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Breathless at the Point of a Sword

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ZYMOSAN-INDUCED PERITONITIS: EFFECTS ON CARDIAC FUNCTION, TEMPERATURE REGULATION, TRANSLOCATION OF BACTERIA, AND ROLE OF DECTIN-1

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ABSTRACT—Zymosan-induced peritonitis is a model commonly used to study systemic inflammatory response syndrome and multiple organ dysfunction syndrome. However, effects of zymosan on cardiac function have not been reported. We evaluated cardiac responses to zymosan in mice and the role of β-glucan and dectin-1 in mediating these responses. Temperature and cardiac function were evaluated before and after intraperitoneal (i.p.) injection of zymosan (100 or 500 mg/kg) or saline. Chronotropic and dromotropic functions were measured using electrocardiograms (ECGs) collected from conscious mice. Cardiac inotropic function was determined by echocardiography. High-dose zymosan caused a rapid and maintained hypothermia along with visual signs of illness. Baseline heart rate (HR) was unaffected but HR variability (HRV) increased, and there was a modest slowing of ventricular conduction. High-dose zymosan also caused prominent decreases in cardiac contractility at 4 and 24 h. Because zymosan is known to cause gastrointestinal tract pathology, peritoneal wash and blood samples were evaluated for bacteria at 24 h after zymosan or saline injection. Translocation of bacterial occurred in all zymosan-treated mice (n = 3), and two had bacteremia. Purified β-glucan (50 and 125 mg/kg, i.p.) had no effect on temperature or ECG parameters. However, deletion of dectin-1 modified the ECG responses to high-dose zymosan; slowing of ventricular conduction and the increase in HRV were eliminated but a marked bradycardia appeared at 24 h after zymosan treatment. Zymosan-treated dectin-1 knockout mice also showed hypothermia and visual signs of illness. Fecal samples from dectin-1 knockout mice contained more bacteria than wild types, but zymosan caused less translocation of bacteria. Collectively, these findings demonstrate that zymosan-induced systemic inflammation causes cardiac dysfunction in mice. The data suggest that dectin-1-dependent and -independent mechanisms are involved. Although zymosan treatment causes translocation of bacteria, this effect does not have a major role in the overall systemic response to zymosan.

KEYWORDS—β-Glucan, bacterial translocation, cardiac conduction, cardiac contractility, heart rate, heart rate variability, hypothermia, systemic inflammatory response syndrome, zymosan

INTRODUCTION

Systemic inflammatory response syndrome (SIRS) is a hyperactive, injurious inflammatory response that is a key component of the septic disease process (1, 2). This inflammatory response is mediated by the release of proinflammatory cytokines, which include interleukin 1 beta, tumor necrosis factor alpha (TNF-α), high mobility group box 1, and other mediators (3, 4). SIRS can occur as a result of infectious and noninfectious processes, including burns, trauma, ischemia, hemorrhagic shock, or administration of endotoxin or zymosan (1, 2, 5). The innate immune system can be activated via a myriad of pattern recognition receptors (PRRs), depending on which modality causes SIRS (6). Ligands for these PRRs include pathogen-associated molecular patterns (PAMPs) derived from invading microbes and danger-associated molecular patterns (DAMPs), which are released either passively or actively from host cells (7). The relative contributions of PAMPs and DAMPs to the nature and scope of inflammatory responses and pathophysiological outcomes undoubtedly depend on the specific insult or experimental model.

Cecal ligation and puncture (CLP) and zymosan-induced peritonitis are models commonly used for the induction of an exaggerated inflammatory response (5, 8). However, these models differ substantially in the nature of the insult that triggers inflammation. The CLP model requires anesthesia and surgery, produces significant tissue necrosis due to ligation of the cecum, and causes immediate infection due to puncture of the cecum and extrusion of feces into the peritoneal cavity. In contrast, zymosan-induced peritonitis is produced by injection of a sterile zymosan suspension into the peritoneal cavity of conscious animals. Zymosan, a derivative of yeast cell wall, is composed chiefly of β-glucan; however, it also contains a

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nonuniform mixture of mannans, chitin, lipids, and various proteins (9, 10). β-Glucan is known to activate dectin-1 receptor and Toll-like receptor 2 (TLR2) (10, 11), but due to the nonuniform composition of zymosan, it has the potential to activate several other PRRs. Given the marked differences between these models, it is likely that they might exhibit some differences in pathophysiological responses.

It is well known that SIRS and sepsis can lead to pronounced cardiac dysfunction clinically (12, 13). We have shown that CLP in mice can cause significant depression of cardiac chronotropic and dromotropic functions as well as substantial reduction of ventricular contractility (14, 15). Collectively, these effects reduce cardiac output and consequently contribute to impaired tissue perfusion. Our primary goal for this study was to determine the impact of zymosan-induced acute peritonitis on cardiac function in mice. Cardiac chronotropic and dromotropic functions were determined by analysis of electrocardiograms (ECGs) recorded from conscious mice. Ventricular inotropic function was assessed by echocardiography in anesthetized mice. Because zymosan is known to disrupt gut permeability barriers, we cultured samples of peritoneal wash and blood to determine if translocation of bacteria occurred. Lastly, to evaluate the possible contribution of glucan and glucan-specific receptors to the effects of zymosan treatment, we assessed responses to highly purified, particulate β-glucan (16) in normal mice and to zymosan in dectin-1 knockout mice.

MATERIALS AND METHODS

Animals

Adult male ChAT-eGFP mice on a C57BL/6 background (B6.Cg-Tg(RP23-268L19-EGFP)2Mik/J strain, Jackson Labs), C57BL/6 mice (Harlan Laboratories, Indianapolis, Ind), and dectin-1 knockout (KO) mice (clec7a$^{-/-}$, originally from Dr Gordon D. Brown) were used for this study. The ChAT-eGFP mice express eGFP in all cholinergic cells and are being used in ongoing studies to probe cholinergic mechanisms in SIRS. These mice are otherwise indistinguishable from wild type (WT) C57BL/6 and are bred in our animal facility. Dectin-1 KO mice were bred in our animal facility, and their genotype was confirmed using a protocol recommended by The Jackson Laboratory (Bar Harbor, Maine). Mice ranged from 3 to 7 months of age at the time of experiments. Animal protocols were approved by the East Tennessee State University Committee on Animal Care and conformed to guidelines of the National Institutes of Health as published in the *Guide for the Care and Use of Laboratory Animals* (Eighth Edition, National Academy of Sciences, 2011).

Zymosan model of systemic inflammation

In zymosan experiments, ChAT-eGFP mice were randomly divided into two groups (n = 5–6). Baseline rectal temperature and the ECG were measured for each group before injection. In the control group, 200 μL of sterile-filtered saline was administered by intraperitoneal (i.p.) injection using a 22-gauge, 1-inch needle. In the experimental group, 200 μL of zymosan suspension (500 or 100 mg/kg) was injected i.p., also using a 22-gauge needle. Previous studies have established that the larger dose of zymosan, 500 mg/kg, causes peritonitis, systemic inflammation, and multiple organ dysfunction (17–19). Zymosan suspension was prepared in the following manner: zymosan was measured and sterilized using UV light for 30 min. Sterile-filtered saline was added to the zymosan, and the suspension was vortexed extensively, kept at room temperature overnight and vortexed again just before use to ensure thorough mixing. After the injections were performed, rectal temperature and ECG were recorded hourly for the next 4 h and again after 24 h. Sterile saline (1 mL) was administered subcutaneously (s.c.) at the time of initial zymosan injection to maintain adequate fluid volume.

β-Glucan experiments

Experiments involving purified (≥97%), microparticulate β-glucan (16) were performed in a manner similar to the zymosan experiments. Two β-glucan experiments were performed; we used a 50 mg/kg dosage in our first experiment and a 125 mg/kg dosage in our second experiment. These doses of β-glucan were selected because commercial zymosan preparations contain varying amounts (15%–60%) of β-glucan depending on the zymosan preparation (D. Williams, personal communication). β-Glucan was administered i.p. to ChAT-eGFP mice using a 22-gauge needle after baseline rectal temperature and ECG measurements. Sterile D5W (5% dextrose in water) was administered as a control. Sterile saline (1 mL) was also administered s.c. at the time of injection to maintain adequate fluid volume. After injection of β-glucan, rectal temperature and ECG were recorded hourly for the next 4 h and again after 24 h.

Recording of ECG

ECG recordings were obtained noninvasively using the ECGenie apparatus (Mouse Specifics, Inc, Framingham, Mass) as previously described (20, 21). Briefly, mice were removed from their individual cages and placed on the elevated recording platform of the apparatus a few minutes before each data collection to allow time for acclimation. ECG signals were then acquired through disposable foot electrodes located on the floor of a recording platform (22). Approximately 200 raw ECG signals were analyzed per mouse at each time point using e-MOUSE software (Mouse Specifics, Inc), which uses processing algorithms for peak detection, digital filtering, and correction of baseline for motion artifacts. Heart rate (HR) was determined from R-R intervals, and HR variability (HRV) was calculated as the mean difference in sequential HRs for the entire set of ECG signals analyzed (22, 23). The software also determined cardiac intervals (i.e., PR, QRT, and QTc), which were within the normal ranges for our control and experimental mice (22, 23).

Measurement of rectal temperature

Rectal temperature was measured in our experiments to identify and quantify hypothermia. This was accomplished using a MicroTherma 2 T handheld thermometer and a 0.75-inch isolated rectal probe (Braintree Scientific, Braintree, Mass).

Echocardiography

The effect of zymosan on cardiac contractile function was evaluated in a separate group of experiments by echocardiography at 4 and 24 h after treatment of ChAT-eGFP mice with zymosan (500 mg/kg, i.p.) as previously described (14) using a Toshiba Aplio 80 Imaging System (Toshiba Medical Systems, Tochigi, Japan). Briefly, the mice were anesthetized by isoflurane. Transthoracic two-dimensional M-mode echocardiogram and pulsed wave Doppler spectral tracings were obtained with a 12-MHz linear transducer M-mode. Tracings were used to measure left ventricular (LV) end-systolic diameter, and LV end-diastolic diameter. Percent fractional shortening (%FS) and ejection fraction (EF) were calculated as previously described (14).

Bacterial culture from blood, peritoneal washes, and fecal samples

Two groups of C57BL/6 mice (n = 3 per group) were injected i.p. with sterile saline or zymosan (500 mg/kg), and a third group of dectin-1 KO mice (n = 4) was injected with zymosan. Another mouse was subjected to CLP surgery as a positive control for bacteria in the peritoneal cavity. Twenty-four hours after injection or surgery, blood and peritoneal washes (saline) were collected for analysis. Samples of blood and peritoneal wash from each mouse were inoculated onto both sheep's blood agar and anaerobic blood agar plates. The cultures were incubated 18 to 48 h, at 37°C, under aerobic or anaerobic conditions. Anaerobic conditions were created by incubation of cultures in an anaerobic jar with a GasPak EZ Gas Generating Sachet (Benton Dickinson, Franklin Lakes, NJ). The total number of colonies on each plate were counted and colony forming units (CFU)/mL were calculated. Colony morphology and Gram stain reaction were assessed for each predominant colony type.

Fresh fecal pellets were collected from groups of WT C57BL/6 and dectin-1 KO mice (n = 4 each). The specimens were weighed and homogenized in sterile saline. Dilutions of the fecal specimens were cultured under aerobic and anaerobic conditions, as described above. Colony forming units, colony morphology, and Gram stain reaction of predominant colony types were assessed for each sample.

Drugs

Zymosan was purchased from Sigma-Aldrich (St. Louis, Mo). Purified β-glucan was a generous gift from the laboratory of Dr David L. Williams.

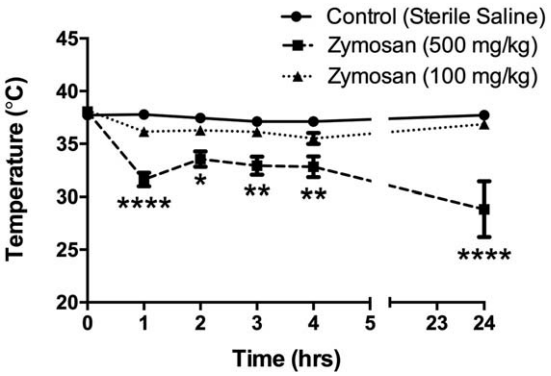

FIG. 1. **Rectal temperature decreases rapidly after injection of zymosan.** Graph shows the time course for changes in rectal temperature after i.p. injection of zymosan (500 and 100 mg/kg) and saline control. Values are the mean ± SE (n = 5-6/group). No differences were determined between groups before injection ($P > 0.05$). Postinjection values were evaluated by two-way ANOVA with repeated measures. With 500 mg/kg zymosan injection, two-way ANOVA with repeated measures showed significant effects of treatment ($F_{2,14} = 18.68$, $P = 0.0001$), time ($F_{5,70} = 10.72$, $P < 0.0001$), and a treatment-time interaction ($F_{10,70} = 6.86$, $P < 0.0001$). With 100 mg/kg zymosan injection, two-way ANOVA with repeated measures showed that there was no significant difference between the two groups during any point in the experiment. Sidak multiple comparisons test was used to identify differences between control and experimental groups at each postinjection time. $^{*}P < 0.05$; $^{**}P < 0.005$; $^{****}P < 0.0001$.

Statistical analysis

Statistical comparisons and graphing of data were accomplished using GraphPad Prism version 6.0 (GraphPad Software, San Diego, Calif). Group data are presented as the arithmetic mean ± SE (n). Statistical comparisons were made using a paired or unpaired *t* test or repeated-measures analysis of variance (ANOVA) as appropriate. The Sidak or Dunnett's multiple comparisons test was used for *post hoc* comparisons after ANOVA. A probability level of 0.05 or smaller was used to indicate statistical significance.

RESULTS

Zymosan causes dose-dependent hypothermia in mice

Septic patients who experience hypothermia have a much greater risk of death (24), and hypothermia is associated with severity of disease in CLP-induced sepsis in mice (25–27). Because of this, rectal temperature of mice was monitored over time after treatment with zymosan (500 and 100 mg/kg) or sterile saline. We found that the higher dose of zymosan lowered rectal temperatures rapidly, beginning 1 h after injection (saline: 37.8 ± 0.1°C, n = 5 and zymosan: 31.6 ± 0.6°C, n = 6; $P < 0.0001$), and the mice remained hypothermic up to 24 h posttreatment when the experiment was terminated (Fig. 1). Mice treated with the higher dose of zymosan experienced orbital tightening and decreased motor activity. The tail and paws were cold to the touch. In marked contrast, the lower dose of zymosan had no significant effect on rectal temperature (Fig. 1), and these mice exhibited no obvious clinical signs of illness.

High-dose zymosan decreases ventricular dromotropic and inotropic functions but increases HRV

Treatment with zymosan at 500 mg/kg, i.p., did not affect HR significantly at times up to 4 h postinjection or at 24 h postinjection (Fig. 2A). However, a significant increase in HRV was observed beginning at 1 h postinjection (saline: 43 ± 20 bpm, n = 5 and zymosan: 267 ± 25 bpm, n = 6; $P < 0.0001$; Fig. 2B). This elevation of HRV was maintained at 24 h postinjection (saline: 33 ± 20 bpm, n = 5 and zymosan: 221 ± 34 bpm, n = 6; $P < 0.0012$; Fig. 2B).

The high dose of zymosan caused modest increases in QRS and QTc intervals compared with baseline values at some early time points and at 24 h postinjection (Fig. 3, B and C). These intervals did not change significantly over time after saline injection. As observed for HR, the PR interval did not change significantly compared with baseline values after injection of saline or zymosan (Fig. 3A).

Cardiac contractile function was evaluated by echocardiography at 4 and 24 h after i.p. injection of 500 mg/kg zymosan (n = 6) or saline (n = 5). Representative echocardiograms are shown in Figure 4A. Compared with saline-treated controls, zymosan decreased EF by 60.6% and %FS by 66.3% at 4 h after injection (Fig. 4B, upper row). At 24 h after zymosan treatment, we also observed significantly impaired cardiac function; EF declined by 45.5% and %FS by 50.8% compared with saline control (Fig. 4B, bottom row). No differences in HR occurred between mice treated with saline or zymosan at 4 h (saline: 438 ± 22 bpm and zymosan: 484 ± 17 bpm) or 24 h (saline: 456 ± 15 bpm and zymosan: 459 ± 17 bpm).

β-Glucan has no effect on body temperature or cardiac function

Mice that received i.p injections of purified, particulate β-glucan (50 and 125 mg/kg) did not experience hypothermia

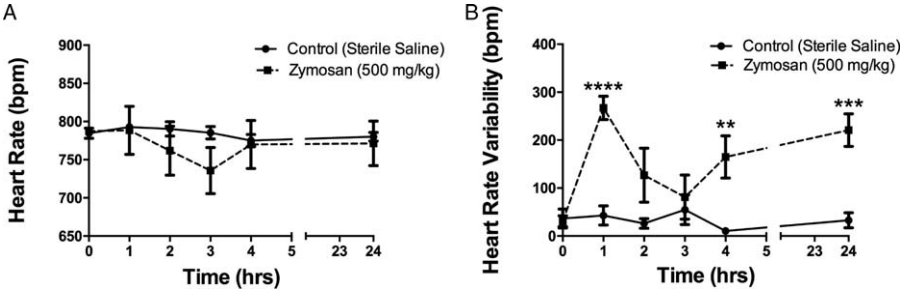

FIG. 2. **Zymosan treatment increases HRV but does not cause bradycardia.** Graphs show the time course for changes in HR (A) and HRV (B). Values are the mean ± SE (n = 5-6/group). For each parameter, no differences were determined between groups before injection ($P > 0.05$). Postinjection values were evaluated by two-way ANOVA with repeated measures. A, HR: two-way ANOVA with repeated measures showed no significant effects of treatment, time, or a treatment-time interaction. B, HRV: two-way ANOVA with repeated measures showed a significant effect of treatment ($F_{1,9} = 21.71$, $P = 0.0022$), time ($F_{5,45} = 4.05$, $P = 0.0040$), and a treatment-time interaction ($F_{5,45} = 4.46$, $P = 0.0022$). Sidak multiple comparisons test was used to identify differences between control and experimental groups at each postinjection time. $^{**}P < 0.005$; $^{***}P < 0.0005$; $^{****}P < 0.0001$. HR, heart rate; HRV, heart rate variability.

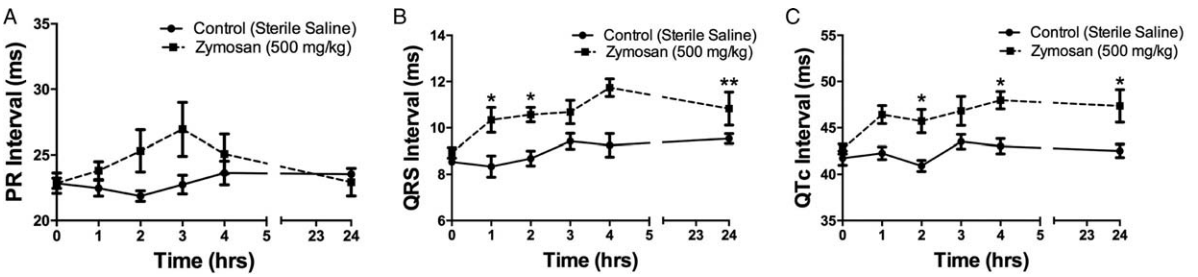

Fig. 3. **Zymosan-induced SIRS causes modest slowing of ventricular conduction.** Graphs show the time course for changes in PR interval (A), QRS interval (B), and QTc interval (C). Values are the mean ± SE (n = 5-6/group). For each parameter, no differences were determined between groups before injection ($P > 0.05$). Postinjection values were evaluated by two-way ANOVA with repeated measures. A, PR interval: two-way ANOVA with repeated measures showed no significant effect of treatment, time, or a treatment-time interaction. B, QRS interval: two-way ANOVA with repeated measures showed a significant effect of treatment ($F_{1,9} = 16.22$, $P = 0.0030$) and time ($F_{5,45} = 0.0003$), but did not show a significant treatment-time interaction. C, QTc interval: two-way ANOVA with repeated measures showed a significant effect of treatment ($F_{1,9} = 17.12$, $P = 0.0025$) and time ($F_{5,45} = 3.53$, $P = 0.0089$), but did not show a significant treatment-time interaction. Sidak multiple comparisons test was used to identify differences between control and experimental groups at each postinjection time. $^{*}P < 0.05$; $^{**}P < 0.005$. SIRS, systemic inflammatory response syndrome.

or alterations of HR at any point during the experiment (Fig. 5). Mice remained warm to the touch and showed no adverse signs.

High-dose zymosan causes translocation of bacteria into the peritoneal cavity and blood

As expected, mice treated with sterile saline did not have significant bacteria present in either the blood or peritoneal wash. In contrast, aerobic and anaerobic bacteria were found in the blood and peritoneal washes of zymosan-treated mice (Fig. 6). Gram staining of bacteria cultivated from zymosan-treated mice revealed a mixture of gram-positive cocci and rods, as well as gram-negative rods and coccobacilli (Table 1). Together these data indicate that zymosan exposure allows translocation of bacteria from the mouse gut into the peritoneal cavity and blood.

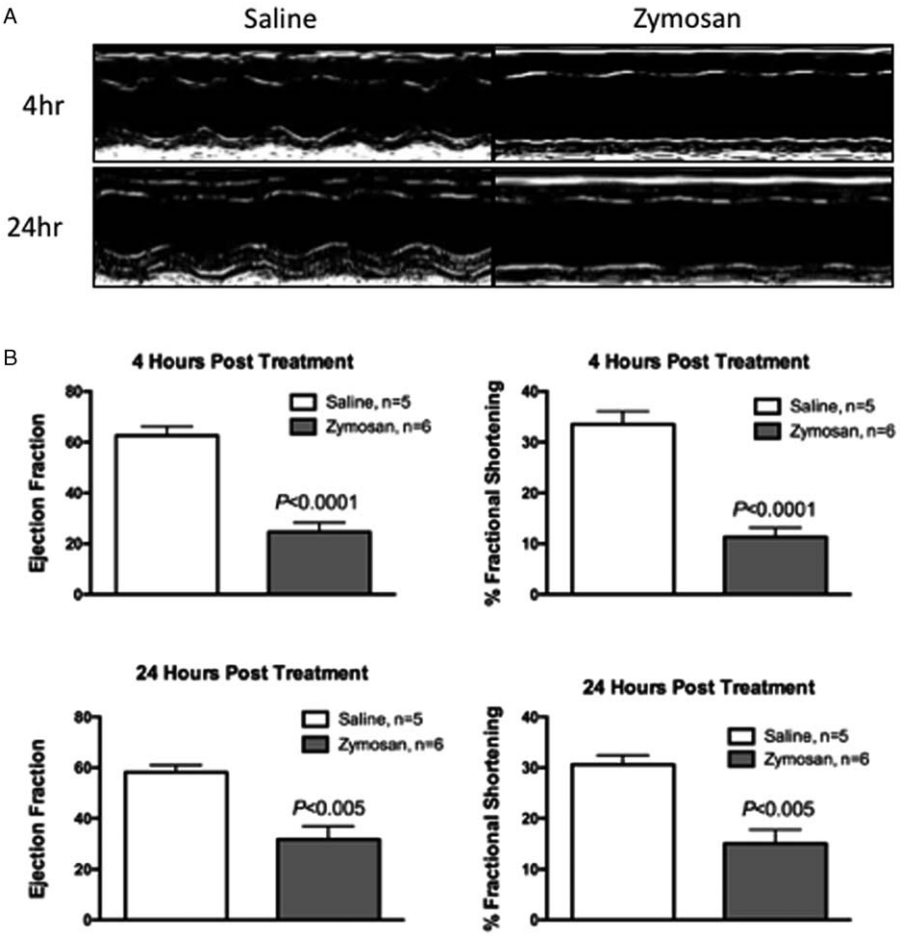

Fig. 4. **Zymosan decreases cardiac contractile function.** Mice were treated with zymosan (500 mg/kg, i.p) or saline and echocardiographic measurements were made 4 and 24 h later under isoflurane anesthesia. A, Representative echocardiograms. B, Graphs showing ejection fraction and % fractional shortening at 4 and 24 h after treatment with zymosan (n = 6) or saline (n = 5). Values are the mean ± SE. Values were compared using unpaired *t* tests.

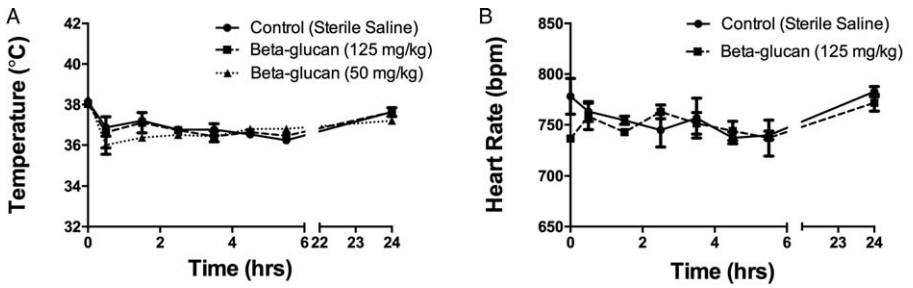

FIG. 5. **Microparticulate β-glucan does not cause hypothermia or affect heart rate.** Graph shows the time course for changes in rectal temperature (A) and heart rate (B) after i.p. injection of pure, particulate β-glucan (50 and/or 125 mg/kg) and D5W control. Values are the mean ± SE (n = 3/group). No differences were determined between groups before injection ($P > 0.05$). With both 50 and 125 mg/kg injections, two-way ANOVAs with repeated measures showed no significant effect of treatment or a treatment-time interaction.

High-dose zymosan causes hypothermia and bradycardia in dectin-1 KO mice but less translocation of bacteria compared with WT

To further evaluate the role of dectin-1 receptors in responses to zymosan, we treated dectin-1 KO mice with the high dose of zymosan (500 mg/kg, i.p) and monitored rectal temperature and ECG as in previous experiments. Peritoneal wash and blood samples were collected from the same mice immediately after collection of data for the 24 h time point. Zymosan caused hypothermia in the dectin-1 KO mice (Fig. 7A), which was comparable in time course and magnitude to that observed after treating ChAT-eGFP mice with the high dose of zymosan (Fig. 1). Zymosan caused a significant decrease in HR at 24 h posttreatment in the dectin-1 KO mice (Fig. 7B), an effect that was not observed in ChAT-eGFP mice (Fig. 2A). However, zymosan did not affect HRV or PR, QRS, and QTc intervals in dectin-1 KO mice (data not shown).

High-dose zymosan caused less translocation of bacteria in dectin-1 KO mice compared with C57BL/6 WT mice. Both the incidence (Tables 1 and 2) and abundance (Figs. 6 and 8A) of translocation were reduced in dectin-1 KO mice. Furthermore, the profile of bacteria that translocated in dectin-1 KO mice differed from WT, with gram-positive cocci being dominant in the KO mice (Tables 1 and 2). Analysis of fecal samples from separate groups of C57BL/6 and dectin-1 KO mice showed that aerobic and anaerobic bacteria were more abundant in samples from KO mice (Fig. 8B). Both gram-positive and gram-negative bacteria of similar morphology to those observed in blood and/or peritoneal wash samples were detected in fecal specimens from WT and dectin-1 KO mice.

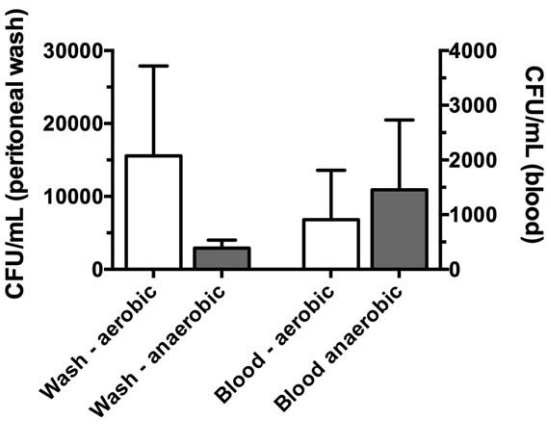

FIG. 6. **Treatment with zymosan causes translocation of gut bacteria into the peritoneal cavity.** Values are the mean colony forming units (CFU)/mL ± SE (n = 3) obtain after incubating samples of peritoneal wash and blood overnight (~18 h) under aerobic and anaerobic conditions. All mice treated with zymosan (500 mg/kg, i.p.) had bacteria in the peritoneal wash but only two mice had bacteremia. Some plates had too many colonies to count and were estimated at 200 colonies.

DISCUSSION

The exaggerated inflammatory response, which is central to SIRS, can be triggered by a diversity of infectious and non-infectious stimuli. Each of these stimuli provides a somewhat unique challenge to the system and, therefore, has the potential for revealing novel information about the diversity of tissue, organ, and system responses. Intraperitoneal injection of zymosan is a model, which has been widely used to induce a generalized inflammatory response that can progress to multiple organ dysfunction syndrome (MODS). However, the effects of zymosan on cardiac function have not been reported. Our results demonstrate that treatment of mice with zymosan, at a dose known to cause MODS (i.e., 500 mg/kg), elicits a rapid and maintained reduction of ventricular contractile function and modest slowing of ventricular conduction, the latter effect revealed by increases of the QRS and QTc intervals. Surprisingly, average HR was not affected by zymosan even though recordings of rectal temperature indicated that mice were already hypothermic by 1 h posttreatment. Nevertheless, zymosan treatment did increase HRV, suggesting that the dynamics of HR control were affected. Putting these observations in the context of global response to zymosan, other investigators have established that the same dose of zymosan causes elevation of proinflammatory cytokines in the plasma (17, 19), hypotension (28), and pathological changes in the intestines, liver, lung, and kidney (19). Consistent with the reported intestinal pathology, we found that zymosan caused translocation of bacteria, which resulted in bacteremia.

The biochemical basis for triggering of pathology by zymosan is clearly multifactorial because zymosan comprises a complex mixture of fungal cell wall components. These agents, which initiate pathophysiological responses, are soon followed

Table 1. **Gram stain reaction and morphology of bacteria isolated from zymosan-treated mice**

	Aerobic growth		Anaerobic growth	
Zymosan-treated mouse	Blood	Wash	Blood	Wash
1	—	Gram-positive cocci	Gram-positive cocci	Gram-positive cocci
2	Gram-positive rods	Gram-positive rods	Gram-positive rods, gram-positive cocci, gram-negative coccobacilli	Gram-positive cocci, gram-positive rods
3	—	Gram-positive rods, gram-negative rods	—	Gram-positive rods, gram-negative rods

by DAMPs that are released secondary to tissue injury. β-Glucans are a major component of fungal cell walls and of zymosan preparations. Accordingly, we evaluated rectal temperature and ECG responses to i.p. injection of purified, particulate β-glucan at doses of 50 and 125 mg/kg. On the basis of lack of response to these doses of β-glucan, we considered that the severe hypothermia, mild cardiac dromotropic changes, and increased HRV observed in zymosan-treated mice might not be due to the activation of glucan specific receptors. However, we were not able to evaluate higher doses of β-glucan, which could be present in some zymosan preparations. Accordingly, we took the opposite approach and evaluated responses to the high dose of zymosan in mice that lacked dectin-1, the primary receptor for β-glucan. These experiments proved that dectin-1 receptors are not required for the hypothermic response to zymosan. This conclusion is supported by recent work, which showed that zymosan produces robust release of inflammatory cytokines in macrophages obtained from dectin-1 KO mice (29), and other work has linked these cytokines to hypothermia in mice (26, 30, 31). Furthermore, in the same study, highly purified preparations of β-glucan caused substantially less activation of TLR2 and TLR4 compared with zymosan. Thus, β-glucans and dectin-1 do not seem to be major contributors to the inflammatory cytokine response evoked by zymosan.

The situation differs regarding the contribution of β-glucan receptors to the cardiac effects of zymosan. Results observed after treatment of ChAT-eGFP mice with purified, particulate β-glucan suggest that the activation of β-glucan receptors does not affect cardiac chronotropic and dromotropic functions. However, it remained possible that higher doses of β-glucan could reproduce the negative dromotropic response evoked by high-dose zymosan in ChAT-eGFP mice. The latter possibility is supported by the lack of dromotropic response to zymosan in dectin-1 KO mice. Deletion of dectin-1 also eliminated the effect of zymosan to increase HRV and resulted in the appearance of a marked bradycardia at 24 h after zymosan treatment. Collectively, these findings suggest that activation of dectin-1 influences the ability of zymosan to affect cardiac rate, ventricular conduction, and cardiovascular regulatory mechanisms.

Given the marked hypothermia that zymosan (500 mg/kg) produced in ChAT-eGFP mice, it was surprising that bradycardia and prominent negative dromotropic effects did not occur as observed after CLP (20). Because all aspects of cardiac function are very temperature-dependent, especially below 34°C (32–34), we believe that rectal temperatures might not accurately reflect intrathoracic temperatures in zymosan-treated ChAT-eGFP mice. We have no direct evidence in this regard; however, previous studies have shown that mesenteric blood flow is reduced by 65% in mice at 18 h after i.p. treatment with 500 mg/kg zymosan (35). In marked contrast, mice with CLP and sham surgery have comparable levels of mesenteric artery blood flow and intestinal microcirculatory blood flow at 18 h (36). Collectively, these observations suggest that intestinal blood flow is better maintained in mice after CLP compared with zymosan treatment. Marked reduction of intestinal blood flow in zymosan-treated mice would likely result in lower values of rectal temperature. It could also contribute

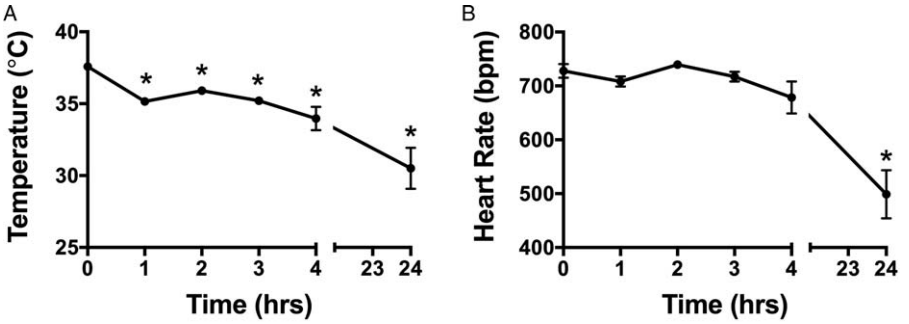

Fig. 7. **Zymosan treatment causes hypothermia and bradycardia in dectin-1 KO mice.** Graphs show the time course for changes in rectal temperature (A) and HR (B) after i.p. injection of zymosan (500 mg/kg) in dectin-1 KO mice. Values are the mean ± SE (n = 4) for serial recordings from the same mice. Repeated measures ANOVA without sphericity assumption showed a significant effect of time on rectal temperature ($F_{1.633,\ 4.899} = 11.30$, $P = 0.0162$) and HR ($F_{1.699,\ 5.097} = 12.97$, $P = 0.0110$). Dunnett's multiple comparison test was used identify significant differences from baseline values. $^{*}P < 0.05$. HR, heart rate; KO, knockout.

TABLE 2. **Gram stain reaction and morphology of bacteria isolated from zymosan-treated dectin-1 KO mice**

	Aerobic growth		Anaerobic growth	
Zymosan-treated mouse	Blood	Wash	Blood	Wash
1	Gram-positive cocci	Gram-positive cocci	Gram-positive cocci	Gram-positive cocci
2	—	—	—	—
3	—	—	—	—
4	—	—	—	Gram-positive rods

KO, knockout.

to the early decline in temperature, but we do not know how soon after zymosan treatment the decline in mesenteric blood flow occurs. The ability of zymosan to evoke a prominent bradycardia in dectin-1 KO mice but not in ChAT-eGFP mice suggests that activation of dectin-1 might affect HR indirectly by influencing the intrathoracic environment.

Our discovery of bacteria in the blood and peritoneal wash of zymosan-treated WT mice indicates that zymosan-induced peritonitis is not a sterile model of SIRS. The gastrointestinal microbiota of the mouse is composed of many diverse groups of bacteria, including *Bacteroides*, *Enterobacteriaceae*, fusobacteria, enterococci, and lactobacilli (37). The various growth patterns, Gram stain reactions, and morphologies of bacteria isolated from blood and/or peritoneal washes of zymosan-treated mice are consistent with those bacteria found in the gut microbiota, as evidenced by our observation of similar bacteria present in mouse feces. Thus, our data demonstrate that zymosan treatment causes translocation of normal bacterial flora from the gut into the peritoneal cavity, suggesting that zymosan-induced inflammation alters gut permeability. Furthermore, bacteremia occurred in two zymosan-treated mice, suggesting that hepatic clearance mechanisms were overwhelmed. Our findings agree with several previous studies showing that zymosan causes bacterial translocation in the rat (5). This effect was dose-dependent in the rat (38) and bacteria were not essential for the development of MODS (39). Likewise, we observed that zymosan exposure still produced detrimental physiological effects in dectin-1 KO mice even though there was reduced bacterial translocation in zymosan-treated dectin-1 KO mice compared with WT mice. Interestingly, Tang et al. reported that the absence of dectin-1 in mice alters the gut microbiota in a manner that favors decreased inflammation and epithelial barrier damage in colitis (40). Thus, it is possible that dectin-1 signaling is required for zymosan-induced changes in gut permeability.

In conclusion, the present study expands our understanding of the zymosan model of SIRS and MODS in several ways. We show that zymosan causes a prominent decrease in cardiac contractile function, an effect that also occurs in the CLP model. Impaired inotropic function might be mediated by cytokines such as TNF-α and induced factors such as nitric oxide because they have known cardiac suppressant actions (41, 42) and are elevated after zymosan treatment and CLP. This change is expected to decrease cardiac output, and thereby contributes to reduced tissue perfusion and ischemia during SIRS. In addition, zymosan-induced peritonitis had a negative dromotropic effect on the ventricles. Zymosan treatment did not alter baseline HR but increased HRV, suggesting that dynamic regulation of HR was affected. The latter effect and decreased dromotropy could be mediated by dectin-1 because these responses were absent in dectin-1 KO mice. Activation of dectin-1 also seems to contribute to the maintenance of HR in zymosan-induced peritonitis because deletion of this receptor unmasked bradycardia associated with hypothermia. Finally, our data suggest that dectin-1 contributes to the translocation of bacteria in zymosan-induced peritonitis.

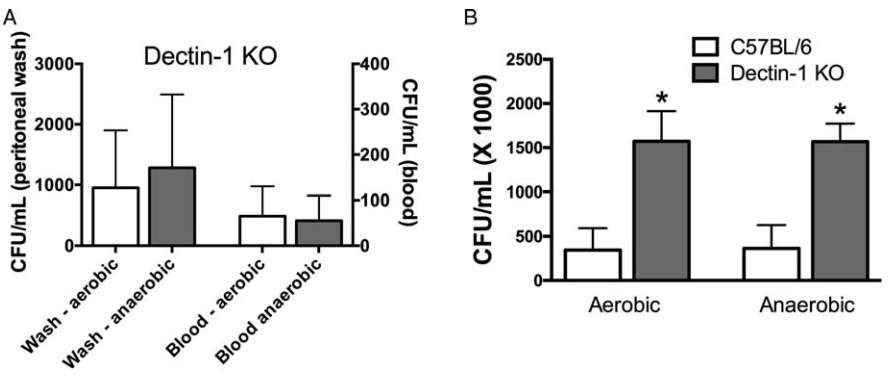

FIG. 8. **Dectin-1 KO mice have more bacteria in fecal samples compared to WT but zymosan causes less translocation of gut bacteria.** A, Dectin-1 KO mice were treated with zymosan (500 mg/kg, i.p.) 24 h before collection of samples. Values are the mean colony forming units (CFU)/mL ± SE (n = 4) obtained after incubating samples of peritoneal wash and blood overnight (~18 h) under aerobic and anaerobic conditions. Two mice had bacteria in peritoneal wash and only one of these had bacteremia. B, Fecal samples were collected from normal WT (C57BL/6) and dectin-1 KO mice, homogenized in sterile saline, and diluted for culture. Values are the mean CFU/mL ± SE (n = 4/group) obtained after incubating samples of peritoneal wash and blood overnight (~18 h) under aerobic and anaerobic conditions. Values for dectin-1 KO and C57BL/6 mice were compared using a two-tailed *t* test for unpaired samples. $^{*}P < 0.05$. KO, knockout; WT, wild type.

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