Secreted Group IIA Phospholipase A₂ Protects Humans Against the Group B Streptococcus: Experimental and Clinical Evidence

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Group B streptococcus (GBS) is a leading neonatal pathogen and a growing cause of invasive disease in the elderly, with clinical manifestations such as pneumonia and sepsis. Despite its clinical importance, little is known about innate immunity against GBS in humans. Here, we analyze the role of human group IIA secreted phospholipase A₂ (sPLA₂-IIA), a bactericidal enzyme induced during acute inflammation, in innate immunity against GBS. We show that clinical GBS isolates are highly sensitive to killing by sPLA₂-IIA but not by human antimicrobial peptides. Using transgenic mice that express human sPLA₂-IIA, we demonstrate that this enzyme is crucial for host protection against systemic infection and lung challenge by GBS. We found that acute sera from humans diagnosed with invasive GBS disease contain increased levels of sPLA₂-IIA compared with normal sera from healthy individuals, indicating that GBS induces an sPLA₂-IIA response in blood during human infection. We demonstrate that clinically relevant GBS strains are rapidly killed in these acute sera. We also demonstrate that the bactericidal effect is entirely due to sPLA₂-IIA, showing that sPLA₂-IIA might represent an important component of humoral innate immunity against GBS. Our data provide experimental and clinical evidence that sPLA₂-IIA protects humans against GBS infections.

Keywords. group B streptococcus; antimicrobial molecules; bactericidal; innate immunity.

When a pathogenic microbe enters the human body, it is targeted by innate immunity. An important arm of this response is represented by various secreted antimicrobial molecules that protect the human host by direct killing of the invading microbe [1–3]. Of these, it has been suggested that the antimicrobial peptides (AMPs), such as the human α- and β- defensins and the cathelicidin LL-37, play an important role in innate immunity against bacterial pathogens [1, 2, 4], but less is known about secreted host enzymes. Here, we analyze a bactericidal human enzyme, the group IIA secreted phospholipase A₂ (sPLA₂-IIA), in host protection against the group B streptococcus (GBS) and present both experimental and clinical evidence that this enzyme is important for innate immunity against GBS in humans.

GBS is an encapsulated gram-positive bacterium recognized as the most common cause of invasive bacterial disease in the neonatal period [5, 6]. The majority of neonatal GBS infections are acquired in connection with childbirth when the child is exposed to the bacterium present in the mother’s vaginal microflora. In most cases, GBS colonizes the infant without any clinical signs of illness; in some cases, colonization of the neonatal lung develops into pneumonia. Subsequently, GBS may spread to the blood stream, and cause sepsis, and finally cross the blood–brain barrier to give rise to meningitis [7]. In recent years, GBS has also gained attention as a cause of morbidity and mortality in adults, in particular, among the elderly [8, 9]. In these individuals, GBS most commonly gives rise to bacteremia, sepsis, skin and/or soft tissue infection, pneumonia,
and urinary tract infection. Thus, in both newborns and adults, GBS must manage several critical steps in order to establish an infection, such as colonization and breaching of mucosal surfaces and systemic spread in the blood. However, little is known about the role of different antibacterial molecules in the innate immune defense against GBS at these sites. We recently showed that GBS strains representing the 3 clinically most important serotypes (Ia, III, and V) are highly sensitive to killing by low concentrations of human sPLA2-IIA, suggesting that this enzyme might represent a major bactericidal component of the innate immune response against GBS [10].

Human-secreted phospholipases A2 (sPLA2s) form a family of enzymes that catalyze the hydrolysis of membrane glycerophospholipids which generates free fatty acids and lysophospholipids [11–13]. Most studies of these enzymes have focused on their role in inflammatory diseases [13]. However, a growing body of evidence suggests that 1 member of this family, sPLA2-IIA, play an important, direct role in innate immunity [3, 14]. Indeed, recombinant sPLA2-IIA shows bactericidal activity against various bacterial pathogens, in particular, against gram-positive bacteria, but the sensitivity varies greatly between species [15–20]. The mechanism by which sPLA2-IIA kills bacteria has been correlated to hydrolysis of the bacterial membrane phospholipids, suggesting a role for the catalytic activity of the enzyme in this process [3]. However, catalysis-independent killing of bacteria by sPLA2-IIA has also been reported [21]. Human sPLA2-IIA is constitutively expressed by some cells, for example, Paneth cells in the gut. Also, it is induced in a Toll-like receptor (TLR)–dependent manner by several cell types, including macrophages, during an acute inflammatory response [22]. This results in a marked increase in sPLA2-IIA concentrations at the mucosa and in blood where sPLA2-IIA may represent an important first line of defense against invading bacteria [3, 23]. However, the role of sPLA2-IIA in innate immunity against GBS during human infection is unknown.

Here, we investigate the role of human sPLA2-IIA in innate immunity against GBS. We show that human sPLA2-IIA is essential for host protection against both systemic infection and lung challenge by GBS in a transgenic mouse model. We demonstrate that acute sera from adults with invasive GBS disease contain increased levels of sPLA2-IIA compared with serum from healthy individuals, providing evidence of an sPLA2-IIA response to GBS infection in humans. We also show that sPLA2-IIA in these acute sera rapidly kills GBS, indicating that this enzyme might represent an important part of humoral innate immunity against GBS in humans.

**METHODS**

**Ethics Statement**

For research involving human sera, we used samples from an existing serum bank (BD3) at the Department of Infectious Diseases, Skåne University Hospital, Lund University, Sweden. All human samples used were anonymized. The ethical review board of the medical faculty at Lund University (LU 208–99), approved the study. The animal experiments were performed with the permission of the Animal Experimental Ethics Committee, Lund District Court (M297–09).

**Bacterial Strains**

The serotype Ia strain A909 was isolated from a neonate with GBS sepsis [24]. BM110 is a serotype III strain that belongs to the hypervirulent ST-17 clone and was isolated from a case of neonatal meningitis [25]. The serotype V strain 2047–93, which is a blood isolate from an adult diagnosed with bacteremia, was obtained from J. A. Elliot (Centers for Disease Control and Prevention, Atlanta, GA). A collection of clinical GBS isolates representing serotypes Ia, Ib, II, III, IV, V, and VIII was available in our laboratory. The *Streptococcus pyogenes* M6 strain JRS4 has been previously described [26]. GBS strains were grown in Todd-Hewitt (TH) broth (Oxoid, Basingstoke, UK) at 37°C without shaking. *S. pyogenes* was grown in TH broth supplemented with 0.2% yeast extract (THY) and grown at 37°C in 5% CO2.

**Proteins, Antibodies, and Reagents**

Recombinant human sPLA2-IIA wild-type (WT) was produced in *Drosophila* S2 cells essentially as described [27] and following the protocol used in insect cells for the structurally related human group IID sPLA2 [28]. The catalytically deficient sPLA2-IIA mutant H48Q was constructed and purified as described [29]. The rabbit anti-human sPLA2-IIA antibodies (α-sPLA2-IIA) were raised against recombinant human sPLA2-IIA, purified as a total immunoglobulin-G fraction on protein A-Sepharose [30], and shown to specifically block the catalytic activity of sPLA2-IIA (Supplementary Figure 1). Compstatin was from Tocris Bioscience (Bristol, UK). The sPLA2 inhibitor LY311727 has been described previously [31]. The human cathelicidin LL-37, the human α-defensins HNP-1 and HNP-2, and the human β-defensins hBD-3 and hBD-4 were from Sigma (St. Louis, MO).

**Bactericidal Analysis With Recombinant Human sPLA2-IIA and AMPs**

Overnight cultures of streptococci (OD620 = 1.1) were diluted 20-fold into fresh broth, and bacteria were grown at 37°C to mid-log phase (OD620 = 0.4). Bacteria were subsequently diluted 1000-fold into hpes buffer (20 mM hpes, 2 mM Ca2+, 1% bovine serum albumin, pH 7.4) to give a bacterial concentration of approximately 1 × 10⁵ CFU/mL. Recombinant human sPLA2-IIA or human AMPs was serially diluted in hpes buffer, and small aliquots of 10 μL were added in triplicate to the wells of a sterile 96-well polypropylene plate (Costar) and mixed with 10 μL of the bacterial suspension. After incubation at 37°C for 1 h, the samples were serially
diluted in phosphate-buffered saline (PBS), plated onto blood agar plates, and incubated overnight to determine the bacterial viability by colony counting. Experiments comparing the bactericidal activity of WT sPLA2-IIA and the H48Q mutant enzyme were performed as described above. For inhibition of sPLA2-IIA activity, LY311727 was added at a final concentration of 100 μM.

Analysis of Bacterial Phospholipid Degradation
Bacterial phospholipids were labeled with [3H]-oleic acid. Bacterial phospholipid degradation after incubation with sPLA2-IIA was analyzed essentially as described [10, 16].

Mice
Female human sPLA2-IIA transgenic mice (C57BL/6NTac-TgN(sPLA2)) and C57BL/6 WT mice were constructed and maintained as described [32]. Human sPLA2-IIA is constitutively expressed in the transgenic mice. The WT C57BL/6 mice have a natural mutation in the gene encoding murine sPLA2-IIA (Pla2g2a) and are therefore deficient for the mouse enzyme [33]. Expression of human sPLA2-IIA in the Tg sPLA2-IIA mice has been verified as described previously [10].

Mouse Infection Experiments
In the intraperitoneal infection model, groups of WT C57BL/6 or Tg sPLA2-IIA mice were anesthetized and infected with a sublethal dose of GBS strain A909 grown to mid-log phase (A620 = 0.5). Each mouse received approximately 1 × 10⁶ CFU. At 18 hours after challenge, the mice were sacrificed, and the livers were collected, weighed, homogenized, serially diluted in PBS, and plated onto blood agar for viable counting. In the intranasal infection model, groups of WT C57BL/6 or Tg sPLA2-IIA mice were anesthetized and challenged with a suspension of GBS strain A909 in TH broth (30 μL) grown to mid-log phase (A620 = 0.5). Each mouse received approximately 2 × 10⁷ CFU. At 18 hours after challenge, the mice were sacrificed, and the lungs were collected, weighed, homogenized, serially diluted in PBS, and plated onto blood agar for viable counting. In both models, data are given as CFU/g of tissue analyzed.

Acute Sera From Patients With Invasive GBS Disease
Acute sera from 4 adults (2 females, 2 males; mean age 70 years) diagnosed with invasive GBS disease were obtained from patients admitted to the Department of Infectious Diseases at Skåne University Hospital (Table 1). Normal serum was obtained from healthy volunteers (n = 3). All sera were free of antibiotics. The concentration of human sPLA2-IIA in acute and normal serum was measured using a human sPLA2-IIA enzyme-linked immunosorbent assay (ELISA) kit from Cayman Chemicals (Ann Arbor, MI). All human samples used in the study were anonymized.

GBS Killing Assay With Acute Sera
Overnight cultures of GBS were diluted 20 times into fresh TH broth, and bacteria were grown to mid-log phase (OD620 = 0.4). Bacteria were then directly diluted into hepes buffer to give a bacterial concentration of approximately 1 × 10⁵ CFU/mL. Aliquots of 10 μL of acute or normal sera were added to sterile 96-well polystyrene plates and mixed with 10 μL of bacterial suspension. After incubation at 37°C for 30 minutes, the samples were diluted in PBS, plated onto blood agar plates, and incubated overnight to determine the bacterial viability by colony counting. To specifically inhibit sPLA2-IIA activity, sera were preincubated with rabbit anti-hGIIA sPLA2 (final concentration 100 μg/mL) or LY311727 (final concentration 100 μM), respectively, at 37°C for 30 minutes before adding the bacteria. Compstatin was used at a final concentration of 100 μg/mL for preinhibition.

Statistical Analysis
The Mann–Whitney U test was used to calculate differences in bacterial load in organs in the in vivo experiment comparing WT C57BL/6 and Tg sPLA2-IIA mice for sensitivity to GBS infection.

RESULTS
GBS Is Highly Sensitive to Killing by Human sPLA2-IIA But Not by Human AMPs
The relative ability of sPLA2-IIA to kill GBS compared to other human antimicrobial molecules implicated in innate immunity

Table 1. Patients Diagnosed With Group B Streptococcus Infection and Measurement of Human Group IIA Secreted Phospholipase A2 Concentration in Their Acute Sera

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Time Until Sampling After Hospital Admission (d)</th>
<th>Concentration of Human Group IIA Secreted Phospholipase A2 in Serum (ng/mL)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>Male</td>
<td>Sepsis</td>
<td>Unknown</td>
<td>35.6</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>Male</td>
<td>Urosepsis</td>
<td>0</td>
<td>402.7</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>Female</td>
<td>Endocarditis</td>
<td>0</td>
<td>423.9</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>Female</td>
<td>Urosepsis</td>
<td>0–1</td>
<td>56.2</td>
</tr>
</tbody>
</table>

² Measured by human group IIA secreted phospholipase A2–specific enzyme-linked immunosorbent assay (see Materials and Methods section).
is unclear. As several human AMPs, including the cathelicidin LL-37 and the α- and β-defensins, have been shown to possess bactericidal activity against many bacterial species [1, 4, 34], we first compared the ability of these human AMPs and sPLA2-IIA to kill GBS using 3 clinical isolates representing the most important serotypes: Ia, III, and V. As shown in Figure 1, we found a striking difference among the GBS strains for sensitivity to sPLA2-IIA compared with the human AMPs analyzed. Human sPLA2-IIA exhibits 50% killing against GBS at around 5 ng/mL (0.34 nM). In contrast, HNP-2 and hBD-4 did not show any killing of GBS, even at concentrations up to 25 μg/mL (7.4 μM). Three of the human AMPs (LL-37, HBP-1, and hBD-3) exhibited 50% killing of GBS bactericidal activity only at concentrations of 5–25 μg/mL (1.5–7.6 μM). Similar results were obtained when incubation in hepes buffer, PBS, and human plasma were compared (Supplementary Figure 2). These data indicate that human sPLA2-IIA may be a major bactericidal component against GBS and focused our interest on investigating the role of sPLA2-IIA in innate immunity against GBS.

To determine if the sensitivity to sPLA2-IIA is a general property among GBS isolates, we extended the analysis [10] to include clinical isolates representing serotypes Ib, II, IV, and VIII, as well as additional strains of serotypes Ia, III, and V. For comparison, we included a strain of S. pyogenes, which showed a high degree of resistance against sPLA2-IIA [10]. As shown in Figure 2, all GBS strains analyzed were highly sensitive to sPLA2-IIA. Thus, clinical GBS isolates are generally highly sensitive to the bactericidal activity of sPLA2-IIA.

### Catalytic Function of sPLA2-IIA Is Required for Its Bactericidal Activity Against GBS

The ability of sPLA2-IIA to kill bacteria has been correlated to hydrolysis of the bacterial membrane phospholipids, indicating that the catalytic activity of sPLA2-IIA is required for its

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**Figure 1.** Bactericidal activity of human group IIA secreted phospholipase A2 (sPLA2-IIA) and different antimicrobial peptides (AMPs) against group B streptococcus (GBS). Overnight cultures of GBS strains A909 (A), BM110 (B), and 2047–93 (C) were grown to mid-log phase (OD600 = 0.4). Bacteria were directly diluted into hepes buffer (20 mM hepes, 2 mM Ca2+, 1% bovine serum albumin, pH 7.4) to give a bacterial concentration of approximately 1 × 10⁵ CFU/mL. Human sPLA2-IIA and human AMPs (LL37, HBP-1, HBP-2, hBD-3, and hBD-4) were added in triplicate to sterile 96-well polypropylene plates and mixed with the bacterial suspension. After incubation at 37°C for 60 minutes, the samples were diluted, plated onto blood agar plates, and incubated overnight to determine the bacterial viability by colony counting. The results are means ± standard deviation of triplicates. The killing is presented as the percentage of colony-forming units for each bacterial strain remaining in the wells after incubation with the different molecules compared with the inoculum.
bactericidal property [3]. However, sPLA$_2$-IIA can kill bacteria independently of its catalytic activity [21], suggesting an alternative killing mechanism by sPLA$_2$-IIA. To analyze the killing mechanism of sPLA$_2$-IIA against GBS, we used the H48Q active site mutant of sPLA$_2$-IIA, which has only about 2%–4% of WT enzymatic activity [29]. Whereas clear killing was observed with WT sPLA$_2$-IIA, the H48Q mutant did not show bactericidal activity against any of the 3 GBS strains analyzed (Figure 3A–C), indicating that the catalytic activity of sPLA$_2$-IIA is required for killing GBS. An inhibitor of the sPLA$_2$-IIA catalytic activity, LY311727 [31], completely inhibited the killing of GBS by sPLA$_2$-IIA (Figure 3A–C). The higher sensitivity to sPLA$_2$-IIA in GBS compared with that of $S$. pyogenes was correlated with an increased hydrolysis of the membrane phospholipids in GBS (Figure 3D), supporting our finding that the catalytic activity of sPLA$_2$-IIA is essential for its ability to kill GBS.

**Human sPLA$_2$-IIA Is Crucial for Host Protection Against Experimental Systemic GBS Infection**

To investigate the in vivo role of sPLA$_2$-IIA in GBS infection, we used transgenic that mice overexpress human sPLA$_2$-IIA (sPLA$_2$-IIA Tg) [32, 33]. To determine whether sPLA$_2$-IIA can protect mice against systemic infection caused by GBS, we compared WT C57BL/6 and sPLA$_2$-IIA Tg mice in a model of intraperitoneal infection using a strain (A909) of serotype Ia, which is a common serotype among GBS infections in both neonates and adults [5, 9]. As shown in Figure 4A, the bacterial load was significantly lower in livers from the sPLA$_2$-IIA Tg mice compared with that of the WT C57BL/6 mice ($P < .001$), indicating that sPLA$_2$-IIA is a major host-protective factor against GBS infection during systemic infection. Interestingly, GBS was rapidly killed in serum from sPLA$_2$-IIA Tg mice but not from WT C57BL/6 mice (Figure 4B), indicating that sPLA$_2$-IIA most likely protects mice against GBS through direct killing.

**Human sPLA$_2$-IIA Protects Mice Against Lung Infection by GBS**

To determine whether sPLA$_2$-IIA can protect against GBS growth in lung tissue, we used a model of intranasal GBS challenge, resulting in inhalation of the bacteria. At 18 hours after challenge, the bacterial load in the lungs of the sPLA$_2$-IIA Tg mice was significantly lower than that in WT C57BL/6 mice ($P < .05$), indicating that sPLA$_2$-IIA promotes clearance of GBS from the lung (Figure 4C), probably through direct killing of the bacteria (Figure 4D). This result, together with our data showing that sPLA$_2$-IIA is host protective against systemic GBS infection, suggests that human sPLA$_2$-IIA is important for innate immunity against experimental GBS infection.
Acute Sera From Adult Humans With Invasive GBS Disease Contain High Levels of sPLA2-IIA

To determine whether GBS infections in humans trigger an sPLA2-IIA response, we measured the serum levels of sPLA2-IIA by ELISA in acute sera from 4 adult patients diagnosed with invasive GBS disease (Table 1). The levels of sPLA2-IIA in acute sera from the GBS patients ranged from 35.6 ng/mL to 423.9 ng/mL, whereas the sPLA2-IIA concentration in normal serum from healthy individuals (n = 3) was in the range of 1.5–3.9 ng/mL. These data indicate that invasive GBS disease in adults trigger an sPLA2-IIA response, leading to a marked increase in serum levels of sPLA2-IIA.

GBS Is Rapidly Killed by sPLA2-IIA Present in Acute Sera From GBS Patients

To determine whether the serum sPLA2-IIA response during a GBS infection protects the host against GBS, we analyzed killing of clinically relevant GBS strains in the acute sera. For comparison, we analyzed killing in normal serum from a healthy individual. Normal serum failed to kill GBS (Figure 5A–C).

Figure 3. The catalytic activity of human group IIA secreted phospholipase A2 (sPLA2-IIA) is required for its bactericidal activity against group B streptococcus (GBS). GBS strains A909 (A), BM110 (B), and 2047–93 (C) were grown to mid-log phase and subsequently diluted in hepes buffer to give a bacterial concentration of approximately 1 × 10⁶ CFU/mL. Recombinant human WT sPLA2-IIA or H48Q sPLA2-IIA mutant protein were serially diluted in hepes buffer, mixed with bacterial suspension, and incubated at 37°C for 30 minutes. For inhibition, sPLA2-IIA was preincubated with the inhibitor LY311727 at a final concentration of 100 μM at 37°C for 30 minutes before addition of the GBS suspension. The samples were subsequently diluted, plated onto blood agar plates, and incubated overnight to determine the bacterial viability by colony counting. The results are means (± standard deviation) of triplicates. D. Comparison of membrane phospholipid hydrolysis of GBS (strain A909) and Streptococcus pyogenes (strain JRS4) by sPLA2-IIA. [3H]-oleic acid–labeled bacteria (10⁸ CFU) were incubated with different concentrations of human sPLA2-IIA for 2 hours. The membrane phospholipid hydrolysis of bacteria by sPLA2-IIA was detected by monitoring the release of free fatty acids from the bacteria using liquid scintillation spectroscopy. Data are presented as the percentage of cell-bound [3H]-oleic acid released by each bacterium after incubation with sPLA2-IIA.
Figure 4. Human group IIA secreted phospholipase A2 (sPLA2-IIA) protects mice against experimental infection with group B streptococcus (GBS). A, Groups of WT C57BL/6 (n = 6) or Tg sPLA2-IIA mice (n = 7) were infected intraperitoneally with mid-log phase A909 bacteria diluted in fresh Todd-Hewitt (TH) broth. Mice were sacrificed 18 hours after challenge; livers were collected and weighed. The livers were homogenized, serially diluted in phosphate-buffered saline (PBS), and plated onto blood agar. Each point represents a mouse. The bars indicate the median value for each group. The difference in colony-forming units per gram of liver between wild-type (WT) C57BL/6 and Tg sPLA2-IIA mice were calculated with Mann–Whitney U test (***P < .001).

B, Analysis of sPLA2-IIA–mediated killing in sera from female sPLA2-IIA Tg (n = 6) and C57BL/6 WT mice (n = 1), respectively. An overnight culture of strain BM110 was grown to mid-log phase (OD620 = 0.4). Bacteria were then directly diluted into hepes buffer and mixed with aliquots of mouse serum. For inhibition with LY311727 (final concentration of 100 μM), samples were preincubated with LY311727 at 37°C for 30 minutes. The plate was incubated at 37°C for another 30 minutes, and the samples were diluted, plated onto blood agar plates, and incubated overnight to determine the bacterial viability by colony counting. The killing is presented as the percentage of colony-forming units for the bacterial strain remaining in the wells after incubation compared with the inoculum.

C, Wild-type C57BL/6 (n = 11) or Tg sPLA2-IIA (n = 11) mice were challenged intranasally with mid-log phase A909 in TH broth. Mice were sacrificed 18 hours after challenge. The lungs were homogenized, serially diluted in PBS, and plated onto blood agar. Each point represents a mouse. The bars indicate the median value for each group. The difference in colony-forming units per gram of lung between WT C57BL/6 and Tg sPLA2-IIA mice was calculated with Mann–Whitney U test (*P < .05).

D, Analysis of sPLA2-IIA–mediated killing in BALF from female sPLA2-IIA Tg (n = 6) and C57BL/6 WT mice (n = 1), respectively. BALFs were obtained by flushing of mouse lung tissue with 1 mL of PBS. BALF was mixed and incubated with GBS strain BM110 as described above and plated onto blood agar to determine the viability. The killing is presented as the percentage of colony-forming units for the bacterial strain remaining in the wells after incubation compared with the inoculum.
Rather, all GBS strains analyzed exhibited growth during incubation in normal serum as the number of bacteria recovered after incubation was higher than that of the inoculum. In contrast, all 3 GBS strains were rapidly killed when incubated with the acute sera from infected patients (Figure 5A–C). Rabbit anti-human sPLA2-IIA antibodies, which block the catalytic activity of sPLA2-IIA (Supplementary Figure 1), completely inhibited the killing of GBS, demonstrating that the bactericidal activity against GBS in the acute sera is entirely mediated by sPLA2-IIA. In contrast, antibodies against human group X sPLA2 did not inhibit killing of GBS in the acute sera (Supplementary Figure 3A). As expected, the anti-sPLA2-IIA antibodies completely inhibited killing of GBS by pure recombinant sPLA2-IIA (Figure 5A–C). Importantly, similar data were obtained using the specific sPLA2 inhibitor LY311727 (Figure 6A–C). Compstatin, an inhibitor of complement C3, did not inhibit the bactericidal activity against GBS in the acute sera (Supplementary Figure 3B), showing that complement does not contribute to killing of GBS in these samples. No killing of GBS was seen in convalescence serum from a case of GBS infection in an elderly adult, showing that sPLA2-IIA-mediated killing is not a general property in sera from elderly patients (Supplementary Figure 3C). Together, these data indicate
that the acute sPLA₂-IIA response in human serum during invasive GBS disease is important for killing and clearance of GBS.

**DISCUSSION**

Whereas most studies on innate immunity against GBS have dealt with the role of the complement system [35, 36] and the TLRs [37, 38], less is known about the role of different secreted bactericidal molecules. Moreover, little is known about host factors that affect sensitivity to GBS during infection in humans. Previous studies have shown that LL-37 and β-defensins are expressed in neonatal skin and that these AMPs show some bactericidal activity against GBS at high concentrations in vitro [39]. However, there is no evidence to indicate that human AMPs play a role in host protection against GBS infection in humans. Our data demonstrate that recombinant sPLA₂-IIA is by far more bactericidal against GBS compared with human AMPs and that low concentrations of sPLA₂-IIA kill GBS isolates, representing all clinically important serotypes. Although this does not exclude a role for AMPs in the innate immune defense against GBS, it focused our interest on the role of human sPLA₂-IIA in host protection against GBS infection.
To analyze the role of sPLA2-IIA in host defense against GBS in vivo, we compared Tg sPLA2-IIA and WT C57BL/6 mice for sensitivity to experimental GBS infection. In a model of intraperitoneal infection, we found that the bacterial load in the liver was significantly lower in the Tg sPLA2-IIA mice compared with WT mice, indicating that sPLA2-IIA protects mice against systemic GBS infection in this model. Moreover, we found that Tg sPLA2-IIA mice cleared GBS from lungs to a significantly greater extent compared with WT mice after intranasal challenge. This suggests that sPLA2-IIA mediates killing of GBS in the lung, providing a first line of defense against GBS growth in lung tissue, which may be important in protecting the host against GBS pneumonia. It is believed that alveolar macrophages represent the primary cellular source of sPLA2-IIA in the lungs, upregulating sPLA2-IIA production upon bacterial challenge [22, 40–42] or during acute inflammatory diseases such as acute respiratory distress syndrome, resulting in strongly increased sPLA2-IIA levels in bronchoalveolar lavage fluid [43]. Although it is not known if GBS can induce sPLA2-IIA secretion from human alveolar macrophages, such a response could potentially contribute to innate immunity against GBS in the lungs.

Since our in vivo data suggest that sPLA2-IIA is important for innate immunity against GBS infection, we determined whether sPLA2-IIA may be involved in host defense against GBS during infection in humans. Adults with invasive GBS disease have increased serum levels of sPLA2-IIA as compared with WT mice, indicating that sPLA2-IIA protects mice completely mediated by sPLA2-IIA activity. Therefore, we conclude that an sPLA2-IIA response in blood against GBS during acute inflammation may be important for humoral innate immunity against GBS in humans.

The experimental and clinical data presented in this study imply that sPLA2-IIA may also provide protection against GBS in neonates. It is not known whether sPLA2-IIA is induced by GBS during neonatal infection. However, studies have shown that sPLA2-IIA is expressed in the neonatal lung [44] and that bacterial infection triggers a substantial sPLA2 response in infants (mean age 7 months) [45], implying that an sPLA2-IIA response may protect neonates against GBS challenge at these sites.

In conclusion, we provide evidence for an important host-protective role of sPLA2-IIA against GBS in humans. Our data focus general interest on this secreted host enzyme in innate immunity against bacterial infections [10, 14, 19]. Interestingly, a role of human sPLA2-IIA in innate immunity against bacteria is further supported by the finding that some bacterial pathogens appear to have developed mechanisms to evade sPLA2-IIA, either through inhibition of direct killing by sPLA2-IIA [10, 46] or by downregulation of sPLA2-IIA secretion from macrophages [16, 47]. Thus, the interplay between pathogenic bacteria and sPLA2-IIA will be an important area of future research in order to fully understand the role of this secreted bacterial host enzyme in host defense against bacterial infections.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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**Potential conflicts of interests.** All authors: No reported conflicts.

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