Geranylgeranyl transferase regulates CXC chemokine formation in alveolar macrophages and neutrophil recruitment in septic lung injury

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Overwhelming accumulation of neutrophils is a significant component in septic lung damage, although the signaling mechanisms behind neutrophil infiltration in the lung remain elusive. In the present study, we hypothesized that geranylgeranylation might regulate the inflammatory response in abdominal sepsis. Male C57BL/6 mice received the geranylgeranyl transferase inhibitor, GGTI-2133, before cecal ligation and puncture (CLP). Bronchoalveolar lavage fluid and lung tissue were harvested for analysis of neutrophil infiltration, as well as edema and CXC chemokine formation. Blood was collected for analysis of Mac-1 on neutrophils and CD40L on platelets. Gene expression of CXC chemokines, tumor necrosis factor-α (TNF-α), and CCL2 chemokine was determined by quantitative RT-PCR in isolated alveolar macrophages. Administration of GGTI-2133 markedly decreased CLP-induced infiltration of neutrophils, edema, and tissue injury in the lung. CLP triggered clear-cut upregulation of Mac-1 on neutrophils. Inhibition of geranylgeranyl transferase reduced CLP-evoked upregulation of Mac-1 on neutrophils in vivo but had no effect on chemokine-induced expression of Mac-1 on isolated neutrophils in vitro. Notably, GGTI-2133 abolished CLP-induced formation of CXC chemokines, TNF-α, and CCL2 in alveolar macrophages in the lung. Geranylgeranyl transferase inhibition had no effect on sepsis-induced platelet shedding of CD40L. In addition, inhibition of geranylgeranyl transferase markedly decreased CXC chemokine-triggered neutrophil chemotaxis in vitro. Taken together, our findings suggest that geranylgeranyl transferase is an important regulator of CXC chemokine production and neutrophil recruitment in the lung. We conclude that inhibition of geranylgeranyl transferase might be a potent way to attenuate acute lung injury in abdominal sepsis.

isoprenylation; chemokines; leukocytes; lung; sepsis

INTESTINAL PERFORATION is a feared condition in which toxins and microbes contaminate the abdominal cavity (31). Fecal bacteria trigger local production of proinflammatory compounds, which are subsequently released into the circulation, causing a systemic inflammatory reaction. Lung injury is a common feature and the most frequent cause of mortality in patients with systemic inflammation (5). In fact, the mortality rate of septic patients has remained high (30–70%) despite substantial investigative efforts, and management is largely limited to supportive care (6, 12). Convincing data have documented that pulmonary recruitment of neutrophils is a rate-limiting step in septic lung injury. For example, blocking neutrophil accumulation by targeting specific adhesion mole-

cules, such as LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), effectively attenuates lung injury in abdominal sepsis (4). Chemokines constitute a family of molecules regulating trafficking of circulating leukocytes into inflamed tissues (30). Based on their amino acid sequences they are divided into subfamilies, such as CC chemokines, which mainly attract monocytes and lymphocytes, and CXC chemokines, cytokine-induced neutrophil chemotactrant (CXCL1), and macrophage inflammatory protein-2 (CXCL2), which primarily attract neutrophils (30, 45). CXC chemokines are known to regulate pulmonary infiltration of neutrophils in septic lung damage (4). Activated neutrophils release tissue destructive substances, such as reactive oxygen species and proteolytic enzymes, which, in turn, cause lung edema and impaired gaseous exchange (13, 22). One study reported that platelets exert a key role in activating neutrophils in polymicrobial sepsis (2). This platelet-dependent activation of neutrophils appears to be mediated by CD40 ligand (CD40L) secreted from activated platelets (26). Thus the roles of adhesion molecules and chemokines in mediating leukocyte trafficking into the lung are relatively well known, whereas the signaling pathways regulating neutrophil recruitment and tissue damage in septic lung injury remain elusive.

Although statins are mainly used to lower lipid levels in patients with cardiovascular diseases, recent investigations have shown that statins exert potent anti-inflammatory effects, including inhibition of adhesion molecule expression and cytokine formation (35, 38). We have recently reported that simvastatin effectively inhibits neutrophil recruitment and lung injury in abdominal sepsis (41) and clinical data indicate that statins may reduce mortality in patients with severe infections and sepsis (23, 24) although the protective mechanisms of statins remain elusive. Statins regulate cholesterol levels by inhibiting the rate-limiting enzyme, HMG-CoA reductase, in the synthesis of mevalonate (1, 32). Mevalonate is not only a precursor for the formation of cholesterol but also for the generation of geranylgeranyl pyrophosphate (1, 7), which is used for protein geranylgeranylation catalyzed by geranylgeranyl transferase (15, 39). Protein geranylgeranylation modifies small G-proteins, including Rho A-C, Cdc42, and Rac1, which is critical for their function (33). For example, geranylgeranylation of Rho proteins facilitates interactions with their effector, Rho-kinase (9, 36). Rho-kinase signaling has been shown to play a significant role in polymicrobial and endotoxemia sepsis (17, 40). However, the potential role of geranylgeranyl transferase in controlling CXC chemokine formation, neutrophil infiltration, and lung damage in abdominal sepsis has not been investigated.

On the basis of the above, the aim of the present study was to define the role of geranylgeranyl transferase in systemic activation and recruitment of neutrophils into the lung in a murine model of polymicrobial sepsis with particular focus on
the potential role of platelet-derived CD40L and CXC chemokine formation in the lung.

**MATERIALS AND METHODS**

**Animals.** Male C57BL/6 mice (21–27 g) were housed on a 12:12-h light-dark cycle and fed a laboratory diet and water ad libitum. All experimental procedures were approved by the ethical committee at Lund University. Mice were anesthetized intraperitoneally (ip) with 75 mg of ketamine hydrochloride (Hoffman-La Roche, Basel, Switzerland) and 25 mg of xylazine (Janssen Pharmaceutica, Beerse, Belgium) per kilogram body weight.

**Experimental protocols.** Abdominal sepsis was induced by puncture of the cecum as described previously (17). In brief, the abdomen was opened and the exposed cecum was filled with feces by milking stool backward from the ascending colon. A ligature was placed below the ileocecal valve. The cecum was soaked with phosphate-buffered saline (PBS; pH 7.4) and punctured twice with a 21-gauge needle. This cerclage ligature and puncture (CLP) protocol is associated with less than 10% mortality within 24 h. The cecum was then pushed back into the abdominal cavity and the abdominal incision was sutured. To delineate the role of geranylgeranyl transferase, vehicle, dimethyl sulfoxide, or the geranylgeranyl transferase inhibitor, GGTI-2133 \[\text{N-[4-(imidazol-4-yl)methylamino]-2-(naphthylbenzoyl)l}-\text{leucine trifluoroacetate salt, G5294, Sigma Aldrich, St. Louis, MO, was treated with 10 mg/kg of GGTI-2133. Pulmonary levels of MPO were into the lungs via the trachea immediately after CLP in mice pre-

**MPO activity.** MPO activity in the lung was determined 6 h after CLP induction. Blood samples were incubated with an anti-CD16/CD32 antibody (10 min at RT) blocking Fcy II receptors to reduce nonspecific labeling and then incubated with phycoerythrin (PE)-conjugated anti-Gr-1 (clone RB6-8C5, rat IgG2b, eBioscience, San Diego, CA), APC-conjugated anti-CD14 (Sal4-2, rat IgG2a, Biotest, Täby, Sweden) and FITC-conjugated anti-Mac-1 (clone M1/70, integrin \(\alpha_{M}\) chain, rat IgG2b) antibodies. Another set of samples was stained with FITC-conjugated anti-CD41 (clone MWReg30, integrin \(\alpha_{M}\) chain, rat IgG1) and PE-conjugated anti-

**Flow cytometry.** For analysis of surface CD40L expression on platelets as well as Mac-1 and CXCR2 expression on circulating neutrophils, blood was collected into syringes containing 1:10 acid citrate dextrose 6 h after CLP induction. Blood samples were incubated with an anti-CD16/CD32 antibody (10 min at RT) blocking Fcy III/II receptors to reduce nonspecific labeling and then incubated with phycoerythrin (PE)-conjugated anti-Gr-1 (clone RB6-8C5, rat IgG2b, eBioscience, San Diego, CA), APC-conjugated anti-CD14 (Sal4-2, rat IgG2a, Biotest, Täby, Sweden) and FITC-conjugated anti-Mac-1 (clone M1/70, integrin \(\alpha_{M}\) chain, rat IgG2b) antibodies. Another set of samples was stained with FITC-conjugated anti-CD41 (clone MWReg30, integrin \(\alpha_{M}\) chain, rat IgG1) and PE-conjugated anti-

**Histology.** Lung samples were fixed by immersion in 4% formaldehyde phosphate buffer overnight and then dehydrated and paraffin embedded. Six-micrometer sections were stained with hematoxylin and eosin. Lung injury was quantified in a blinded manner by using a modified scoring system based on two previous publications (10, 19), including alveolar collapse, thickness of alveolar septae, alveolar fibrin deposition, and neutrophil infiltration graded on a scale of zero (absent) to four (extensive). In each tissue sample, five random areas were scored and mean value was calculated. The histology score is the sum of all four parameters.

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**Neutrophil isolation and activation.** Bone marrow neutrophils were freshly extracted from femurs and tibias of healthy mice by aseptically flushing the bone marrow with complete culture medium RPMI 1640 and then subsequently isolated by using Ficoll-Paque Research Grade (Amersham Pharmacia Biotech, Uppsala, Sweden). The purity of the isolated neutrophils was higher than 70% as assessed in a hemacytometer. Neutrophils were then resuspended in PBS to 10⁷ cells per milliliter and coincubated with 3 μg/ml recombinant mouse CXCL2 (R & D Systems) for 10 min at 37° C. Neutrophils were preincubated with GGTI-2133 (1 or 10 μM) 20 min before challenge with CXCL2.
Cells were stained and fixed for flow cytometric analysis of Mac-1 expression on neutrophils as described above.

Isolation of alveolar macrophages and quantitative RT-PCR. In separate experiments, gene expression of CXCL1, CXCL2, TNF-α, and CCL2 was quantified in alveolar macrophages isolated from sham mice (n = 5) and CLP animals pretreated with vehicle or 10 mg/kg of GGTI-2133 ip 30 min prior to CLP (n = 5). Alveolar macrophages were isolated from BALF as described in detail (42). Briefly, 30 min after induction of CLP, lungs were flushed three times with 1 ml of PBS supplemented with 0.5 mM EDTA. Alveolar fluid collections were then centrifuged at 1,400 RPM, 10 min, 18°C. The cells were then resuspended in RPMI 1640 complete culture medium and incubated at 37°C, 5% CO₂ in a 48-well plate. After 2 h, nonadherent cells were washed away by PBS. A total of 2–3 × 10⁵ macrophages were obtained per mice and the purity of macrophages was higher than 97%. Total RNA was isolated from the alveolar macrophages using an RNeasy Mini Kit (Qiagen, West Sussex, UK) following the manufacturer’s protocol and treated with RNase-free DNase (DNase I; Amersham Pharmacia Biotech, Sollentuna, Sweden) to remove potential genomic DNA contaminants. RNA concentrations were determined by measuring the absorbance at 260 nm spectrophotometrically. Each cDNA was synthesized by reverse transcription from 10 μg of total RNA using the StrataScript First-Strand Synthesis System and random hexamer primers (Stratagene; AH diagnostics, Stockholm, Sweden). Real-time PCR was performed using a Brilliant SYBRgreen QPCR master mix and MX 3000P detection system (Stratagene). The primer sequences of CXCL1, CXCL2, and β-actin were as follows: CXCL1 (forward) 5′-GCC AAT GAG CTG CGC TGT CAA TGC-3′, CXCL1 (reverse) 5′-CTT GGG GAC ACC TTT TAG CAT CTT-3′; CXCL2 (forward) 5′-GCT TCC TCG GGC ACT CCA GAC-3′.

<table>
<thead>
<tr>
<th>GGTI-2133 (mg/kg)</th>
<th>Sham</th>
<th>Vehicle</th>
<th>CLP+Vehicle</th>
<th>CLP+GGTI-2133 (10 mg/kg)</th>
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<td>6.5</td>
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<td>3.5</td>
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Table 1. Systemic leukocyte differential counts

<table>
<thead>
<tr>
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<th>MNL</th>
<th>PMNL</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>5.1 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>6.6 ± 0.2</td>
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<tr>
<td>Vehicle + CLP</td>
<td>0.8 ± 0.1*</td>
<td>0.6 ± 0.1*</td>
<td>1.4 ± 0.2*</td>
</tr>
<tr>
<td>GGTI-2133 1 mg/kg + CLP</td>
<td>1.9 ± 0.2*</td>
<td>1.0 ± 0.1</td>
<td>2.9 ± 0.1*</td>
</tr>
<tr>
<td>GGTI-2133 10 mg/kg + CLP</td>
<td>2.4 ± 0.2†</td>
<td>1.2 ± 0.1†</td>
<td>3.6 ± 0.3†</td>
</tr>
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Blood was collected from vehicle- and GGTI-2133-treated mice exposed to cecal ligation and puncture (CLP) for 24 h as well as sham-operated animals. Cells were identified as monomorphonuclear leukocytes (MNLs) and polymorphonuclear leukocytes (PMNLs). Data represent means ± SE and n = 5.

*P < 0.05 vs. Sham, †P < 0.05 vs. Vehicle + CLP.
Fig. 2. Geranylgeranylation transferase controls CLP-induced infiltration of neutrophils in the lung. Lung MPO levels (A) and number of BALF neutrophils (B) were determined 6 h and 24 h, respectively, after CLP induction. Animals were treated with GGTI-2133 (1 or 10 mg/kg) or vehicle prior to CLP induction. Mice treated with PBS served as sham animals. Data are presented as means ± SE and *P < 0.05 vs. Sham and #P < 0.05 vs. Vehicle + CLP.

CXCL2 (reverse) 5'-TTA GCC TTG CCT TTC AGT AT-3'; TNF-α (forward) 5'-CTT CAC ACT CAT CTC ATC TTC TC-3'; TNF-α (reverse) 5'-AGA CCC ATG CCC ATA GCC AT-3'; CCL2 (forward) 5'-TGT GAG TTA CAT ACC CCG GC-3'; CCL2 (reverse) 5'-GCC TGA ACA GCA GCC ATA GA-3'; and β-actin (forward) 5'-ATG TTT GAG ACC TTC AAC ACC-3'; β-actin (reverse) 5'-TCT CCA GGG AGG AAG AGG AT-3'. Standard PCR curves were generated for each PCR product to establish linearity of the RT-PCR reaction. PCR amplifications were performed in a total volume of 50 µl containing 25 µl of SYBRgreen PCR 2× master mix, 2 µl of 0.15 µM each primer, 0.75 µl of reference dye, and one 1-µl cDNA as a template adjusted up to 50 µl with water. PCR reactions were started with 10 min of denaturing temperature of 95°C, followed by a total of 40 cycles (95°C for 30 s and 55°C for 1 min), and 1 min of elongation at 72°C. Cycling time values for the specific target genes were related to that of β-actin in the same sample.

Chemotaxis assay. Neutrophils isolated from bone marrow by use of Ficoll-Paque were preincubated with GGTI-2133 (1 or 10 µM) for 30 min, and 1.5 × 10⁶ neutrophils were placed in the upper chamber of the Transwell inserts (5-µm pore size; Corning Costar, Corning, NY). Inserts were placed in wells containing medium alone (control) or medium plus CXCL2 (100 ng/ml; R&D Systems). After 120 min, inserts were removed, and migrated neutrophils were stained with Turks solution. Chemotaxis was determined by counting the number of migrated neutrophils in a Burker chamber.

Bacterial cultures. Blood was taken from the interior vena cava 24 h after CLP and cultured to evaluate the bacterial clearance. Serial logarithmic diluted blood was plated on trypticase soy agar II with 5% sheep blood (Becton Dickinson, Heidelberg, Germany). Plates were incubated under aerobic conditions at 37°C, and colonies were counted after 24 h of incubation. Bacterial counts are expressed as the number of CFU (×10⁵) per milliliter of blood.

Fig. 3. Mac-1 expression on neutrophils in vehicle and GGTI-2133 (1 and 10 mg/kg)-treated animals 6 h after CLP induction (A and B), and on isolated bone marrow neutrophils incubated with CXCL2 (5 µg/ml) and vehicle (PBS) or GGTI-2133 (1 or 10 µM) (C and D). Mean fluorescence intensity (MFI) is shown on the x-axis and cell counts on the y-axis. Figures are representative histograms from 5 samples. Data are presented as means ± SE and n = 5. *P < 0.05 vs. Sham and #P < 0.05 vs. Vehicle + CLP. *P < 0.05 vs. PBS and #P < 0.05 vs. PBS + CXCL2.
Increased lung wet-to-dry ratio (Fig. 1F). CLP triggered a clear-cut edema in the lung, reflected by an increased lung wet-to-dry ratio in CLP-treated mice (Fig. 1F, P < 0.05 vs. Sham, n = 5). Administration of GGTTI-2133 (10 mg/kg) reduced the wet-to-dry ratio by 73% in septic mice (Fig. 1F, P < 0.05 vs. Vehicle + CLP, n = 5). Moreover, CLP caused leukocyteopenia after 24 h (Table 1, P < 0.05 vs. Sham, n = 5). The CLP-induced leukocyteopenia was decreased in mice pretreated with GGTTI-2133 (Table 1). To investigate neutrophil recruitment in septic lung damage, activity of MPO (an indicator of neutrophils) and the number of neutrophils in BALF were determined. CLP-induced MPO levels in the lung and the amount of BALF neutrophils peaked at 6 h and 24 h, respectively (data not shown). It was found that pulmonary levels of MPO activity increased from 1.4 ± 0.1 units/g in sham animals to 10.1 ± 0.7 units/g in CLP mice, corresponding to a seven-fold increase (Fig. 2A, P < 0.05 vs. Sham, n = 5). Treatment with GGTTI-2133 (10 mg/kg) decreased CLP-induced levels of MPO by 61% in the lung (Fig. 2A, P < 0.05 vs. Vehicle + CLP, n = 5). The number of neutrophils in the BALF increased from 5.6 ± 1.0 in sham mice to 126.4 ± 9.4 in CLP animals, corresponding to a 22-fold increase (Fig. 2B, P < 0.05 vs. Sham, n = 5). Inhibition of geranylgeranyl transferase reduced the number of neutrophils in the bronchoalveolar space by 72% in septic mice (Fig. 2B, P < 0.05 vs. Vehicle + CLP, n = 5).

Geranylgeranyl transferase regulates neutrophil recruitment of Mac-1 in vivo. CLP caused a significant upregulation of Mac-1 on circulating neutrophils (Fig. 3, A and B, P < 0.05 vs. Sham, n = 5). Administration of GGTTI-2133 reduced Mac-1 expression on peripheral blood neutrophils in septic mice (Fig. 3, A and B, P < 0.05 vs. Vehicle + CLP, n = 5). Thus mean fluorescence intensity values of Mac-1 on neutrophils decreased from 336.4 ± 17.6 to 189.6 ± 11.5 in CLP mice pretreated with GGTTI-2133 (10 mg/kg), which corresponds to an 84% reduction (Fig. 3, A and B). We next tested whether this inhibitory effect of GGTTI-2133 might be a direct effect on neutrophils. Neutrophils isolated from the bone marrow were treated with GGTI-2133 (1 or 10 mg/kg) for 1 h. The surface expression of CD40L on neutrophils decreased in a dose-dependent manner in a concentration range of 1 to 10 µg/ml (Fig. 4, P < 0.05 vs. Sham, n = 5). The number of neutrophils in the BALF decreased in septic mice (Fig. 4, P < 0.05 vs. Sham, n = 5).}

**RESULTS**

**Geranylgeranyl transferase regulates neutrophil recruitment and lung injury.** Morphological analysis revealed normal lung architecture in sham mice (Fig. 1A). CLP caused severe pulmonary damage, characterized by severe destruction of pulmonary tissue microstructure, extensive edema of interstitial tissue, and massive infiltration of neutrophils (Fig. 1B). Inhibition of geranylgeranyl transferase reduced CLP-evoked tissue destruction and neutrophil infiltration in the lung (Fig. 1, C and D). Quantification of the morphological damage showed that CLP-induced lung injury was markedly attenuated in GGTTI-2133-treated mice (Fig. 1E, P < 0.05 vs. Vehicle + CLP, n = 5). CLP triggered a clear-cut edema in the lung, reflected by an increased lung wet-to-dry ratio (Fig. 1F, P < 0.05 vs. Sham, n = 5). Administration of GGTTI-2133 (10 mg/kg) reduced the wet-to-dry ratio by 73% in septic mice (Fig. 1F, P < 0.05 vs. Vehicle + CLP, n = 5). Moreover, CLP caused leukocyteopenia after 24 h (Table 1, P < 0.05 vs. Sham, n = 5). The CLP-induced leukocyteopenia was decreased in mice pretreated with GGTTI-2133 (Table 1). To investigate neutrophil recruitment in septic lung damage, activity of MPO (an indicator of neutrophils) and the number of neutrophils in BALF were determined. CLP-induced MPO levels in the lung and the amount of BALF neutrophils peaked at 6 h and 24 h, respectively (data not shown). It was found that pulmonary levels of MPO activity increased from 1.4 ± 0.1 units/g in sham animals to 10.1 ± 0.7 units/g in CLP mice, corresponding to a seven-fold increase (Fig. 2A, P < 0.05 vs. Sham, n = 5). Treatment with GGTTI-2133 (10 mg/kg) decreased CLP-induced levels of MPO by 61% in the lung (Fig. 2A, P < 0.05 vs. Vehicle + CLP, n = 5). The number of neutrophils in the BALF increased from 5.6 ± 1.0 in sham mice to 126.4 ± 9.4 in CLP animals, corresponding to a 22-fold increase (Fig. 2B, P < 0.05 vs. Sham, n = 5). Inhibition of geranylgeranyl transferase reduced the number of neutrophils in the bronchoalveolar space by 72% in septic mice (Fig. 2B, P < 0.05 vs. Vehicle + CLP, n = 5).

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Notably, surface expression of CXCR2 on circulating neutrophils decreased in septic mice (Fig. 4, P < 0.05 vs. Sham, n = 5). However, GGTTI-2133 had no effect on this CLP-
induced downregulation of CXCR2 expression on neutrophils (Fig. 4, P > 0.05 vs. Vehicle + CLP, n = 5).

Platelet shedding of CD40L is independent of geranylgeranyl transferase. CLP triggered a significant reduction in platelet expression of CD40L in vivo (Fig. 5A, P < 0.05 vs. Sham, n = 5). Administration of GGTI-2133 had no effect of CLP-triggered CD40L shedding (Fig. 5A, P > 0.05 vs. Vehicle + CLP, n = 5). Expression of CD40L was induced on isolated platelets by the thrombin receptor activating peptide, AYPGKF (Fig. 5B, P < 0.05 vs. PBS, n = 5). It was found that inhibition of geranylgeranyl transferase had no effect on AYPGKF-induced platelet expression of CD40L in vitro (Fig. 5B, P > 0.05 vs. PBS + AYPGKF, n = 5).

Geranylgeranyl transferase regulates pulmonary production of CXC chemokines. CXC chemokines, such as CXCL1 and CXCL2, are critical in directing neutrophils to extravascular sites of inflammation (43). Lung levels of CXCL1 and CXCL2 were low but detectable in sham animals (Figs. 6 and 7). CLP increased plasma and pulmonary levels of CXCL1 by 245-fold and 5-fold, respectively (Fig. 6, A and B, P < 0.05 vs. Sham, n = 5).

Geranylgeranyl transferase inhibition reduced CLP-evoked formation of CXCL1 by 58% in plasma and by 82% in the lung (Fig. 6, A and B, P < 0.05 vs. Vehicle + CLP, n = 5). CLP enhanced plasma and pulmonary levels of CXCL2 by 530-fold and 19-fold, respectively (Fig. 7, A and B, P < 0.05 vs. Sham, n = 5). GGTI-2133 treatment decreased CLP-induced production of CXCL1 by 92% in plasma and by 80% in the lung (Fig. 7, A and B, P < 0.05 vs. Vehicle + CLP, n = 5). We next isolated alveolar macrophages from the BALF in sham and CLP animals with or without pretreatment with GGTI-2133. CLP increased mRNA expression of CXCL1 and CXCL2 by more than 26-fold and 5-fold, respectively (Figs. 6C and 7C, P < 0.05 vs. Sham, n = 5). It was found that GGTI-2133 markedly decreased mRNA levels of CXCL1 and CXCL2 in the alveolar macrophages in CLP-induced animals (Figs. 6C and 7C, P < 0.05 vs. Vehicle + CLP, n = 5). Moreover, we found that CLP markedly increased plasma formation and gene expression in alveolar macrophages of TNF-α and CCL2 (Fig. 8, P < 0.05 vs. Sham, n = 5). Inhibition of geranylgeranyl transferase significantly decreased the CLP-evoked increase in plasma levels and mRNA levels in alveolar macrophages of TNF-α and CCL2 (Fig. 8, P < 0.05 vs. Vehicle + CLP, n = 5). In separate experiments, we observed that local administration of CXC chemokines in the lung increased MPO levels from 5.9 ± 1.2 units/g to 9.7 ± 0.8 units/g in CLP animals treated with 10 mg/kg of GGTI-2133 (P < 0.05 vs. Vehicle + GGTI-2133 + CLP, n = 5).

Neutrophil migration. We found that 100 ng/ml CXCL2 triggered a significant increase in neutrophil chemotaxis over a time period of 120 min (Fig. 9, P < 0.05 vs. Control, n = 5).
Coincubation of neutrophils with GGTI-2133 abolished CXCL2-provoked neutrophil chemotaxis in vitro (Fig. 9, P < 0.05 vs. PBS + CXCL2, n = 5).

Systemic bacteremia. CLP markedly increased the number of bacteria in the blood (Fig. 10, P < 0.05 vs. Sham, n = 5). Treatment with GGTI-2133 had no effect on the number of bacteria in the blood of septic animals (Fig. 10, P > 0.05 vs. Vehicle + CLP, n = 5).

DISCUSSION

Patients with abdominal sepsis pose a significant challenge to clinicians, which is partly due to an incomplete understanding of the pathophysiology. Herein, we show that geranylgeranyl transferase plays a key role in mediating lung damage in septic lung disease. Our findings show that geranylgeranyl transferase regulates pulmonary formation of CXC chemokines and infiltration of neutrophils in abdominal sepsis. In fact, inhibition of geranylgeranyl transferase not only decreased inflammation but also protected against tissue damage in the septic lung.

Sepsis is characterized by a generalized activation of the host innate immune system, including platelets and neutrophils, in which the most insidious feature is lung injury and impaired gaseous exchange (2, 28). In the present study, we show that the geranylgeranyl transferase inhibitor protects against pulmonary edema and tissue damage in polymicrobial sepsis, suggesting that geranylgeranyl transferase signaling plays an important role in abdominal sepsis. This finding extends on previous studies reporting that geranylgeranyl transferase appears to be critical in diseases, such as carcinogenesis, viral infection, rheumatoid arthritis, and glaucoma (8, 14, 18, 27). It is widely held that neutrophil infiltration is a key feature in the pathophysiology of septic lung damage (4, 16, 40). In the present study, we could demonstrate that inhibition of geranylgeranyl transferase reduced lung activity of MPO, a marker of neutrophil recruitment, by more than 61% in abdominal sepsis. This inhibitory effect on MPO correlated well with our observation that administration of GGTI-2133 decreased sepsis provoked neutrophil accumulation in the bronchoalveolar space by 72%, indicating that geranylgeranyl transferase is a potent regulator of neutrophil recruitment in septic lung injury. In fact, our investigation represents the first study to demonstrate that targeting geranylgeranyl transferase can attenuate pulmonary recruitment of neutrophils. Keeping in mind the tight relationship between neutrophil accumulation and lung injury, it might be suggested that the protective effect of GGTI-2133 is related to the reduction in pulmonary neutrophilia. Two previous studies have shown that inhibition of geranylgeranyl transferase reduces eosinophil and T-lymphocyte accumulation in the lung (11) and brain (37), respectively, suggesting that geranylgeranyl transferase might control extravascular trafficking of multiple subtypes of
that the reduction of CLP-evoked Mac-1 expression on neutrophils, it is plausible to conclude that geranylgeranyl transferase exert proinflammatory functions in the lung. Moreover, we have recently reported that simvastatin treatment decreases CLP-provoked lung injury (41). With the knowledge that statins prevent formation of geranylgeranylprophosphate and isoprenylation of Rho proteins (44), our present findings might help explain the protective effects of simvastatin against lung damage in abdominal sepsis. It is interesting to note that although inhibition of geranylgeranyl transferase protected against neutrophil accumulation and tissue injury in the lung the number of bacteria in the blood was not altered in CLP animals receiving GGTI-2133. Considering recent data indicating that platelets mediate neutrophil activation via secretion of CD40L in abdominal sepsis (26), it was of great interest to examine the role geranylgeranyl transferase in regulating platelet shedding of CD40L. We observed that platelet surface expression of CD40L was not altered by inhibition of geranylgeranyl transferase activity in septic animals. Moreover, agonist-induced expression of CD40L on isolated platelets was not altered by GGTI-2133. Together, these findings indicate that geranylgeranyl transferase-dependent pulmonary recruitment of neutrophils is independent of CD40L in abdominal sepsis.

Inflammatory infiltration of neutrophils at extravascular sites is a multistep process supported by adhesion molecules on neutrophils, such as P-selectin glycoprotein ligand-1 (PSGL-1) and Mac-1 (3, 4). Therefore, we next asked whether geranylgeranyl transferase might regulate neutrophil activation and expression of Mac-1. Indeed, treatment with GGTI-2133 significantly reduced expression of Mac-1 on the surface of neutrophils in mice exposed to CLP. However, inhibition of geranylgeranyl transferase had no effect on CXCL2-induced upregulation of Mac-1 on isolated neutrophils in vitro, suggesting that geranylgeranyl transferase regulates Mac-1 expression on neutrophils in an indirect manner in vivo. Neutrophil activation and trafficking in the extravascular space are coordinated by secreted CXC chemokines, such as CXCL1 and CXCL2, being murine homologues of human interleukin-8 (34). A functional role of CXC chemokines has been proposed in abdominal infections (40, 41). Herein, it was observed that inhibition of geranylgeranyl transferase markedly reduced pulmonary and plasma levels of CXC chemokines in septic animals. An important role of CXC chemokines was emphasized by our observation that local administration of CXCL1 and CXCL2 reversed the inhibitory effect of GGTI-2133 on pulmonary accumulation of neutrophils in CLP animals. Moreover, we found that CLP markedly enhanced CXCL1 and CXCL2 mRNA levels in alveolar macrophages. Notably, administration of GGTI-2133 abolished CLP-provoked gene expression of CXC chemokines in alveolar macrophages, indicating that geranylgeranyl transferase signaling is a key feature in macrophage production of CXC chemokines in abdominal sepsis. Knowing that CXC chemokines effectively increase Mac-1 expression on neutrophils, it is plausible to conclude that the reduction of CLP-evoked Mac-1 expression on neutrophils observed in CLP mice herein might be due to the profound attenuation of CXC chemokine formation in GGTI-2133-treated animals. Considering that CXC chemokines are potent inducers of neutrophil migration (43), it was also of interest to evaluate the role of geranylgeranyl transferase in regulating chemokine-dependent chemotaxis herein. Notably, we observed that inhibition of geranylgeranyl transferase dose dependently decreased CXCL2-induced neutrophil migration in vitro. Given that Rho-kinase signaling is instrumental in actin cytoskeletal organization and cell motility (21, 25), it is plausible that geranylgeranyl transferase-mediated isoprenylation of Rho proteins upstream of Rho-kinase activation might help to explain the potent role of geranylgeranyl transferase in chemokine-mediated neutrophil migration. Nonetheless, these results indicate that geranylgeranyl transferase regulate sepsis-evoked pulmonary infiltration of neutrophils at two distinct levels, i.e., both formation and function (i.e., migration) of CXC chemokines. Taken together, these inhibitory effects of GGTI-2133 on neutrophil expression of Mac-1 and CXC chemokine formation in the lung mimic the actions exerted by simvastatin in the same model (41), suggesting that reduced formation of geranylgeranyl pyrophosphate might, at least in part, explain certain anti-inflammatory effects of statins in abdominal sepsis. Moreover, we confirmed in the present study that the neutrophil expression of CXCR2 is downregulated in sepsis (29) but inhibition of geranylgeranyl transferase had no effect on the surface level of CXCR2 on neutrophils in CLP mice, indicating that modulation of CXCR2 expression is not involved in the anti-inflammatory effects of GGTI-2133.

We conclude that geranylgeranyl transferase is a potent regulator of acute lung injury in abdominal sepsis. Inhibition of geranylgeranyl transferase decreases CLP-induced pulmonary recruitment of neutrophils via reduction of CXC chemokine production in lung macrophages. Our findings suggest that targeting geranylgeranyl transferase might be an effective way to ameliorate respiratory failure in polymicrobial sepsis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

GERANYLGERANYL TRANSFERASE AND ABDOMINAL SEPSIS


