Increased Susceptibility of ST2-Deficient Mice to Polymicrobial Sepsis Is Associated with an Impaired Bactericidal Function

Julliette M. Buckley, Jing Hua Liu, Chong Hui Li, Siobhan Blankson, Qiong Di Wu, Yong Jiang, H. Paul Redmond and Jiang Huai Wang

*J Immunol* 2011; 187:4293-4299; Prepublished online 12 September 2011; doi: 10.4049/jimmunol.1003872

http://www.jimmunol.org/content/187/8/4293

© 2011 by The American Association of Immunologists, Inc. All rights reserved.
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2011 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

**Why *The JI***?
- **Rapid Reviews!** 30 days* from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References** This article cites 44 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/187/8/4293.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Increased Susceptibility of ST2-Deficient Mice to Polymicrobial Sepsis Is Associated with an Impaired Bactericidal Function

Julliette M. Buckley,*1 Jing Hua Liu,*1† Chong Hui Li,*2 Siobhan Blankson,* Qiong Di Wu,* Yong Jiang,† H. Paul Redmond,* and Jiang Huai Wang*

ST2, a member of the Toll/IL-1R superfamily, negatively regulates both TLR2 and TLR4 signaling. In this study, we report that ST2-deficient mice were more susceptible to polymicrobial sepsis than their wild-type littermates, with increased production of proinflammatory cytokines. Bacterial clearance from the circulation and visceral organs following polymicrobial infection was markedly impaired in ST2-deficient mice. This was associated with substantially reduced uptake, phagocytosis, and intracellular killing of both Gram-positive and Gram-negative bacteria by ST2-deficient phagocytes. Consistent with a reduced antimicrobial response, phagocytes lacking ST2 displayed a defect in bactericidal activity in response to bacterial challenges with severely impaired phagosome maturation and NOX2 function. Thus, ST2-deficient mice exhibit an increased susceptibility to polymicrobial infection with impaired bacterial clearance, which is associated with defects in phagosome maturation and NOX2-derived production of reactive oxygen species characterized in ST2-deficient phagocytes. The Journal of Immunology, 2011, 187: 4293–4299.

T
he orphan receptor ST2 (also known as DER4, Fit-1, or T1) was originally identified as a serum-induced late response gene in murine fibroblasts (1–3) and has been of considerable scientific interest since its discovery. ST2 is encoded from a single st2 gene by mRNA splicing and exists in two main forms, a transmembrane receptor and a soluble form (1, 2, 4). ST2 in its type I transmembrane form is expressed selectively by mast cells (5) and by Th2, but not Th1, cells (6–8). In addition to being a specific cellular marker on Th2 effector cells, ST2 promotes predominance of the Th2 response to inflammatory stimuli, while suppressing the Th1 response (6, 7, 9, 10). This defines the ST2 receptor as a therapeutic target in the management of allergic diseases specifically (11). Blockage of ST2 by a specific anti-ST2 Ab or by an IgG-ST2 fusion protein attenuates the Th2 response and enhances the Th1 activity, thus ameliorating Th2-associated allergic airway inflammation (6, 7, 12). IL-33, a new member of the IL-1 family, has been recently identified as the natural and functional ligand for ST2 (13), which provides new insights into the contribution of IL-33/ST2 signaling in T cell-mediated immune responses.

More recently, studies have emerged on the role of ST2 in the TH1 response in macrophage-mediated inflammation. ST2 is a member of the Toll/IL-1R (TIR) superfamily; however, in contrast to all the well-characterized members of the TIR family that induce the inflammatory response via activation of NF-κB, the TIR domain-containing ST2 receptor, although homologous in this way with the TLR and IL-1R, was unable to activate NF-κB, although it can activate MAPKs (14). Subsequently, it was found that ST2 negatively regulates TLR and IL-1R signaling and attenuates LPS-stimulated proinflammatory cytokine production by inhibition of TLR4 expression (15) and IκBα degradation (16) or by sequestration of MyD88 and MyD88 adaptor-like through its TIR domain (17). As a result, ST2-deficient macrophages produced markedly more proinflammatory cytokines in response to stimulation with LPS, bacterial lipoprotein, and CpG (17, 18). Furthermore, mice with targeted deletion of ST2 failed to mount endotoxin/LPS tolerance, indicating that ST2 is necessary for the development of LPS tolerance (17).

In the absence of the ST2 receptor, the TLR signal-mediated proinflammatory response to pathogenic stimuli proceeds unchecked. As a result, macrophages from ST2-deficient mice produce significantly increased amounts of proinflammatory cytokines in response to bacterial cell wall components (17, 18). However, it remains undefined whether ST2 influences the phagocyte-mediated antimicrobial response that is essential for host innate immunity to limit microbial infection. Using a clinically relevant model of polymicrobial sepsis induced by cecal ligation and puncture (CLP), we show that mice lacking ST2 are more susceptible to polymicrobial sepsis, which is associated with a defect in bacterial clearance due to impaired phagosome maturation and NOX2-derived production of reactive oxygen species, two major bactericidal processes, in ST2-deficient phagocytes.

Materials and Methods

Mice and polymicrobial sepsis

Eight- to 10-wk-old ST2-deficient mice that were backcrossed to the BALB/c background for eight generations and their wild-type littermates were maintained in the University Biological Services Unit, University College
Cork/National University of Ireland. Mice were housed in barrier cages under controlled environmental conditions (12/12 h of light/dark cycle, 55 ± 5% humidity, 23°C) and had free access to standard laboratory chow and water. Animals were fasted 12 h before experiments and allowed water ad libitum. All animal procedures were carried out in the University Biomedical Services Unit under a license from the Department of Health (Republic of Ireland). All animal studies were conducted with ethical approval granted from the University College Cork Ethics Committee.

BALB/c mice were purchased from Harlan (Oxon, U.K.), and ST2-deficient mice were originally provided by Dr. Andrew McKenzie (Med- ical Research Council Laboratory of Molecular Biology, Cambridge, U.K.).

Polymermic sepsis was induced using a CLP method (19, 20). Briefly, age- and weight-matched wild-type and ST2-deficient mice were anesthetized by i.m. injection of 150 μl ketamine/xylazine admixture (150 μl ketamine + 150 μl xylazine made up to 1 ml with 0.9% saline). A midline laparotomy was performed at which the cecum was delivered and ligated at the base just distal to the ileocaecal juncture with a 2/0 mersilk tie. A single through puncture was then made distal to the ligature with a 17G needle. The cecum was returned to the peritoneal cavity, and the abdomen was closed by 6/0 prolene sutures. Survival was monitored for at least 14 d.

Serum cytokine measurement

Wild-type and ST2-deficient mice underwent CLP-induced polymicrobial sepsis, and blood samples were collected via retinal artery puncture at different time points postseptic challenge. Serum TNF-α and IL-6 were assessed by cytometric bead array (BD Biosciences, San Jose, CA).

Enumeration of bacteria in blood and visceral organs

Wild-type and ST2-deficient mice were culled at 12 and 24 h after induction of polymicrobial sepsis. Blood samples were obtained by retinal artery puncture, and the dissected liver and spleen were homogenized in sterile PBS. Serial 10-fold dilutions of whole blood and organ homogenates in sterile water containing 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO) were plated on brain heart infusion agar (BD Biosciences), and cultured for 24 h at 37°C for determination of bacterial CFU.

FACSscan analysis of immunofluorescence

Heparinized blood and peritoneal lavage were collected from wild-type and ST2-deficient mice, and dual stained with anti–Ly-6G (BD Pharmingen, San Diego, CA), anti-F4/80 Ag (Serotec, Oxford, U.K.), anti-complement receptor type 3 (CR3; BD Pharmingen), and anti-FcyIII/II receptor (FcγR; BD Pharmingen) mAbs conjugated with PE or FITC. PE- or FITC-conjugated anti-mouse isotype-matched mAbs (BD Pharmingen; Serotec) were used as negative controls. Erythrocytes were lysed using lysis buffer (BD Biosciences). FACSscan analysis was performed from at least 10,000 events for detecting the surface expression of CR3 and FcγR on macrophages (F4/80-positive cells) and polymorphonuclear neutrophils (PMNs) (Ly-6G–positive cells) using CellQuest software (BD Biosciences).

Bacterial uptake, ingestion, and intracellular bacterial killing

Gram-positive Staphylococcus aureus and Gram-negative Salmonella typhimurium were obtained from American Type Culture Collection (Manassas, VA) and the National University of Ireland Culture Collection, respectively. Bacteria were cultured at 37°C in trypticase soy broth (Merck, Darmstadt, Germany) harvested at the mid-logarithmic growth phase, washed twice, and resuspended in PBS for in vitro use. The concentration of resuspended bacteria was determined and adjusted spectrophotometrically at 550 nm.

Bacterial uptake, phagocytosis, and intracellular bacterial killing were determined, as described previously (20, 21). Briefly, S. aureus and S. typhimurium were heat killed at 95°C for 20 min and labeled with 0.1% FITC (Sigma-Aldrich). Heparinized blood and peritoneal lavage collected from wild-type and ST2-deficient mice were incubated with ≥ 1 × 106 CFU/ml heat-killed, FITC-labeled S. aureus or S. typhimurium at 37°C for 15 min. Bacterial uptake by PMNs and peritoneal macrophages was assessed by FACSscan analysis. PMN and peritoneal macrophage populations were identified by their positive staining for anti–Ly-6G and anti-F4/80, respectively. Bacterial ingestion was further determined after the external fluorescence of 150 μl of the bound, but noningested, bacteria was quenched with 0.025% crystal violet (Sigma-Aldrich). Intracellular bacterial killing was determined by incubation of macrophages with live S. aureus or S. typhimurium (macrophage/bacteria = 1:20) at 37°C for 60 min in the presence or absence of cytochalasin B (5 μg/ml; Sigma-Aldrich). After macrophages were lysed, total and extracellular bacterial killing were determined by incubation of serial 10-fold dilutions of the lysates on tryptone soy agar (Merck) plates at 37°C for 24 h. Intracellular bacterial killing was calculated according to the total and extracellular bacterial killing.

NADPH oxidase assay

The NADPH oxidase NOX2 activity of macrophages and PMNs was assessed with the fluorogenic substrate lucigenin (bis-N-methylacridinium nitrate; Sigma-Aldrich), as described previously (22, 23). Briefly, peritoneal macrophages and bone marrow-derived PMNs isolated from wild-type and ST2-deficient mice were incubated with heat-killed, opsonized S. aureus or S. typhimurium (phagocytes/bacteria = 1:20 or 1:50) at 37°C for various time periods. Cells were also incubated with 1.0 μg/ml PMA (Sigma-Aldrich) to measure the maximal receptor-independent NOX2 activation.

Measurement of phagosomal pH

Phagosome luminal pH was assessed, as described previously (24, 25), Briefly, heat-killed S. aureus and S. typhimurium were doubly labeled with 5 μg/ml carboxyfluorescein-SE (a pH-sensitive fluorescent probe; Molecular Probes, Eugene, OR) and 10 μg/ml carboxytetramethylrhodamine-SE (a pH-insensitive fluorescent probe; Molecular Probes). Isolated peritoneal macrophages were pulsed with the labeled bacteria (macrophage/bacteria = 1:20) for 20 min, and then chased at 37°C for the indicated time periods. Macrophage-based mean fluorescence intensity (MFI) of fluorescein on FL1 and rhodamine on FL2 was simultaneously analyzed by a FACScan flow cytometer (BD Biosciences). Phagosomal pH was calculated according to the ratio of fluorescein/rhodamine fluorescence using a calibration curve.

Phagosome/lysosome fusion assay

Peritoneal macrophages were harvested and plated into 8-well chamber slides (Lab-Tek; Nunc, Rochester, NY) at 1 × 105 cells/well. After resting in RPMI 1640 containing 1% FCS for 6 h, cells were loaded with Lyso-Tracker red (50 nM) (Molecular Probes) at 37°C for 30 min and further incubated with FITC-conjugated bacteria (Molecular Probes) of either S. aureus or Escherichia coli (macrophage/bacteria = 1:20) for various time periods. LysoTracker red was replenished every hour of incubation. After each time point, slides were vigorously washed five times in cold PBS and fixed in 2% paraformaldehyde (Sigma-Aldrich). Cell nuclei were stained with DAPI (Molecular Probes). Slides were mounted with coverslips and examined under a fluorescent Olympus BX61-TRF microscope (Olympus, Tokyo, Japan). Fluorescent images were acquired using the cell imaging software for life sciences microscopy (Olympus Soft Imaging Solutions, Munster, Germany). Unfused phagosomes containing FITC-bacteria and lysosomes labeled with LysoTracker red were stained in green and red, respectively, whereas phagosomes containing FITC-bacteria after being fused with LysoTracker red-labeled lysosomes were stained in yellow due to the coexistence of the two fluorochromes.

Assessment of phagosome maturation in a cell-free organelle system

Peritoneal macrophages isolated from wild-type and ST2-deficient mice were labeled with a red fluorescent cell membrane linker PKH26 (20 μM; Sigma-Aldrich) for subsequent phagosome recognition, as described previously (26). PKH26-labeled macrophages were pulsed and chased with heat-killed S. aureus or S. typhimurium (macrophage/bacteria = 1:20) at 37°C for the indicated time periods. Cells were lysed in a hypotonic buffer, and phagosomes were prepared by centrifugation. The isolated phagosomes were permeabilized with 0.2% saponin (Sigma-Aldrich) and stained with FITC-conjugated anti–LAMP-1 mAb (Abcam, Cambridge, U.K.) that specially recognizes late endosomes/lysosomes or FITC-conjugated isotype-matched mAb (Abcam) as the negative control. The green MFI of LAMP-1 on the positive red fluorescent events (phagosomes that have ingested bacteria), representing phagolysosome fusion and/or phagosome maturation, was quantitatively assessed by FACSscan analysis using CellQuest software (BD Biosciences).

Statistical analysis

All data are presented as the mean ± SD. Statistical analysis was performed using the log-rank test for survival and the Mann-Whitney U test for all others, with GraphPad software, version 5.01 (Prism, La Jolla, CA). A p value < 0.05 was judged statistically significant.
Results

ST2-deficient mice are more susceptible to polymicrobial sepsis

Polymicrobial sepsis was induced in wild-type and ST2-deficient mice, and survival was monitored for at least 14 d. All ST2-deficient mice succumbed within 60 h of septic challenge, whereas their wild-type littermates were more resistant to CLP-induced polymicrobial sepsis ($p = 0.0009$ versus ST2-deficient mice) (Fig. 1A).

Blood samples were collected at different time points post-polymicrobial sepsis from wild-type and ST2-deficient mice for cytokine analysis. Serum peak levels of TNF-α at 2 h ($p = 0.021$) and IL-6 at 6 h ($p = 0.004$) postseptic challenge were much higher in ST2-deficient mice than those in wild-type mice (Fig. 1A).

Bacterial CFU at 12 h postseptic challenge was significantly greater in the blood ($p = 0.017$) and the liver ($p = 0.014$) of ST2-deficient mice compared with wild-type mice (Fig. 1C). At 24 h postseptic challenge, significantly higher bacterial counts were observed in the blood, liver, and spleen of ST2-deficient mice ($p < 0.05$ versus wild-type mice) (Fig. 1C). This suggests that in addition to substantially higher serum levels of proinflammatory cytokines, ST2-deficient mice exhibit delayed bacterial clearance in response to polymicrobial infection.

Bacterial uptake, phagocytosis, and killing by phagocytes are impaired in ST2-deficient mice

Uptake of Gram-positive S. aureus ($p = 0.013$) and Gram-negative S. typhimurium ($p = 0.027$) by ST2-deficient macrophages was significantly less than that observed in wild-type macrophages (Fig. 1D). PMNs from ST2-deficient mice also took up less S. aureus ($p = 0.039$) and S. typhimurium ($p = 0.030$) than did wild-type PMNs (Fig. 1E). Phagocytosis of S. aureus and S. typhimurium was impaired in both ST2-deficient macrophages ($p < 0.05$) (Fig. 1D) and PMNs ($p < 0.05$) (Fig. 1E) compared with wild-type phagocytes. Furthermore, intracellular killing of the ingested live S. aureus and S. typhimurium by ST2-deficient macrophages was markedly reduced ($p < 0.05$ versus wild-type macrophages) (Fig. 1F). Thus, ST2-deficient phagocytes display an impaired antimicrobial response to both Gram-positive and Gram-negative bacteria.

Peritoneal leukocyte accumulation and phagocytic receptor expression are identical between wild-type and ST2-deficient mice

There were few PMNs detectable in the peritoneal cavity of both wild-type and ST2-deficient mice prior to septic challenge (Fig. 2A). Following induction of CLP-induced polymicrobial sepsis, a substantially increased accumulation of leukocytes in the peritoneal cavity was observed, and the predominantly recruited cells were PMNs; however, similar to total leukocyte accumulation and peritoneal macrophage population, we didn’t find any differences in PMNs recruited into the peritoneal cavity between wild-type and ST2-deficient mice challenged with polymicrobial infection (Fig. 2A).

To find out the cause responsible for the reduced bacterial uptake, ingestion, and killing by ST2-deficient phagocytes, we measured the surface expression of two phagocytic receptors, CR3 and FcγR, on peritoneal macrophages and circulating PMNs from wild-type and ST2-deficient mice. FACS analysis demonstrated that levels of constitutively expressed CR3 and FcγR on wild-type and ST2-deficient phagocytes were identical (Fig. 2B).

ST2-deficient macrophages exhibit a defect in phagosome maturation

Phagosome maturation of professional phagocytes after ingestion of microbial bacteria is characterized by phagosomal acidification and phagosome/lysosome fusion (27). A significantly delayed and reduced phagosomal acidification after ingestion of Gram-positive S. aureus was observed in ST2-deficient macrophages compared with wild-type macrophages ($p = 0.017$) (Fig. 3A). A similar defect in phagosomal acidification was also found in ST2-deficient macrophages after ingestion of Gram-negative S. typhimurium ($p = 0.014$ versus wild-type macrophages) (Fig. 3B).

Consistent with an impaired phagosomal acidification, ST2-deficient macrophages showed a markedly reduced phagoacellular fusion after ingestion of either S. aureus or S. typhimurium ($p < 0.05$ vs. wild-type macrophages) (Fig. 3B).

**FIGURE 1.** Susceptibility to polymicrobial sepsis and impaired bactericidal function in ST2-deficient mice and phagocytes. A, Kaplan-Meier survival curve shows that ST2-deficient (KO) mice ($n = 28$) were more susceptible to polymicrobial sepsis than their wild-type (WT) littermates ($n = 28$) ($p = 0.0009$). B, Data shown are the results of peak serum levels of TNF-α at 2 h and IL-6 at 6 h postseptic challenge. C, Bacterial clearance from the blood and visceral organs collected at 12 and 24 h postseptic challenge was expressed as CFU/ml. Data in B and C are mean ± SD of five to eight mice per time point. D and E, Uptake and phagocytosis of S. aureus and S. typhimurium (S. typhi) by peritoneal macrophages (D) and circulating PMNs (E) were expressed as mean channel fluorescence (MCF) per cell. Data in D and E are mean ± SD of four to six independent experiments. F, Intracellular killing of ingested bacteria by peritoneal macrophages is mean ± SD of triplicate samples from at least four to six separate experiments. *$p < 0.05$ compared with WT.
versus wild-type macrophages) (Fig. 3C), as determined in a cell-free organelle system. We further loaded peritoneal macrophages with LysoTracker red that selectively labels late endosomes/lysosomes, and monitored the maturation of phagosomes that have ingested \textit{S. aureus}-FITC by examining their ability to colocalize with LysoTracker red over time. A nearly completed colocalization of \textit{S. aureus}-FITC with LysoTracker red was observed at 60 min in wild-type macrophages; by contrast, most \textit{S. aureus}-FITC at this time point did not colocalize with LysoTracker red in ST2-deficient macrophages (Fig. 3D). A considerably less colocalization of \textit{E. coli}-FITC with LysoTracker red was also found in ST2-deficient macrophages compared with wild-type macrophages (Fig. 3E). These results indicate that ST2-deficient macrophages have a defect in phagosome maturation after ingestion of microbial bacteria.

\textit{ST2 deficiency impairs the NOX2 function in both macrophages and PMNs}

The NADPH oxidase complex NOX2 located in the phagosomal membrane is responsible for the production of superoxide anion, one of the bactericidal reactive oxygen species, thus contributing to an efficient killing of the ingested microbial bacteria by phagocytic cells (28, 29). To determine the NOX2 activity in phagocytes, we assessed the production of superoxide anion in macrophages and PMNs isolated from wild-type and ST2-deficient mice. When compared with wild-type macrophages, ST2-deficient macrophages displayed an impaired NOX2 activity with substantially reduced production of superoxide anion in response to either Gram-positive or Gram-negative bacterial challenge (p < 0.05) (Fig. 4A). Similarly, ST2-deficient PMNs, after being chased with \textit{S. aureus} or \textit{S. typhi}, produced much less superoxide anion than did wild-type PMNs (p < 0.05) (Fig. 4B). However, in response to PMA stimulation, the amounts of superoxide anion produced by both ST2-deficient macrophages and PMNs were equivalent to those produced by wild-type phagocytes (Fig. 4), indicating that the deficiency of ST2 rather than an inherent inability to produce superoxide anion is responsible for the impaired NOX2 activity observed in ST2-deficient phagocytic cells after ingestion of microbial bacteria.

\textbf{Discussion}

Since its discovery, ST2 has been identified as a specific cellular marker that differentiates Th2 from Th1 cells, and as a promoter that facilitates the Th2 response by inducing Th2-associated
cytokine production (6, 7, 9, 10). This link the contribution of ST2 to the pathogenesis of allergic diseases, in particular pulmonary allergic inflammation (11, 12, 30–33). More recently, as a member of the TIR superfamily, ST2 has been demonstrated to function as a negative regulator for both TLR4 (17) and TLR2 (18) signaling. ST2 attenuates TLR4-mediated proinflammatory response in a negative feedback manner (34), as stimulation with the TLR4 ligand LPS led to ST2 expression on macrophages and the induced ST2, in turn, downregulated the TLR4 signal transduction pathway by sequestration of the TLR proximal signaling components MyD88 and MyD88 adaptor-like (17). ST2 also negatively regulates TLR2 signaling by affecting the formation of both TLR2–MyD88 and MyD88–IL-1R–associated kinase immunocomplexes (18). Consequently, macrophages from ST2-deficient mice produced markedly more proinflammatory cytokines in response to either LPS (17) or bacterial lipoprotein (18) stimulation, whereas administration of the soluble ST2 fusion protein, by attenuation of proinflammatory cytokine production, protected mice against endotoxin-induced shock (15) and ischemia/reperfusion-related lethality (35).

In the current study, we demonstrated that mice lacking ST2 were more susceptible to polymicrobial sepsis. When challenged with CLP-induced polymicrobial sepsis, ST2-deficient mice had a significantly higher mortality rate than wild-type mice, which is consistent with the previously published work in which Il1rl1−/− (ST2-deficient) mice when challenged with a mild form of CLP displayed a reduced survival rate compared with wild-type mice (36). ST2-deficient mice also produced substantially more proinflammatory cytokines in response to polymicrobial infection, which is in line with the notion of ST2 acting as a negative regulator for both TLR4 (17) and TLR2 (18) signaling. As one of the fundamental functions of host innate immunity to microbial infection is rapid clearance of the invading pathogens from the body (37), we further examined bacterial clearance in septic challenged ST2-deficient mice. Consistent with an increased susceptibility to polymicrobial sepsis, ST2-deficient mice displayed impaired bacterial clearance from the circulation and visceral organs post-septic challenge, with significantly higher bacterial counts in the blood, liver, and spleen compared with wild-type mice. This finding suggests that not only is the proinflammatory response to microbial infection uncontrolled in these animals, the antimicrobial response of the host innate immunity is dysregulated and thus less effective.

A rapid recruitment of PMNs into the peritoneal cavity is a characteristic in several experimentally established murine polymicrobial sepsis models, and plays a key role in host innate immunity against microbial infection (38, 39). Therefore, an impaired and/or reduced recruitment of PMNs into the peritoneal cavity may account for an increased susceptibility of ST2-deficient mice to CLP-induced polymicrobial sepsis. In response to septic challenge, substantial amounts of PMNs were rapidly recruited into the peritoneal cavity in both wild-type and ST2-deficient mice; however, no significant differences were found in numbers of the recruited PMNs and peritoneal macrophages between wild-type and ST2-deficient mice.

The antimicrobial response of innate immunity is initiated by the receptor-associated recognition of invading pathogens, and subsequently, these invaded pathogens are engulfed by the professional phagocytes, including macrophages and PMNs via phagocytic receptors and killed within phagocytes through a process of phagosome/lysosome fusion (27, 40). To elucidate the underlying mechanisms that might be responsible for an inability in bacterial clearance observed in septic challenged ST2-deficient mice, we assessed phagocytic receptor expression, bacterial uptake, phagocytosis, and intracellular bacterial killing in macrophages and PMNs from ST2-deficient mice and compared them with wild-type controls. We observed no difference in surface expression of phagocytic receptors, CR3 and FcγR, between ST2-deficient phagocytic cells and those from wild-type mice. When exposed to either Gram-positive or Gram-negative bacterial challenge, however, bacterial uptake, phagocytosis, and intracellular bacterial killing by macrophages and PMNs were all significantly reduced in ST2-deficient mice compared with wild-type controls. Both CR3 and FcγR contribute to the phagocyte-associated uptake, ingestion, and killing of the invading bacteria (41, 42). As a result, any defects in CR3 and/or FcγR may cause a downregulated antimicrobial response, whereas overexpression of these receptors leads to the enhanced bacterial clearance in a murine model of
acute polymicrobial peritonitis (38). With no obvious alterations in CR3 and FcγR expression, we propose that the impairment of bacterial internalization and/or interruption of the processing of ingested microbial pathogens by ST2-deficient phagocytes, such as a defect in phagosome formation and maturation, may offer a plausible explanation for our observed results.

Phagosome maturation after ingestion of microbial pathogens is a critical step in the killing and degradation of internalized pathogens by the professional phagocytes, and thus plays a key role in innate immunity against microbial infection (27, 43). During engulfment by phagocytes, the internalized bacteria are delivered to a de novo membrane-limited organelle, the phagosome. Once formed, the phagosome undergoes maturation to remodel its membrane and contents through a complex series of fusion events with early endosomes, and then late endosomes and lysosomes. This maturation process culminates in the formation of the phagolysosome, a highly acidic organelle in which most of the internalized bacterial killing and degradation occurs. Thus, phagosome maturation of phagocytes after ingestion of bacteria is characterized by phagosomal acidification and phagosome/lysosome fusion (27, 40). We observed a substantially delayed and reduced phagosome acidification in ST2-deficient macrophages after ingestion of either S. aureus or S. typhimurium. Furthermore, ST2-deficient macrophages also exhibited severely impaired phagolysosome formation in response to both Gram-positive and Gram-negative bacterial challenges. These data indicate a defect in phagosome maturation into the late lysosomal stage in the absence of ST2.

One of the key mechanisms by which phagocytic cells are capable of killing the ingested microbial bacteria is through the production of reactive oxygen species. We propose that the impaired phagosome acidification and phagosome/lysosome fusion in response to both Gram-positive and Gram-negative bacterial challenges is a plausible explanation for our observed results.

TLRs, in particular TLR4 and TLR2, play a key role in host defense against microbial infection by sensing pathogen-associated molecular patterns and triggering the transcriptional inflammatory response in phagocytes that encounter invading pathogens. However, TLR activation is a double-edged sword. Although normally helping to eradicate pathogens from a local infection, a persistent activation of the TLR-mediated signal transduction pathway, with excessive release of proinflammatory cytokines, can lead to the development of septic shock. Therefore, TLR signaling must be tightly regulated (34, 44). Recent studies have shown that ST2 negatively regulates both TLR4 and TLR2 signaling pathways and attenuates proinflammatory cytokine production (17, 18). In the current study, we further demonstrated that ST2 is essential for phagosome maturation and NOX2-derived production of reactive oxygen species in phagocytes challenged with either Gram-positive or Gram-negative bacteria. Thus, ST2 possesses a novel role in host innate immunity against microbial infection by promoting bacterialidal functions in phagocytes to facilitate an efficient killing of invading pathogens on the one hand, and, in contrast, by modulating both TLR4 and TLR2 signaling to avoid detrimental and inappropriate inflammatory responses.

Taken together, our data establish ST2 as a molecule contributing to the two major bacterial processes, that is, phagosome maturation and NOX2-derived production of reactive oxygen species. We conclude that ST2, in addition to acting as a negative regulator for TLR-mediated proinflammatory response, is necessary for efficient functioning of the host innate immunity-initiated antimicrobial response against microbial infection, as both ST2-deficient phagocytes and mice display impaired bacterial activity and increased susceptibility to polymicrobial sepsis.

Disclosures

The authors have no financial conflicts of interest.

References


2. Tominaga, S. 1989. A putative receptor for an activation of a growth specific cDNA from BALB/c

3. ST3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. FEBS Lett. 258: 301–304.


Downloaded from http://www.jimmunol.org/ by guest on January 14, 2018


