Protection from Lethal Gram-positive Infection by Macrophage Scavenger Receptor–dependent Phagocytosis

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Abstract

Infections with gram-positive bacteria are a major cause of morbidity and mortality in humans. Opsonin-dependent phagocytosis plays a major role in protection against and recovery from gram-positive infections. Inborn and acquired defects in opsonin generation and/or recognition by phagocytes are associated with an increased susceptibility to bacterial infections. In contrast, the physiological significance of opsonin-independent phagocytosis is unknown. Type I and II class A scavenger receptors (SR-AI/II) recognize a variety of polyanions including bacterial cell wall products such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), suggesting a role for SR-AI/II in innate immunity to bacterial infections. Here, we show that SR-AI/II–deficient mice (MSR-A$^{-/-}$) are more susceptible to intraperitoneal infection with a prototypic gram-positive pathogen, *Staphylococcus aureus*, than MSR-A$^{+/+}$ control mice. MSR-A$^{-/-}$ mice display an impaired ability to clear bacteria from the site of infection despite normal killing of *S. aureus* by neutrophils and die as a result of disseminated infection. Opsonin-independent phagocytosis of gram-positive bacteria by MSR-A$^{-/-}$ macrophages is significantly decreased although their phagocytic machinery is intact. Peritoneal macrophages from control mice phagocytose a variety of gram-positive bacteria in an SR-AI/II–dependent manner. Our findings demonstrate that SR-AI/II mediate opsonin-independent phagocytosis of gram-positive bacteria, and provide the first evidence that opsonin-independent phagocytosis plays a critical role in host defense against bacterial infections in vivo.

Key words: scavenger receptor • macrophage • phagocytosis • gram-positive bacteria • *Staphylococcus aureus*

Introduction

Phagocytosis of microorganisms is a key element in host defense against bacterial infections (1). Two principal mechanisms of phagocytosis have been described, opsonin-dependent phagocytosis (2) and opsonin-independent phagocytosis (for a review, see reference 3). In opsonin-dependent phagocytosis, immunoglobulin or complement molecules bind to microorganisms, thereby promoting ingestion via Fcγ or complement receptors on phagocytic leukocytes (2). In contrast, in opsonin-independent phagocytosis, ligands on the surfaces of microorganisms are directly recognized by receptors on the plasma membranes of phagocytes (3).

Several lines of evidence link defects in opsonin-dependent phagocytosis to an increased susceptibility to infection. First, immunoglobulin- or complement-deficient animals (4–6) and humans (7) exhibit an increased incidence of bacterial infections. Second, administration of antibodies against bacterial capsular antigens (8–10), or immunization against bacterial capsular antigens (11), protects against infections with bacteria expressing these antigens. Third, Fcγ receptor polymorphisms in humans (12, 13) are associated with increased susceptibility to infection.

Much less is known about the physiological roles of op-

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Published January 3, 2000

sonin-independent phagocytosis in host defense against bacterial infections. Types I and II class A macrophage scavenger receptors (SR-AI/II)[1] are homotrimeric membrane proteins (14) of mononuclear phagocytes that mediate phagocytosis of apoptotic thymocytes (15), endocytosis of modified lipoproteins (16), and adhesion of macrophages to surfaces coated with serum proteins (17), glucose-modified basement membrane proteins (18), and β-amyloid fibrils (19). The demonstration that macrophage scavenger receptors bind bacterial cell wall components such as LPS from gram-negative bacteria (20) or lipoteichoic acid (LTA) from gram-positive bacteria (21), and that SR-AI/II–deficient mice (MSR-A$^{-/-}$) exhibit increased susceptibility to infection with *Listeria monocytogenes* (22) and to LPS-mediated shock (23) suggested that these receptors function in the absence of serum opsonins in host defense against bacterial infections.

We hypothesized that SR-AI/II mediate opsonin-independent phagocytosis of bacteria via a direct interaction of SR-AI/II with bacterial cell wall products such as LTA, leading to clearance of bacteria from sites of infection. To test this hypothesis, we chose *Staphylococcus aureus*, a prototypical gram-positive microorganism and an important cause of life-threatening bacterial infections in humans (24–26). Since SR-AI/II are expressed mainly on mononuclear phagocytes, and these cells are the first line of antimicrobial defense in the peritoneal cavity (27–29), we compared the susceptibility of MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice to intraperitoneal challenge with two strains of *S. aureus*. Here, we show that intraperitoneal injection of *S. aureus* Cowan strain I leads to overwhelming infection and death in MSR-A$^{-/-}$ mice at a significantly lower dose than in wild-type mice. The impaired ability of MSR-A$^{-/-}$ mice to clear *S. aureus* from the peritoneal cavity is associated with a marked decrease in the ability of their peritoneal macrophages to phagocytose nonopsonized *S. aureus* as well as several other gram-positive bacteria. This is the first direct evidence that opsonin-independent phagocytosis of bacteria is a critical determinant of host survival in bacterial infection.

Materials and Methods

Mice and Bacteria. SR-AI/II knockout mice (MSR-A$^{-/-}$) are described in detail elsewhere (22). MSR-A$^{-/-}$ or BALB/c control mice (The Jackson Laboratory) were kept in a germ-free barrier facility with free access to autoclaved water and irradiated Purina-Pico mouse diet (W.F. Fisher & Son, Inc.). *S. aureus* (Wood strain, 10832), *S. aureus* (Cowan I strain, 12598), *S. aureus* (Smith diffuse strain, 13709), *Streptococcus agalactiae* (12386), *Streptococcus pyogenes* (10403), *L. monocytogenes* (43251), and *Enterococcus hirae* (9790) were from the American Type Culture Collection. *S. aureus* (strain DB) was a gift from Dr. A. Cheung, Rockefeller University, New York, NY. Heat-inactivated, BODIPY® fluorophore–labeled *S. aureus* (Wood strain) and zymosan particles were from Molecular Probes.

Infection Assay. *S. aureus* (Cowan I) was grown overnight in Brain Heart broth (Difco) in a bacterial shaker at 37°C. On the day of an experiment, bacteria were washed three times in phosphate-buffered saline (PD) without Ca^{2+}/Mg^{2+} and spectrophotometrically adjusted to OD ≈ 2.0 (10^8 CFU/ml). The number of viable bacteria was confirmed by plating serial dilutions on Brain Heart agar plates and counting bacterial colonies after overnight incubation at 37°C. For each experiment, four MSR-A$^{-/-}$ or four MSR-A$^{+/+}$ control mice (4–6 wk of age, 20–25 g) were injected intraperitoneally with 1 ml PD containing 2×10^7–10^9 CFU of the indicated microorganism and observed for 72 h. Moribund animals were killed with CO_2. At various time points after injection, mice were killed and blood and peritoneal fluid were harvested and assayed for the presence of viable bacteria by plating serial dilutions on agar plates. Blood was obtained by cardiac puncture. Three to six experiments of this type were performed, as indicated in the figure legends.

Analysis of Peritoneal Cells of Mice Inoculated Intraperitoneally with S. aureus. The total number of white blood cells in the peritoneal fluid was determined using a hemocytometer. Leukocytes were typed by evaluating a Wright's stained smear of peritoneal fluid.

Killing of S. aureus by Peritoneal Neutrophils In Vitro. Neutrophil bactericidal activity was assayed in a modified tumble assay (30). In brief, MSR-A$^{+/+}$ control mice or MSR-A$^{-/-}$ mice were inoculated intraperitoneally with 1 ml of 2% sodium caseinate (Sigma Chemical Co.) (31), and the resulting neutrophil-rich exudate was harvested 6 h later by lavage as described above. The exudate cells (75% neutrophils) were washed, counted, and suspended at a concentration of 2.6×10^6 exudate cells/ml in PBS (with Ca^{2+} and Mg^{2+}) containing 0.1% human serum albumin and 5 mM glucose. *S. aureus* (Cowan I) was grown overnight, washed, suspended at a concentration of 2×10^5 CFU/ml in PBS containing 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+}, 5 mM glucose, 0.1% human serum albumin, and 20% mouse serum (as a source of complement; Sigma Chemical Co.), and incubated for 10 min at 37°C to opsonize the bacteria. 250 μl aliquots of this bacterial suspension were mixed with 250 μl of the neutrophil suspension, and the mixture was incubated at 37°C for 1 h on a rotary shaker. The mixture was then diluted sixfold in sterile distilled water, incubated for 5 min to lyse the neutrophils, and the number of CFU of *S. aureus* was determined by plating serial dilutions on agar plates. Data are reported as percent reduction in CFU of *S. aureus* incubated with neutrophils, and were calculated as 1 − no. of CFU recovered at 1 h/no. of CFU in the inoculum at time 0 (i.e., 1×10^5 CFU). In the absence of neutrophils, the number of CFU of *S. aureus* increased by 1.5-fold over 1 h. No *S. aureus* killing occurred when neutrophils and bacteria were incubated in medium containing mouse serum lacking complement activity.

Fluorescent Labeling of Bacteria. Bacteria were labeled with BODIPY® FL (Molecular Probes) according to the manufacturer's specifications. Bacteria were grown overnight in 5–10 ml Brain Heart broth, washed twice, and resuspended in 0.2 ml buffer (0.1 M $NaHCO_3$, 125 mM NaCl). 100 μg BODIPY® FL was added slowly, and bacteria were incubated under constant stirring in the dark at room temperature for 1 h. The reaction was stopped by dropwise addition of 1.5 M hydroxylamine (Sigma Chemical Co.). Bacteria were washed and resuspended in PD at OD = 2.0 (≈10^8 CFU/ml). Bacterial viability was checked by plating serial dilutions and was typically >80%.

Phagocytosis of Bacteria by Macrophages In Vitro. Thioglycollate broth–elicited peritoneal macrophages (TMϕ) were harvested by irrigating the peritoneal cavity of mice with cold PD 4 d after in-

[1]*Abbreviations used in this paper:* LTA, lipoteichoic acid; MSR-A$^{-/-}$ mice, macrophage scavenger receptor–deficient mice; MSR-A$^{+/+}$ mice, wild-type mice; SR-AI/II, type I and/or II class A macrophage scavenger receptors; TMϕ, thioglycollate broth–elicited peritoneal macrophage(s).

Published January 3, 2000

traperitoneal injection of 2 ml aged thioglycollate broth (Sigma Chemical Co.). TMϕ were plated in 96-well plates at 10^5 cells/well in 100 μl RPMI 1640/10% fetal calf serum and incubated overnight. TMϕ were washed once with PD, and labeled bacteria were added at ≈100 CFU/macrophage for 30 min at 37°C. Phagocytosis was stopped by addition of cold PD. Fluorescence of extracellular bacteria was quenched by incubation with PD containing 1.0 mg/ml trypan blue for 20 min at room temperature as described (32, 33). Intracellular fluorescence of phagocytosed bacteria was measured at λ = 485 nm (excitation) and λ = 530 (emission), using a fluorescence plate reader (Cytofluor II; PerSeptive Biosystems). To determine background fluorescence from noningested extracellular bacteria, TMϕ were incubated with 1.0 μM cytochalasin D (Sigma Chemical Co.) to inhibit phagocytosis, and fluorescence was measured after trypan blue quenching (32–34). Phagocytosis is expressed as the ratio of fluorescence of phagocytosed bacteria to fluorescence of adherent but uningested bacteria: a value of 1 is equal to background fluorescence; values >1.0 indicate the presence of intracellular (phagocytosed) bacteria. Results were confirmed by fluorescence microscopy. In some experiments, TMϕ were preincubated for 30 min (at room temperature) with the antibodies 2F8 (Serotec) or EM-34.1 (Sigma Chemical Co.), or 500 μg/ml of one of the following SR-AI/II ligands: polyinosinic acid, polyguanylic acid, fucoidan, or LTA, or the control reagent polycytidylic acid in the presence of a ribonuclease inhibitor (all from Sigma Chemical Co.).

Results

Decreased Clearance of S. aureus by MSR-A$^{-/-}$ Mice. MSR-A$^{+/+}$ and MSR-A$^{-/-}$ mice were injected intraperitoneally with 10^7 CFU *S. aureus* Cowan I, and viable bacteria were quantified in blood and peritoneal lavage samples at the indicated time points. The number of viable bacteria recovered from the peritoneum of MSR-A$^{+/+}$ mice decreased to ∼0.01% of the inoculum and to <0.3% of the number of bacteria recovered from the peritoneum immediately after infection (i.e., from 4.0×10^5 to 1.1×10^3 CFU/mouse) within 24 h (Fig. 1 A). No bacteria were detected in the blood of MSR-A$^{+/+}$ mice by 12 h (Fig. 1 B). In contrast, the number of viable bacteria in the peritoneum of MSR-A$^{-/-}$ mice was 20% of the inoculum at 24 h (Fig. 1 A) and increased 200-fold in the blood at 12 h compared with 5 min after inoculation (Fig. 1 B).

Decreased Survival of MSR-A$^{-/-}$ Mice after Infection with S. aureus. Impaired ability to eliminate *S. aureus* may lead to increased mortality from disseminated infection. To test this, we injected MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice with increasing numbers of *S. aureus*. All control mice survived intraperitoneal infection with 2×10^7 and 2×10^8 CFU of *S. aureus*, whereas 40 and 60% of MSR-A$^{-/-}$ mice became moribund within 24 h (Fig. 2, A and B) after inoculation with 2×10^7 and 2×10^8 CFU of *S. aureus*, respectively. 20% of control mice became moribund within 24 h of infection with 10^9 CFU/mouse (Fig. 2 C). In contrast, 90% of MSR-A$^{-/-}$ mice became moribund within 12 h of infection with 10^9 CFU of *S. aureus* (Fig. 2 C). No further deaths were observed in either group after 48 h. Thus, targeted disruption of the SR-AI/II gene significantly increased the susceptibility of mice to infection with *S. aureus.*

Normal Recruitment of Inflammatory Cells by MSR-A$^{-/-}$ Mice after Infection with S. aureus. Atherosclerotic lesions in MSR-A$^{-/-}$ mice contain significantly fewer macrophages compared with lesions in MSR-A$^{+/+}$ mice (22). To examine whether the increased susceptibility of MSR-A$^{-/-}$ mice to *S. aureus* reflected a similar defect in recruitment of macrophages or other leukocytes to the site of infection, peritoneal cells from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice were harvested at various times after intraperitoneal injection of *S. aureus*. Before infection, the peritoneal cavities of MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice contained similar numbers of resident peritoneal cells, >99% of which were mononuclear leukocytes, and the majority of which were mononuclear phagocytes (not shown). As described previously (27), intraperitoneal injection of *S. aureus* induced a marked influx of leukocytes, >99% of which were neutrophils (Fig. 3). There were no significant differences between MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice in either the number or types of leukocytes that were recovered from the peritoneal cavity after intraperitoneal injection of *S. aureus* (Fig. 3). Thus, the increased susceptibility of MSR-A$^{-/-}$ mice to *S. aureus* infection cannot be explained by lack of recruitment of neutrophils or mononuclear phagocytes to the peritoneal cavity.

SR-AI/II Mediate Opsonin-independent Phagocytosis of Gram-positive Bacteria. SR-AI/II bind LPS from gram-negative bacteria (20), LTA and gram-positive bacteria (21), and possibly *Mycobacterium tuberculosis* (35) to macrophages. We hypothesized that SR-AI/II play an important role in host defense against intraperitoneal infection with *S. aureus* by mediating opsonin-independent phagocytosis of this bacte-

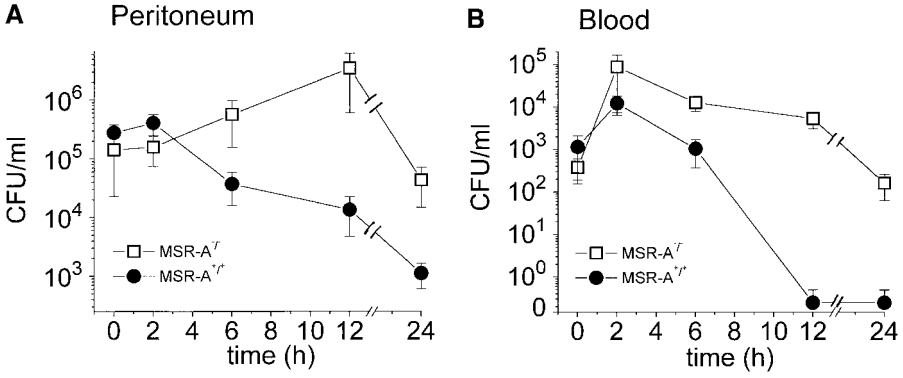

Figure 1. Clearance of *S. aureus* by MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice. 10^7 CFU *S. aureus* (Cowan I) were injected into the peritoneal cavity of four MSR-A$^{-/-}$ and four MSR-A$^{+/+}$ mice. (A) At the indicated times, the peritoneum was lavaged and its content of viable bacteria was determined by plating serial dilutions on agar plates. (B) Viable bacteria in the blood were determined after collecting blood via cardiac puncture of killed animals and subsequent plating on agar plates at the indicated times. Data represent the mean of three experiments.

Published January 3, 2000

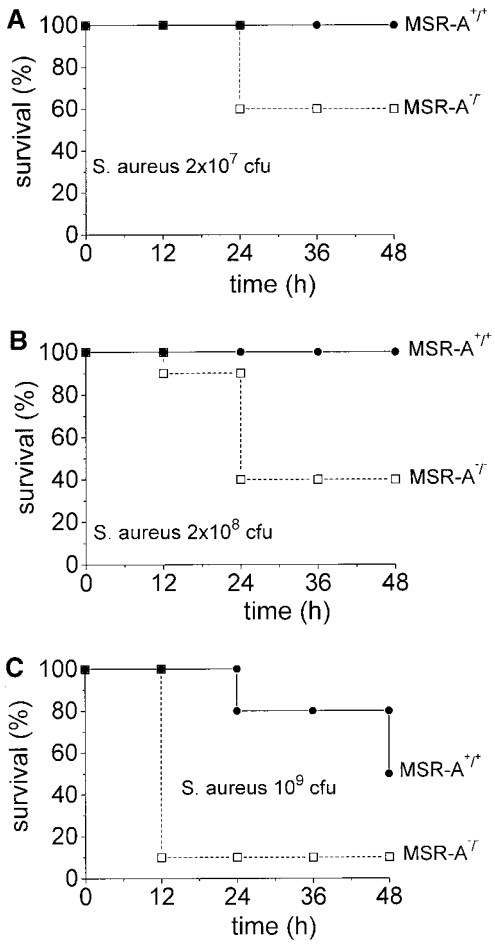

Figure 2. Mortality of MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice after *S. aureus* challenge. Four MSR-A$^{-/-}$ and four MSR-A$^{+/+}$ mice were injected intraperitoneally with 1 ml of buffer containing *S. aureus* (Cowan I): (A) 2×10^7 CFU, (B) 2×10^8 CFU, (C) 10^9 CFU. Mice were observed for signs of systemic infection, and moribund animals were killed. Data represent the mean of six experiments.

rium. To test this, we compared phagocytosis of four strains of *S. aureus*, as well as of three other gram-positive microorganisms, by TMϕ from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice. All gram-positive bacteria, with the exception of an encapsulated *S. aureus* strain (Smith diffuse), were efficiently ingested by TMϕ from MSR-A$^{+/+}$ mice in the absence of added opsonins (Fig. 4 A). TMϕ from MSR-A$^{-/-}$ mice phagocytosed 72–83% fewer bacteria than TMϕ from MSR-A$^{+/+}$ mice (Fig. 4 A). In contrast, zymosan, a particle whose uptake is mediated by mannose and β-glucan receptors (36), was phagocytosed with equal efficiency by TMϕ from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice, indicating that macrophages from MSR-A$^{-/-}$ mice possess intact phagocytic machinery.

Macrophages have been shown to secrete sufficient complement to opsonize zymosan for phagocytosis (37). To test the possibility that complement receptors or other membrane integrins played a role in phagocytosis of gram-positive bacteria, we compared phagocytosis of gram-positive bacteria by incubating TMϕ from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice in medium with and without Ca^{2+} and Mg^{2+}. Ca^{2+} and Mg^{2+} are required for complement activation and for most integrin-mediated functions, but not for interactions of SR-AI/II with their ligands (14, 17–19). TMϕ from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice ingested 62–95% as many organisms in Ca^{2+}/Mg^{2+}-free medium (Fig. 4 B) as in Ca^{2+}/Mg^{2+}-containing medium, demonstrating that the phagocytosis of these bacteria was both complement independent and integrin independent.

LTA, a major cell wall component of gram-positive bacteria (38) and a known ligand of SR-AI/II (21), inhibited opsonin-independent phagocytosis of *S. aureus* and other gram-positive bacteria (Fig. 4, C and E), suggesting that soluble LTA was competing with LTA on the surfaces of these bacteria for receptors on the macrophage plasma membrane. In addition to LTA, phagocytosis of *S. aureus* was inhibited by other SR-AI/II ligands (polyinosinic acid, polyguanylic acid, fucoidan), whereas a control reagent (polycytidylic acid) that does not block SR-AI/II receptors had no effect (Fig. 4 C). Furthermore, 2F8, an mAb that specifically recognizes murine SR-AI/II (17) and that has been shown to block SR-AI/II–mediated phagocytosis of apoptotic thymocytes (15), inhibited phagocytosis of three different strains of *S. aureus* and of *E. hirae* and *B. subtilis* by 56–95% (Fig. 4 D). An isotype-matched control antibody (EM-34.1) had no effect (Fig. 4 D). In contrast, phagocytosis of unopsonized zymosan was unaffected by 2F8 (Fig. 4 C). Both 2F8 and LTA inhibited *S. aureus* phagocytosis by TMϕ from wild-type mice in a dose-dependent manner (Fig. 4 E). Interestingly, LTA inhibited *S. aureus* phagocytosis to a greater extent than 2F8 (Fig. 4 E). This was similar to a previous report that 2F8 incompletely blocks phagocytosis of apop-

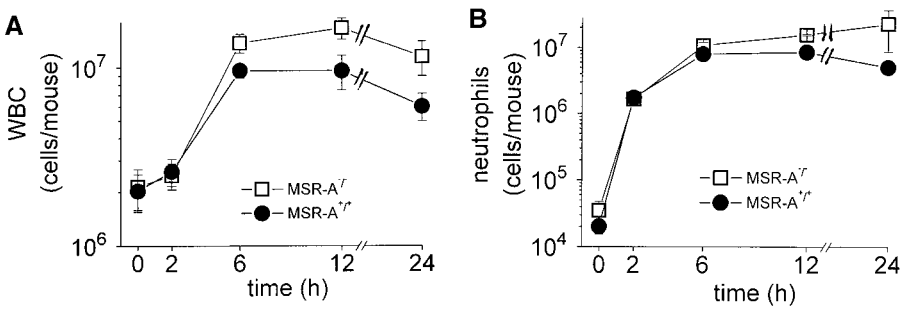

Figure 3. Recruitment of peritoneal leukocytes. Four MSR-A$^{-/-}$ mice and four MSR-A$^{+/+}$ mice were injected intraperitoneally with 10^7 CFU of *S. aureus* (Cowan I). At the indicated times, mice were killed and their peritoneal cavities were irrigated with cold buffer. (A) The total number of leukocytes (WBC) in peritoneal lavage fluids of each mouse was determined microscopically. (B) The total number of neutrophils in the peritoneal lavage fluid was calculated after the percentage of neutrophils was determined microscopically by Wright's stain. Data represent the mean of three experiments.

Published January 3, 2000

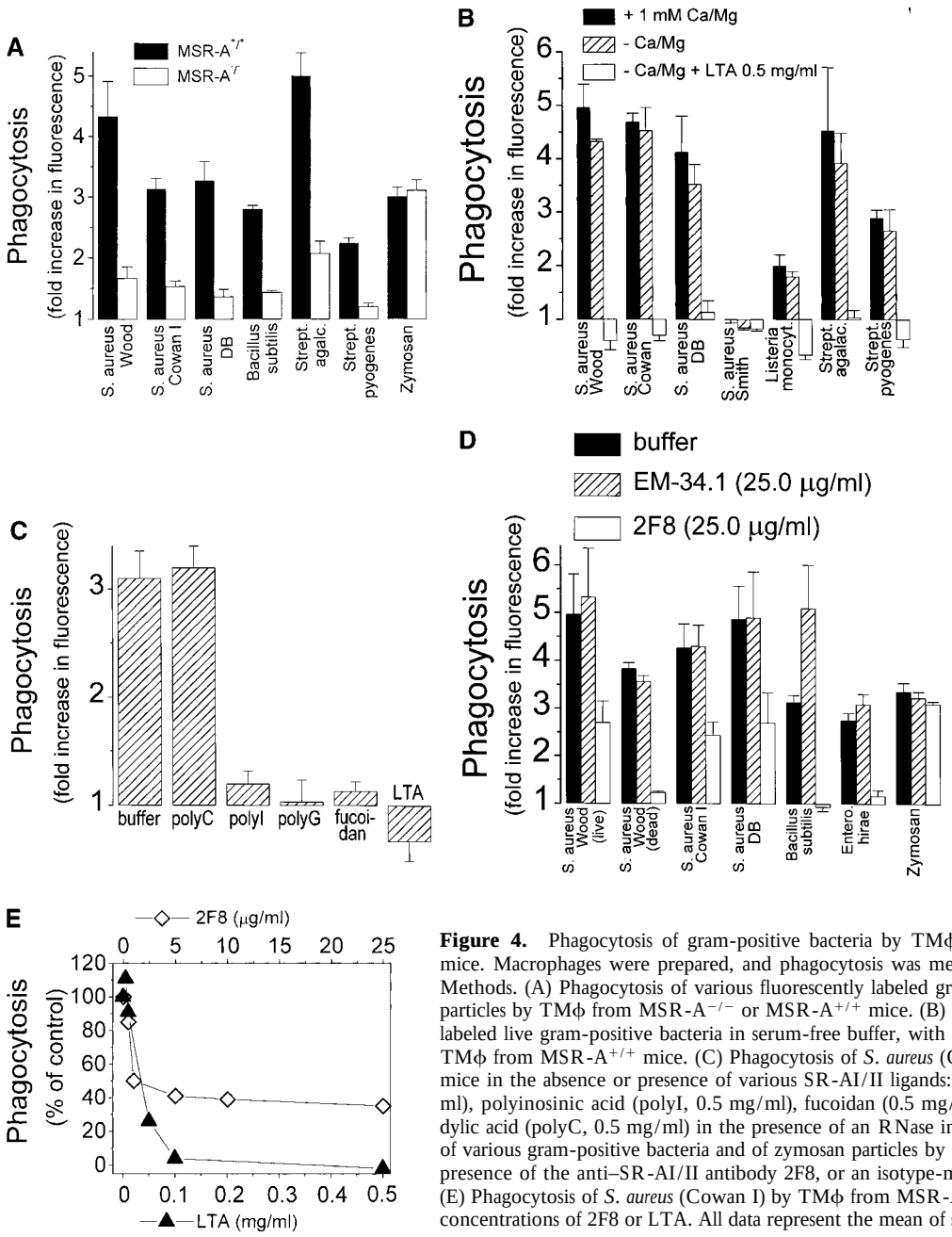

Figure 4. Phagocytosis of gram-positive bacteria by TMϕ from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice. Macrophages were prepared, and phagocytosis was measured as described in Materials and Methods. (A) Phagocytosis of various fluorescently labeled gram-positive bacteria and of zymosan particles by TMϕ from MSR-A$^{-/-}$ or MSR-A$^{+/+}$ mice. (B) Phagocytosis of various fluorophore-labeled live gram-positive bacteria in serum-free buffer, with or without Ca^{2+}, Mg^{2+}, or LTA, by TMϕ from MSR-A$^{+/+}$ mice. (C) Phagocytosis of *S. aureus* (Cowan I) by TMϕ from MSR-A$^{+/+}$ mice in the absence or presence of various SR-AI/II ligands: polyguanylic acid (polyG, 0.5 mg/ml), polyinosinic acid (polyI, 0.5 mg/ml), fucoidan (0.5 mg/ml), LTA (1.0 mg/ml), or polycytidylic acid (polyC, 0.5 mg/ml) in the presence of an RNase inhibitor (10 U/ml). (D) Phagocytosis of various gram-positive bacteria and of zymosan particles by TMϕ from MSR-A$^{+/+}$ mice in the presence of the anti–SR-AI/II antibody 2F8, or an isotype-matched control antibody (EM-34.1). (E) Phagocytosis of *S. aureus* (Cowan I) by TMϕ from MSR-A$^{+/+}$ mice in the presence of various concentrations of 2F8 or LTA. All data represent the mean of six to eight experiments.

totic thymocytes (15). It may reflect the inability of 2F8 to fully mask the binding site(s) for *S. aureus* in the collagenous domain of SR-AI/II, and/or the participation of other scavenger receptors, such as MARCO (39) or CD36 (40), in phagocytosis of gram-positive bacteria. Regardless of the reasons for the incomplete inhibition of binding and ingestion of gram-positive bacteria by 2F8, it is likely that LTA on the surface of these bacteria interacts directly with SR-AI/II (21), and that this interaction is an essential first step in initiating opsonin-independent phagocytosis of unencapsulated gram-positive bacteria.

Killing of S. aureus (Cowan I) by Neutrophils from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ Mice. Approximately equal numbers of neutrophils were elicited after intraperitoneal injection of *S. aureus* (Cowan I) into the peritoneal cavities of MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice (Fig. 3 B). However, ~12-fold more viable bacteria (4×10^5 vs. 3×10^4) were recovered from the peritoneal cavity of MSR-A$^{-/-}$ than MSR-A$^{+/+}$ mice (Fig. 1 A). Although neutrophils do not express SR-AI/II (41), it was possible that genetic disruption of the class A scavenger receptor impaired in some unknown manner the ability of neutrophils from MSR-A$^{-/-}$ mice to phagocytose and kill bacteria. To exclude this possibility, we compared killing of *S. aureus* (Cowan I) by peritoneal exudate neutrophils from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice. *S. aureus* (Cowan I) bacteria were killed equally by neutrophils from

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MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice (Table I). These results are in agreement with previous findings that macrophages are the first line of defense against *S. aureus* infection in the mouse peritoneal cavity (27, 28).

Ineffective Phagocytosis of an Encapsulated S. aureus Strain (Smith Diffuse). The data presented thus far indicate that *S. aureus* strain Cowan I is phagocytosed by macrophages by an opsonin-independent mechanism that involves interactions of ligands on the surfaces of these bacteria, presumably LTA, with SR-AI/II. They suggest that SR-AI/II–mediated opsonin-independent phagocytosis of *S. aureus* strain Cowan I by macrophages in the peritoneum of MSR-A$^{+/+}$ mice permits these mice to resist intraperitoneal infection with a dose of this bacterium that is lethal for MSR-A$^{-/-}$ mice. If this interpretation is correct, then MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice should be equally susceptible to infection with an encapsulated *S. aureus* strain, such as Smith diffuse, which is not phagocytosed in the absence of opsonins by TMϕ from either MSR-A$^{-/-}$ or MSR-A$^{+/+}$ mice (Fig. 4 A). Indeed, MSR-A$^{-/-}$ and MSR-A$^{+/+}$ mice were equally susceptible to intraperitoneal challenge with *S. aureus* Smith diffuse (Table II), confirming that the increased susceptibility of MSR-A$^{-/-}$ mice to infection with *S. aureus* Cowan I is related to the inability of macrophages lacking SR-AI/II to phagocytose *S. aureus* Cowan I in an opsonin-independent manner, and not to other immune defects.

Discussion

The inability of peritoneal macrophages from MSR$^{-/-}$ mice to phagocytose *S. aureus* (Cowan I), the impaired ability of MSR-A$^{-/-}$ mice to clear *S. aureus* from their peritoneal cavities, and the increased susceptibility of MSR$^{-/-}$ versus MSR$^{+/+}$ mice to lethal intraperitoneal challenge with *S. aureus* provide the first direct evidence that macrophage SR-AI/II play an essential role in host defense against *S. aureus* and other gram-positive microorganisms by promoting opsonin-independent phagocytosis of these bacteria.

The majority of *S. aureus* infections in humans are caused by microencapsulated or unencapsulated strains similar to the Cowan I strain used in the present studies (42). Like other gram-positive bacteria, they express LTA on the surfaces of their cell walls. LTA interacts directly with SR-AI/II on mononuclear phagocytes (21), leading to the opsonin-independent phagocytosis of these bacteria (Fig. 4, A–E). This may explain why shortly after intraperitoneal infection a much larger percentage of *S. aureus* Cowan I was cleared by resident peritoneal macrophages of MSR$^{+/+}$ than of MSR$^{-/-}$ mice (Fig. 1).

In humans, introduction of dialysis fluid into the peritoneal cavity reduces the concentration of antibodies and complement to a level insufficient to opsonize bacteria for phagocytosis (43). In mice, intraperitoneal inoculation of 1 ml of buffered saline containing *S. aureus* dilutes enormously the very small volume of fluid that coats the peritoneal cavity and is likely to have a similar inhibitory effect on the efficiency of opsonization. However, the inflammatory response initiated by peritoneal inoculation of these bacteria promotes the influx of neutrophils, monocytes (Fig. 3), and plasma proteins (e.g., complement, antibodies). We envision that this led to opsonization of *S. aureus* Cowan I and clearance of these bacteria from the peritoneum of MSR$^{-/-}$ mice challenged with sublethal doses of *S. aureus* Cowan I (Fig. 1). Similarly, it is likely that bacteria that entered the bloodstream of sublethally challenged MSR$^{-/-}$ mice (Fig. 1) were opsonized by antibodies and complement and were cleared by Fcγ- and complement receptor–dependent phagocytosis. This is consistent with our observation that even in the absence of opsonins, phagocytosis of gram-positive bacteria by MSR$^{+/+}$ macrophages is somewhat more efficient in the presence of divalent cations than in their absence (Fig. 4 B). Divalent cations are required both for the activation of complement, and for binding of complement-coated bacteria by integrins such as CD11b/CD18 (complement receptor 3). CD11b/CD18 plays an important role in phagocytosis of other bacterial pathogens (for a review, see reference 3). We suggest that these opsonin-dependent mechanisms account for the ability of MSR$^{-/-}$ mice challenged intraperitoneally with sublethal numbers of *S. aureus* Cowan I to clear these bacteria from the peritoneum and blood.

As indicated above, LTA on the surface of unencapsulated *S. aureus* (Cowan I strain) is probably responsible for the phagocytosis of these bacteria by MSR$^{+/+}$ macrophages (Fig. 4). In contrast, capsular polysaccharides mask the LTA of Smith strain *S. aureus* (44, 45). These capsular polysaccharides are not ligands for SR-AI/II on mononuclear phagocytes. Presumably this is the reason why the encapsulated

Table I. *Killing of S. aureus (Cowan I) by Peritoneal Exudate Neutrophils from MSR-A$^{-/-}$ and MSR-A$^{+/+}$ Mice*

PMN/*S. aureus* ratio	MSR-A$^{+/+}$	MSR-A$^{-/-}$
	%	%
6:1	55 ± 7	51 ± 6

Killing of *S. aureus* (Cowan I) by neutrophils was determined in a tumble assay as described in Materials and Methods. Data represent mean percent killing ± SEM for three experiments.

Table II. *Mortality of MSR-A$^{+/+}$ and MSR-A$^{-/-}$ Mice after Injection with Encapsulated S. aureus*

S. aureus (Smith diffuse)	MSR-A$^{+/+}$	MSR-A$^{-/-}$
CFU/mouse		
5×10^7	6/8 (75%)	7/8 (87.5%)
5×10^8	8/8 (100%)	7/8 (87.5%)

MSR-A$^{+/+}$ and MSR-A$^{-/-}$ mice were infected intraperitoneally by injection of *S. aureus* Smith diffuse (5×10^7 and 5×10^8 CFU). Mice were observed for signs of systemic infections, and sick animals were killed. Data are presented as number of dead mice per total number of mice.

Published January 3, 2000

Smith strain of *S. aureus* was not phagocytosed in the absence of opsonins by $MSR^{+/+}$ macrophages (Fig. 4 A).

Virtually all adult humans express antibodies to *S. aureus* cell wall constituents. However, antibodies against *S. aureus* cell wall constituents do not promote phagocytosis of encapsulated *S. aureus* strains by neutrophils and monocytes. This is because the capsular polysaccharides of *S. aureus* mask antibodies and complement deposited on the *S. aureus* cell wall (45–47), thereby preventing the interaction of these opsonins with Fc and complement receptors on neutrophils and mononuclear phagocytes. This is probably the reason why the LD_{50} of the encapsulated Smith strain of *S. aureus* is substantially lower than that of the unencapsulated Cowan I strain, and why $MSR^{-/-}$ and $MSR^{+/+}$ mice were equally susceptible to lethal infection with the encapsulated Smith strain. The report by Karakawa et al. (48) that specific anticapsular antibodies are required to promote phagocytosis of encapsulated *S. aureus*, and Cohn's (29) observation that antibodies directed against the capsular polysaccharides of Smith strain *S. aureus* protect against intraperitoneal challenge with this bacterium are consistent with this interpretation.

Staphylococcal species from the normal skin flora are the most frequent causative agents of bacterial peritonitis (49), a significant cause of morbidity and mortality in patients undergoing peritoneal dialysis (50). Carrozi and colleagues (51, 52) have shown that intraperitoneal administration of IgG reduces the incidence of bacterial peritonitis in some but not all peritoneal dialysis patients. Peritoneal macrophages of dialysis patients unresponsive to intraperitoneal IgG administration exhibited deficient Fc receptor activity, and this deficit was partially reversed by IFN-γ administration (53). The findings presented here suggest that cytokines such as M-CSF (54) or pharmacologic agents that increase SR-AI/II expression on mononuclear phagocytes may help reduce the incidence of bacterial peritonitis, especially with unencapsulated gram-positive bacteria, in peritoneal dialysis patients.

$MSR^{-/-}$ mice are more susceptible to lethal infection with *S. aureus* (Cowan I) (Fig. 2), *L. monocytogenes*, HSV-1 (22), and to the lethal effects of LPS (23) than $MSR^{+/+}$ mice. Our observations suggest that SR-AI/II exert their protective effect against *S. aureus* infection by promoting the clearance of these bacteria by macrophages (Figs. 1 and 4). SR-AI/II may also protect against the lethal effects of LPS by promoting endocytosis of LPS, which would minimize LPS–CD14 interaction and thereby diminish synthesis and secretion of TNF-α. However, *L. monocytogenes* and HSV-1 are intracellular pathogens, and neither organism requires SR-AI/II to enter or grow within host cells. Thus, increased uptake of *L. monocytogenes* or HSV-1 does not explain the beneficial effect of macrophage SR-AI/II in host defense against infections with these intracellular pathogens.

SR-AI/II mediate phagocytosis of apoptotic thymocytes (15). Phagocytosis of apoptotic *Mycobacterium avium*–infected macrophages by fresh uninfected macrophages leads to the killing of *M. avium* contained within the apoptotic macrophages (55). It is possible that SR-AI/II play a similar role in clearing apoptotic *Listeria*- or HSV-1–infected cells. Two findings are consistent with this suggestion. First, both *Listeria* (56) and HSV-1 (57) induce apoptosis of the cells they infect. Second, mice whose monocytes have been inhibited by antibodies that block CR3 (CD11b/CD18 [58]) or whose macrophages have been depleted die when infected with doses of HSV-1 (59) or *L. monocytogenes* (60) that are tolerated by normal mice. We suggest that SR-AI/II participate in controlling infections with these intracellular pathogens by promoting the phagocytosis and subsequent degradation of infected apoptotic cells and the pathogens they contain.

This study was supported by a grant from the Arthur N. Saydman Trust Fund for Research in Septicemia in honor of Dr. Harold C. Neu (to C.A. Thomas), a Research Fellowship from the Lucille P. Markey Charitable Trust (to C.A. Thomas), and grant AI20516 from the National Institutes of Health (to S.C. Silverstein).

Submitted: 24 July 1998
Revised: 18 August 1999
Accepted: 5 October 1999

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