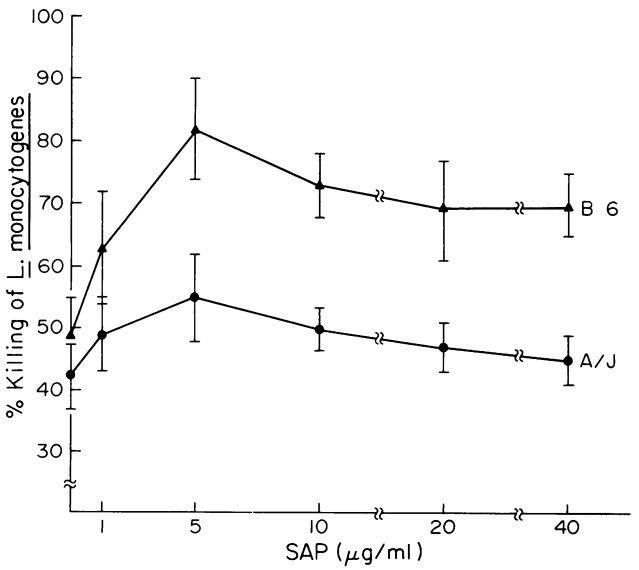

FIG. 1. Listericidal activities of elicited (inflammatory) macrophages from strain A/J and C57BL/6J (B6) mice treated with different concentrations of purified mouse SAP. Data are the means ± standard deviations of five experiments, each of which tested macrophages from three mice separately. Significant increases ($P < 0.05$) in killing by strain C57BL/6J macrophages occurred at concentrations of 5 to 40 μg/ml.

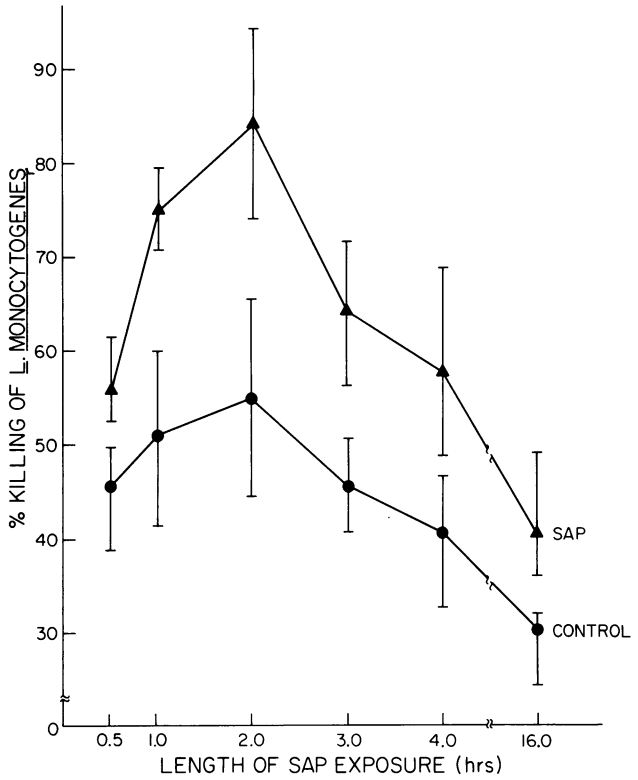

FIG. 2. Effect of incubating elicited macrophages from C57BL/6J mice with purified mouse SAP (5.0 μg/ml) for various periods of time prior to measuring listericidal activity. The means ± standard deviations of three experiments are shown.

Macrophages were also isolated from a subcutaneous inflammatory site induced by sterile polyacrylamide beads. Upon incubation with SAP, these macrophages also displayed enhanced listericidal activity; the treated C57BL/6J macrophages were significantly more responsive than the A/J macrophages (Table 3). Therefore, monocytes or macrophages from different anatomical sites were responsive to mouse SAP.

Effect of sugars on SAP-enhanced listericidal activity. Elicited macrophages from C57BL/6J mice bind SAP that can be readily detected on approximately 30% of the cells by peroxidase staining. Of a large variety of substances tested, only certain mannose-based sugars inhibited the binding of

TABLE 1. Effect of purified mouse SAP on the listericidal activity of resident peritoneal macrophages from C57BL/6J and A/J mice

SAP (μg/ml)	% Killing (mean ± SD) of *L. monocytogenes* by macrophages[a]	
	Strain C57BL/6J	Strain A/J
5.0	58 ± 11	53 ± 9
0	47 ± 6	45 ± 7

[a] Resident macrophages at a concentration of 0.5×10^6 cells per well (24-well plates) were tested for listericidal activity by the procedure used for elicited macrophages. $P > 0.05$.

TABLE 2. Effect of purified mouse SAP on the listericidal activity of BM-derived macrophages from C57BL/6J and A/J mice

No. of BM-derived macrophages per well[a]	No. of *L. monocytogenes* cells per well	% Killing (mean ± SD) of *L. monocytogenes* by macrophages			
		Strain C57BL/6J		Strain A/J	
		SAP treated[b]	Control	SAP treated[b]	Control
10^5	10^6	83 ± 5[c]	55 ± 6	52 ± 7	39 ± 4
10^4	10^5	78 ± 3[c]	50 ± 8	55 ± 3	39 ± 6
10^3	10^4	85 ± 6[c]	53 ± 4	49 ± 5	43 ± 5

[a] Number of macrophages per well in a 24-well culture plate (1.0 ml).
[b] Macrophages were exposed to 5.0 μg of SAP per ml for 2.0 h at 37°C.
[c] $P < 0.05$ for the SAP-treated value compared with the control value.

SAP (33). D-(+)-Mannose, mannose 6-phosphate, and mannose 1-phosphate inhibited binding of SAP to macrophages. The first two sugars also showed selective inhibition of SAP-enhanced listericidal activity at concentrations of 10.0 to 1.0 mM (Table 4). Methyl-alpha-mannopyranoside and *N*-acetylgalactosamine had no significant inhibitory effect on either the binding of purified SAP or the enhanced listericidal activity. Our results show a requirement for SAP binding to macrophages to exert an enhanced listericidal response.

Effect of SAP on the listericidal activity of macrophages from LPS-hyporesponsive mice. To determine whether the SAP-induced enhancement of macrophage listericidal activity was a response to small amounts of LPS in the SAP, macrophages from LPS-responsive mouse strains C57BL/10SNJ (L^r) and C3H/HeN (L^s) were compared with macrophages from LPS-hyporesponsive strains C57BL/10ScN (L^r) and C3H/HeJ (L^s). Our results showed that macrophages from strains C57BL/10SNJ and C57BL/10ScN responded to SAP with a significant increase in listericidal activity, whereas the response of the macrophages from the C3H/HeN and C3H/HeJ mice was minimal (Table 5). The LPS responsiveness of the donor strain of the macrophages had no influence on the SAP-enhanced listericidal activity. Removal of the small amount of LPS from the SAP preparation was attempted by passing the preparation through a Detoxi-gel column (Pierce Chemical Co., Rockford, Ill.) containing polymyxin B linked to agarose beads. Although this procedure lowered the LPS content by 50% to 0.025 to 0.050 ng/ml, it had no significant effect on the ability of SAP to enhance the listericidal activity of macrophages (data not shown).

Effect of SAP on phagocytosis. One possible mechanism by which SAP may increase the listericidal activity as measured by our assay is to enhance phagocytosis. Therefore, macrophages were incubated in suspension with SAP for 90 min and exposed to listeria cells for only 30 min; SAP did not alter the proportion of macrophages ingesting bacteria or the mean number of bacteria per macrophage over a 30-min period (Table 6). In addition, the zero-time number of CFU in the listericidal assay was not altered by the presence of SAP (Fig. 1). In other experiments, SAP was added to suspensions of *L. monocytogenes* to see whether it directly opsonized the bacteria; however, no significant uptake was mediated by SAP. It is unlikely that SAP enhanced the listericidal activity by augmenting the uptake of listeria.

TABLE 4. Effect of simple sugars on the SAP-enhanced listericidal activity of macrophages from C57BL/6J mice

Treatment		% Macrophages binding SAP	% Killing of *L. monocytogenes*		% Inhibition of SAP-enhanced killing[b]
Sugar added	Concn (mM)		SAP treated[a]	Medium	
None		30	83	49	0
D-(+)-Mannose	10.0	2	50	47	40
	1.0		67	50	19
Mannose 6-phosphate	10.0	5	63	46	24
	1.0		73	44	12
Mannose 1-phosphate	10.0	7	81	48	2
	1.0		84	52	(+1)
Methyl-alpha-mannopyranoside	10.0	32	80	51	4
	1.0		83	49	0
N-Acetyl-galactosamine	10.0	24	80	47	4
	1.0		85	51	(+2)

[a] Preparations were incubated with 5.0 μg of SAP per ml for 2 h in the presence of the sugar. The percent killing is the mean of three identical experiments.
[b] The numbers in parentheses indicate enhancement rather than inhibition.

Activity of SAP compared with other serum proteins. To determine whether the activity displayed by SAP was a nonspecific effect by a protein, other serum proteins were tested in the listericidal assay. Macrophages exposed for 2.0 h to purified mouse immunoglobulin G (IgG) (0.1 to 40 μg/ml), mouse IgA myeloma (0.1 to 50 μg/ml), human IgG (1.0 to 40 μg/ml), or human CRP (0.1 to 10 μg/ml) did not display the enhanced listericidal activity observed with purified mouse SAP. Representive data from one of three similar experiments are shown in Table 7.

Effect of SAP on LK-dependent listericidal activity. Since LK preparations can activate elicited macrophages to become bactericidal in vitro, we wished to determine whether SAP influenced this activity. No change in the SAP-enhanced macrophage listericidal activity was observed when macrophages were exposed to 5.0 μg of SAP per ml and suboptimal dilutions of LK for 2 h simultaneously (Fig. 3). However, the activation level was already maximal (approximately 90%), and thus any additional activity could not be detected. Therefore, to determine whether any syn-

TABLE 3. Effect of purified mouse SAP on the listericidal activity of macrophages isolated from granulomas of C57BL/6J and A/J mice

SAP (μg/ml)	% Killing (mean ± SD) of *L. monocytogenes* by macrophages	
	Strain C57BL/6J	Strain A/J
5.0	87 ± 7[a]	58 ± 6
None	51 ± 5	48 ± 4

[a] Value significantly greater than the strain A/J and control values ($P < 0.05$).

TABLE 5. Effect of purified mouse SAP on the listericidal activity of macrophages from LPS-responsive and LPS-hyporesponsive mice

SAP (μg/ml)	% Killing (mean ± SD) of *L. monocytogenes* by macrophages from:			
	LPS-responsive strains		LPS-hyporesponsive strains	
	C57BL10/SNJ	C3H/HeN	C57BL10/ScN	C3H/HeJ
5.0	77 ± 5[a]	53 ± 6	81 ± 9[a]	57 ± 4
0	52 ± 3	45 ± 7	49 ± 6	43 ± 5

[a] $P < 0.05$ for SAP-treated mice compared with controls.

TABLE 6. Effect of mouse SAP on C57BL/6J macrophage phagocytosis of *L. monocytogenes*

Treatment of macrophages[a]	% Macrophages (SD) ingesting bacteria[b]	No. of bacteria/macrophage (SD)
Control	65.5 (7.5)	6.1 (1.3)
SAP (10 μg/ml)	57.3 (6.2)	5.1 (1.5)

[a] Cells were incubated with SAP for 90 min before challenge with listeria; the ratio of bacteria to macrophages was 10:1.
[b] Data from four experiments, each consisting of three slides with 100 cells per slide.

ergy between SAP and LK existed, macrophages were exposed to suboptimal amounts of SAP (≤1.0 μg/ml) and LK (dilution, >1:16) simultaneously. No synergy between LK and SAP was observed (Table 8).

DISCUSSION

Inbred mouse strains vary in both their nonimmune or natural resistance to early stages of infection with *L. monocytogenes* (4, 10, 18, 35, 36) and their endogenous SAP levels (25). We previously reported that induced SAP levels during the first 24 h of infection correlated with the organ burden of listeria in both resistant and susceptible mouse strains (26, 34). Both high endogenous levels and very high induced levels (milligrams per milliliter) of SAP were associated with listeria susceptibility in mouse strains A/J, DBA/2, and C3H/HeJ; however, the blood level of SAP may not reflect its function in the liver and spleen, the two major sites of infection following intravenous challenge with *L. monocytogenes* (28). The high levels observed by days 2 and 3 may reflect the extent of tissue damage by 24 h of infection (26). The apparent contradiction between elevated blood levels of SAP and lack of increased resistance was the basis for these studies since testing of purified SAP and a relatively pure population of macrophages should enable us to detect whether SAP influences macrophage functions clearly related to relative resistance to listeria (5, 6, 18, 28, 36).

The observations reported in this paper demonstrate that purified mouse SAP can enhance the in vitro listericidal activity of inflammatory mononuclear phagocytes. Macrophages from C57BL/6J mice, a strain with relatively high natural resistance to listeria (35), were more responsive to SAP than macrophages from susceptible strain A/J. The enhancement of listericidal activity was dependent on SAP binding to macrophages since both binding and the increase in killing were inhibited in the presence of mannose-based sugars. Monocytes and macrophages obtained from different anatomical sources were equally responsive to SAP; how-

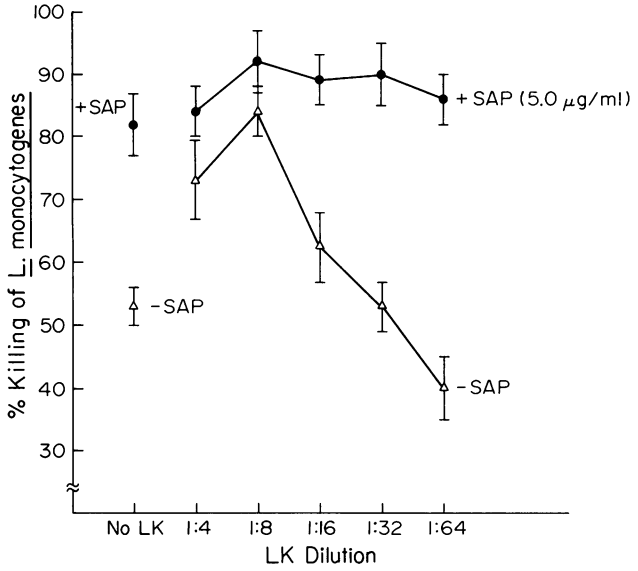

FIG. 3. Effect of SAP (5 μg/ml) on the LK-induced enhancement of the listericidal activity of elicited macrophages. The means ± standard deviations of two similar experiments are shown. Significant differences ($P < 0.05$) were observed at LK dilutions of 1:16, 1:32, and 1:64.

TABLE 7. Selective activation by SAP of C57BL/6J macrophages for listericidal activity compared with other serum proteins

Protein[a]	Concn (μg/ml)	% Killing of *L. monocytogenes* (mean ± SD)
Mouse IgG	0.1	51 ± 4
	5.0	53 ± 6
	10.0	51 ± 7
Mouse IgA	0.1	47 ± 4
	5.0	48 ± 4
	10.0	52 ± 6
Human IgG	1.0	49 ± 4
	5.0	53 ± 5
	10.0	54 ± 2
Human CRP	0.1	49 ± 2
	5.0	58 ± 5
	10.0	57 ± 7
Mouse SAP	0.1	49 ± 4
	5.0	79 ± 8
	10.0	63 ± 6
Medium		48 ± 4

[a] Mouse IgG and human IgG were prepared from serum. Mouse IgA was obtained from the MOPC-315 myeloma. Human CRP was purified as described elsewhere (2).

TABLE 8. Effect of suboptimal concentrations of SAP and LK on the listericidal activity of C57BL/6J macrophages[a]

SAP (μg/ml)	LK dilution	% Killing of *L. monocytogenes*
5.0		86[b]
1.0		59
0.5		50
0.1		48
	1:8	91[b]
	1:16	57
	1:32	55
	1:64	53
1.0	1:16	53
1.0	1:32	55
1.0	1:64	56
0.5	1:16	55
0.5	1:32	53
0.5	1:64	57
0.1	1:16	44
0.1	1:32	50
0.1	1:64	47
		47

[a] SAP and LK were added simultaneously to 10^6 macrophages for 2 h prior to the listericidal assay.
[b] $P < 0.05$ compared with the control.

ever, neither resident nor fully activated (LK-treated) macrophages could respond with increased listericidal activity to SAP. The SAP-driven enhanced bactericidal activity appeared to be an event that was independent of LK-mediated activation and was not related to increased uptake of the bacteria.

The mechanism of enhanced activation of macrophages by APRs is not fully understood, yet several relevant characteristics of the interaction between SAP and macrophages were observed. LPS is widely appreciated as a potent activator of macrophages for a variety of effector functions, including microbicidal activities (24); therefore, we tested whether the subnanogram amount of LPS (0.05 to 0.10 ng/ml) present in the SAP preparation was responsible for the enhanced listericidal response. The listericidal response of SAP-treated macrophages from LPS-nonresponsive mouse strains was not different from that of the syngeneic LPS-responsive counterparts. This result is consistent with an earlier finding of equal susceptibility of C3H/HeJ and C3H/HeN mice to infection with *L. monocytogenes* (35). SAP binding to macrophages was selectively blocked by simple mannose sugars, and therefore we tested the effects of nontoxic concentrations of these sugars on listericidal activity in the presence of SAP and found a correlation between inhibition of binding and inhibition of enhanced listeria killing. Inflammatory macrophages are known to have a binding site(s) on their surface that recognizes glycoproteins with mannose, *N*-acetylglucosamine, or fucose residues (8, 14). Mouse SAP is a glycoprotein that may contain mannose, although the carbohydrate composition has not been determined, and mannose is the most abundant sugar in glycosylated proteins and would be expected to be present (30). Fully activated *Mycobacterium bovis* BCG-induced macrophages failed to bind detectible SAP (33), an observation consistent with the progressive loss of mannose receptors from macrophages during activation (8, 14). Whether SAP is selectively bound by inflammatory macrophages and therefore functions via a specific receptor remains to be determined. It should be noted that binding of human macrophages to immobilized SAP has been reported to activate complement receptors, resulting in the phagocytosis of C3b-coated particles (40). SAP does not contain detectable amounts of mouse gamma-interferon, a well-documented potent activator of macrophage tumoricidal activity (1).

Human CRP is considered the prototype APR because it increases up to 10^3-fold in concentration (19); it is also closely related by several molecular criteria to SAP (30). CRP delivered encapsulated in liposomes has been shown to activate mouse macrophages to display tumoricidal activity (2, 7); however, mouse SAP did not activate when it was tested in the same assays (2). The dissociation between tumoricidal and microbicidal activities by macrophages has been noted before (39) and probably reflects distinct mechanisms for each function (1, 23). CRP occurs in only nanogram-per-milliliter amounts in the blood of mice and is probably not an APR in this species (21, 30). CRP has been shown to enhance mouse macrophage production of superoxide anion (2), a microbicidal chemical; however, in preliminary tests SAP did not enhance hydrogen peroxide production induced by phorbol myristic acetate, but did increase background levels by twofold. Reactive oxygen intermediates generated by an oxidative burst in macrophages can kill intracellular *L. monocytogenes* (22). However, lysosomal enzymes also play a role in the bactericidal activity of macrophages, and the effect of acute-phase proteins on the activity of these enzymes has not been examined.

The infection of mice or macrophages with *L. monocytogenes* has been extensively used as a model of both T-cell (LK)-dependent resistance (5, 15, 22, 28) and natural resistance (4, 6, 10, 18, 36) to intracellular microbial pathogens. Since mononuclear phagocytes, especially monocytes, have been implicated in adoptive transfer of natural or nonspecific resistance to listeria (18, 35), it would be logical to examine blood proteins such as APRs which might influence the behavior of these cells during the early stages of infection. Although SAP has a modest enhancing effect on in vitro listericidal activity, it might influence the behavior of the cells during the early stages of the infection and may be present in sufficient quantities in the in situ environment of the monocytes and fixed macrophages to alter the outcome of the early stages of an acute infection with an intracellular microbial pathogen. Our results indicate that strain C57BL/6J and A/J macrophages differ dramatically in their responses to SAP, with strain A/J macrophages displaying an intrinsic defect in their responsiveness.

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