Cell-specific pattern recognition receptor signaling in antibacterial defense
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Chapter 9

Single immunoglobulin interleukin-1 receptor related molecule impairs host defense during pneumonia and sepsis caused by Streptococcus pneumoniae


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Abstract

*Streptococcus (S.) pneumoniae* is a common cause of pneumonia and sepsis. Toll-like receptors (TLRs) play a pivotal role in host defense against infection. We here sought to determine the role of Single immunoglobulin interleukin-1 receptor-related molecule (SIGIRR a.k.a. TIR8), a negative regulator of TLR signaling, in pneumococcal pneumonia and sepsis. Wild type and SIGIRR deficient (*Sigirr* -/-) mice were infected intranasally (to induce pneumonia) or intravenously (to induce primary sepsis) with *S. pneumoniae* and euthanized after 6, 24 or 48 hours for analyses. Additionally, survival studies were performed. *Sigirr* -/- mice showed a delayed mortality during lethal pneumococcal pneumonia. In accordance, *Sigirr* -/- mice displayed lower bacterial loads in lung and less dissemination of the infection at 24 hours after induction of pneumonia. SIGIRR deficiency was associated with increased interstitial and perivascular inflammation in the lung tissue early after infection without impacting on neutrophil recruitment or cytokine production. *Sigirr* -/- mice also demonstrated reduced bacterial burdens at multiple body sites during *S. pneumoniae* sepsis. *Sigirr* -/- alveolar macrophages and neutrophils exhibited an increased capacity to phagocytose viable pneumococci. These results suggest that SIGIRR impairs antibacterial host defense during pneumonia and sepsis caused by *S. pneumoniae*. 
Introduction

The gram-positive diplococcus *Streptococcus (S.) pneumoniae* is the leading cause of community-acquired pneumonia and the most common cause of death from infection in developed countries today (1, 2). In the United States alone, *S. pneumoniae* is responsible for more than half a million pneumonia cases and 50,000 episodes of bacteremia each year, with case fatality rates of 7 and 20% respectively (3); similar figures have been reported for Europe (4). Globally, the annual pneumococcal related death toll has been estimated at approximately 2 million (2). As such, *S. pneumoniae* represents a major health burden despite vaccination programs and effective antibiotic treatments.

Toll-like receptors (TLRs) form an important part of innate defense against infection (5, 6). TLRs recognize conserved motifs expressed by microbes (pathogen/microbe-associated molecular patterns or PAMPs/MAMPs) and host derived damage-associated molecular patterns (DAMPs), resulting in the recruitment of intracellular adaptor molecules, the activation of nuclear factor (NF)-κB and other signaling pathways, and the production of pro-inflammatory cytokines. Multiple TLRs are involved in the detection of pneumococci. TLR2 is mainly responsible for recognition of *S. pneumoniae* cell wall components (7-9), while TLR4 induces cytokine release in response to pneumolysin, a toxin expressed by all virulent pneumococcal strains (10, 11). During experimental pneumococcal pneumonia protective roles have been reported for TLR4 (10-12) and TLR9 (13), the receptor that recognizes bacterial DNA (5, 6), while TLR2 contributes to the induction of inflammation in the airways (7, 14). All TLRs involved in sensing *S. pneumoniae* signal via a common adapter: myeloid differentiation primary response gene 88 (MyD88), which also mediates the intracellular effects of the interleukin (IL)-1 receptor (R) and IL-18R (15). Not unexpectedly, mice with a genetic deletion of the *Myd88* gene (*Myd88⁻/⁻* mice) showed a strongly impaired host defense during pneumococcal pneumonia, as reflected by enhanced bacterial growth and an increased mortality (16).

Unrestrained activation of TLRs can cause disproportionate inflammation and collateral tissue damage. Therefore, TLR signaling is securely regulated in order to avoid such injurious inflammatory responses (17). Single immunoglobulin interleukin-1 receptor related molecule (SIGIRR or TIR8) has been shown to inhibit NF-κB activation dependent on TLRs and IL-1R like receptors (ILRs) (18). SIGIRR is ubiquitously expressed in different tissues, including the lung, where the main SIGIRR positive cell types are bronchial epithelium, leukocytes and blood endothelial cells (19). Recent research has implicated SIGIRR as an important regulator of inflammation in the respiratory tract. In a model of acute pneumonia caused by *Pseudomonas (P.) aeruginosa*, a gram-negative pathogen primarily affecting immune compromised hosts, *Sigirr⁻/⁻* mice showed increased lethality and higher bacterial burdens together with exaggerated local and systemic inflammation (19). Likewise, in chronic lung infection caused by *Mycobacterium tuberculosis* SIGIRR deficiency was associated with excessive lung and systemic inflammation, and as a consequence thereof increased lethality (20). In accordance, overexpression of SIGIRR in lung epithelial cells attenuated acute lung injury elicited by airway
exposure to LPS, the toxic component of gram-negative bacteria (21). Thus far, the contribution of SIGIRR to the host response during gram-positive infection has not been studied. We here sought to determine the role of SIGIRR in pneumonia and sepsis caused by S. pneumoniae.

Materials and methods

Animals
Specific pathogen free 9-11 week old C57BL/6 wild-type (WT) mice were from Charles River (Maastricht, the Netherlands). Sigirr -/- mice (22), backcrossed six times to a C57BL/6 background, were bred in the animal facility of the Academic Medical Center in Amsterdam. Age- and sex-matched animals were used in all experiments. The Animal Care and Use Committee of the University of Amsterdam approved all experiments.

Experimental infections
The models of pneumococcal pneumonia and pneumococcal sepsis have previously been described (23, 24). In short, mice were inoculated intranasally (to induce pneumonia) with 5x10^4 CFU of S. pneumoniae (serotype 3; American Type Culture Collection, ATCC 6303, Rockville, MD) or intravenously (to induce primary sepsis) with 5x10^5 CFU S. pneumoniae. Lung, blood, spleen and liver were harvested 6, 24, or 48 post infection for quantitative bacterial cultures as described (n = 8 or 16 per group at each time point) (23, 24). Neutrophil counts in bronchoalveolar lavage (BAL) fluid were determined as described (25). In separate studies, mice were followed for 4 days and survival was monitored at least every 12 hours (n = 20 per group).

Histopathological analysis
Lung histopathology was semi-quantitatively analyzed as described (7, 26). In short: the “lung inflammation score” was expressed as the sum of six, on a scale of 0 (‘absent’) to 4 (‘severe’) graded, parameters: pleuritis, bronchitis, edema, interstitial inflammation, percentage of pneumonia, and endothelialitis. Granulocyte staining was done with a Ly-6G monoclonal antibody (BD Pharmingen, San Diego, CA, USA) as described previously (23, 27). The entire Ly-6G stained lung sections were digitized with a slide scanner (Olympus, Tokyo, Japan). Immunopositive (Ly-6G+) areas were analyzed with ImageJ (version 2006.02.01, US National Institutes of Health, Bethesda, MD) and expressed as the percentage of the total lung surface area (26, 27). Analyses were performed in a blinded way, i.e., without knowledge of genotype (n=8 per group at each time point).

Assays
Lung homogenates were prepared as described (7). In lung homogenates, tumor necrosis factor (TNF)-α, IL-1β, IL-6, macrophage inflammatory protein (MIP-2, also known as CXCL2) and cytokine-induced neutrophil chemo attractant (KC, also known as CXCL1) were measured using specific enzyme-linked immunosorbent assays (R&D systems, Abingdon, UK) in accordance with the manufacturer’s
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recommendations. In plasma TNF-α, IL-6 and monocyte chemoattractant protein 1 (MCP1, also known as CCL2) were measured by cytometric bead array multiplex assay (BD Biosciences, San Jose, CA).

Phagocytosis

Heparinized whole blood from WT and Sigirr−/− mice was collected and murine alveolar macrophages (AMs) were obtained by bronchoalveolar lavage and cultured to adhere overnight. Phagocytosis of UV irradiated (254 nm; 30 minutes at 0.12J/cm²; in a BLX-254; Vilber Lourmat, France) CFSE labeled (Invitrogen) opsonized (10% autologous normal mouse serum) S. pneumoniae by alveolar macrophages (MOI 100) and in whole blood by GR-1 identified neutrophils (8x10⁷ CFU/ml blood) was determined with the help of flow cytometry as described previously (23). The percentage phagocytosing cells at 37 °C was corrected for the percentage phagocytosis at 4 °C.

Statistical analysis

Data are expressed as box and whisker diagrams depicting the smallest observation, lower quartile, median, upper quartile and largest observation or as bar graphs depicting means ± SEM. Differences were analyzed by Mann Whitney U test. Survival was compared by Kaplan-Meier analysis followed by a log rank test. A value of p < 0.05 was considered statistically significant.

Results

Sigirr−/− mice show delayed mortality and diminished bacterial outgrowth during S. pneumoniae pneumonia

To obtain insight into the potential role of SIGIRR in the outcome of pneumococcal pneumonia WT and Sigirr−/− mice were infected intranasally with S. pneumoniae and followed for 4 days (Figure 1A). Sigirr−/− mice showed a prolonged survival (median survival time 63 hours) when compared to WT mice (median survival time 54 hours, p < 0.01). In order to determine whether the survival advantage of Sigirr−/− mice corresponded with an improved antibacterial response, we next harvested lungs, blood, livers and spleens from both mouse strains at predefined time points following induction of pneumonia for quantitative cultures (Figure 1B-E). At 6 hours post infection, pneumococci were cultured from lungs only (with the exception of one positive blood culture in each group) and bacterial loads were similar in Sigirr−/− and WT mice. In contrast, at 24 hours after infection, Sigirr−/− mice showed lower bacterial counts in all body sites examined when compared with WT mice (lungs p < 0.05; blood, spleen, liver all p < 0.01). At 48 hours, differences in bacterial loads between mouse strains had subsided in distant organs, whereas at this late time point Sigirr−/− mice still had lower pneumococcal burdens in their lungs (p < 0.01 versus WT mice). Together these data suggest that Sigirr−/− mice show a prolonged survival in this lethal model of pneumococcal pneumonia resulting from a transient limitation of bacterial growth and dissemination.
Figure 1: Survival and bacterial loads in WT and Sigirr⁻/⁻ mice during pneumococcal pneumonia. Wild-type (grey dots & boxes) and Sigirr⁻/⁻ mice (white dots & boxes) were inoculated with S. pneumoniae intranasally. Lack of SIGIRR improved survival following intranasal infection (A). Sigirr⁻/⁻ mice displayed decreased bacterial loads after 24 and 48 hours in lung (B), and after 24 hours in blood (C), liver (D) and spleen (E). CFUs are expressed as box and whisker plots showing the smallest observation, lower quartile, median, upper quartile and largest observation. Survival: N= 20 mice per group, t=6 & 24 hours n=16 mice per group, t=48 hours n=8 mice per group. * p < 0.05, ** p < 0.01
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Sigirr⁻/⁻ mice demonstrate an increased pulmonary inflammatory response early after induction of pneumonia

To obtain insight in the role of SIGIRR in the regulation of lung inflammation induced by *S. pneumoniae*, lung tissue slides prepared 6, 24 and 48 hours after infection were semi-quantitatively analyzed according to the scoring system described in Methods section (Figure 2A-E). As reported earlier, this model of pneumococcal pneumonia is characterized by a gradually developing inflammatory response in lung tissue with typical features of lower respiratory tract infection, including bronchitis, perivascular and interstitial inflammation, edema and, especially during the progressed phase, accumulation of neutrophils (12, 23, 24). At the earliest time point (6 hours) Sigirr⁻/⁻ mice showed significantly increased lung inflammation (*p* < 0.05 relative to WT mice), which was caused by enhanced interstitial and perivascular inflammation. At later time points, when the extent of lung pathology had clearly increased, pathology scores did not differ between Sigirr⁻/⁻ and WT mice. The recruitment of neutrophils to the primary site of infection is a prominent part of the innate immune response to invading respiratory pathogens. We determined the number of neutrophils in lung tissue by quantifying the amount of Ly-6+ cells in lung slides by digital imaging (Figure 3A-E). In both mouse strains, the number of Ly6+ cells in lung tissue gradually increased during the course of the infection; no differences between groups were observed at any time point (6h *p* = 0.5; 24h *p* = 0.9; 48h *p* = 0.13). Considering the importance of early neutrophil influx into the bronchoalveolar space, we also analyzed the number of neutrophils in BAL fluid harvested 6 hours after infection; no difference between Sigirr⁻/⁻ and WT mice was found (*p* = 0.13) (Figure 3F). BAL fluid macrophage, and lymphocytes numbers were also similar between groups at this early time point (data not shown). Together these data argue against an important role for SIGIRR in neutrophil recruitment during pneumococcal pneumonia; SIGIRR deficiency did impact on early interstitial and perivascular inflammation.

Sigirr⁻/⁻ mice demonstrate unaltered lung cytokine and chemokine levels in lungs following *S. pneumoniae* infection

To obtain further insight in the potential role of SIGIRR in the regulation of lung inflammation during pneumococcal pneumonia, we measured proinflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines (MIP-2, KC) in whole lung homogenates harvested 6, 24 or 48 hours after intranasal inoculation with *S. pneumoniae* (Table 1). Lung cytokine and chemokine levels were similar in Sigirr⁻/⁻ and WT mice at all time points. Moreover, as a readout for systemic inflammation, we measured the plasma concentrations of TNFα, IL-6 and MCP-1; no differences were found between groups (Table 1).
Figure 2: Histopathology of lungs from WT and Sigirr\(^{-/-}\) mice during pneumococcal pneumonia. Semi-quantitative histology scores of lung slides as determined by the scoring system described in the Methods section from WT (grey bars) and Sigirr\(^{-/-}\) mice (white bars) (A) and a haematoxylin-eosin staining of the lung 6 (B-C) and 48 hours (D-E) following pneumococcal infection. Representative lung slides of WT (B,D) and Sigirr\(^{-/-}\) mice (C,E); original magnification x10. Histology scores are mean ± SEM of 8 mice per group at each time point. Arrows in C indicate several foci of increased perivascular and interstitial inflammation.
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Figure 3: Neutrophil influx into lungs of WT and Sigirr−/− mice during pneumococcal pneumonia. Neutrophil numbers in WT (grey bars) and Sigirr−/− lung tissue (white bars) were evaluated by Ly-6 staining of lung slides (A-E) during pneumonia. Neutrophil numbers were further counted in bronchoalveolar lavage fluid 6 hours following inoculation (F). Representative Ly-6 stained lung sections of WT (B,D) and Sigirr−/− mice (C,E) are depicted 6 (B-C) and 48 hours (D-E) post induction of pneumonia: original magnification x 10. Data in panels A and F are means ± SEM of 8 mice per group at each time point.
Table 1: Cytokines measured in lung homogenates and plasma during *S. pneumoniae* pneumonia.

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<th>T=6</th>
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<td></td>
<td>WT</td>
<td>Sigirr&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td><strong>Lung</strong></td>
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<tr>
<td>TNF-α</td>
<td>45 ± 17</td>
<td>109 ± 58</td>
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<td>IL-1β</td>
<td>90 ± 45</td>
<td>164 ± 79</td>
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<td>IL-6</td>
<td>48 ± 12</td>
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<tr>
<td>MIP-2</td>
<td>477 ± 78</td>
<td>894 ± 255</td>
<td>1560 ± 694</td>
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<td>KC</td>
<td>1398 ± 290</td>
<td>942 ± 255</td>
<td>8882 ± 3474</td>
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<td><strong>Plasma</strong></td>
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<tr>
<td>TNF-α</td>
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<td>5 ± 1</td>
<td>11 ± 2</td>
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<tr>
<td>IL-6</td>
<td>3 ± 0.4</td>
<td>7 ± 2</td>
<td>133 ± 38</td>
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<tr>
<td>MCP-1</td>
<td>23 ± 2</td>
<td>19 ± 4</td>
<td>157 ± 42</td>
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Mice were infected with *S. pneumoniae* at t=0. Data are means ± SEM of 8 mice per group.
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Enhanced phagocytosis of *S. pneumoniae* by *Sigirr* <sup>−/−</sup> alveolar macrophages and neutrophils ex vivo

Next, we investigated the ability of WT and *Sigirr* <sup>−/−</sup> alveolar macrophages and neutrophils to phagocytose *S. pneumoniae*. For this alveolar macrophages and whole blood were exposed to growth arrested CFSE labeled bacteria for 60 minutes at 4 or 37 °C and internalization was analyzed by flow cytometry (Figure 4). Alveolar macrophages showed a relatively low ability to phagocytose viable *S. pneumoniae*; nonetheless, *Sigirr* <sup>−/−</sup> alveolar macrophages demonstrated an enhanced capacity to internalize *S. pneumoniae* compared to WT macrophages (Figure 4A, *p* < 0.001). Neutrophils of both genotypes readily phagocytosed pneumococci; clearly *Sigirr* <sup>−/−</sup> neutrophils showed an increased ability to phagocytose *S. pneumoniae* relative to WT neutrophils (Figure 4B, *p* < 0.001).

![Figure 4: Phagocytosis of *S. pneumoniae* by alveolar macrophages and neutrophils.](image)

Figure 4: Phagocytosis of *S. pneumoniae* by alveolar macrophages and neutrophils. Alveolar macrophages (AM) and whole blood were exposed to fluorescently labelled *S. pneumoniae* for 60 minutes at 4 or 37 °C. Depicted are percentages of phagocytosing AM (A) and neutrophils (B) at 37 °C when corrected for their 4 °C controls. Data are means ± SEM (AM: *n* = 6 WT vs 8 *Sigirr* <sup>−/−</sup>, PMN n= 8 vs 8). *** *p* < 0.001
Sigirr⁻/⁻ mice show diminished bacterial outgrowth during primary S. pneumoniae sepsis

We wondered whether the improved antibacterial defense in Sigirr⁻/⁻ mice after induction of pneumonia was primarily caused by limitation of bacterial growth in the lungs and as a consequence thereof impeded dissemination and/or by an additional reduction of pneumococcal multiplication in body sites distant from the lungs. To address this question, Sigirr⁻/⁻ and WT mice were infected with S. pneumoniae by intravenous injection via the tail vein, thereby by passing the initial interaction between host and pathogen in the respiratory tract, and euthanized 6, 24 or 48 hours later for quantitative cultures of multiple body sites. Once more lower bacterial counts were observed in Sigirr⁻/⁻ mice, especially at 48 hours after infection when SIGIRR deficiency was associated with reduced bacterial loads in blood (p < 0.05 versus WT mice), spleen (p < 0.05), liver (p < 0.01) and lungs (p < 0.01, Figure 5). Notably, in these experiments SIGIRR deficiency did not significantly influence the plasma concentrations of TNFα, IL-6 or MCP-1 (Table 1).

Figure 5: Bacterial loads in WT and Sigirr⁻/⁻ mice during pneumococcal sepsis. WT (grey boxes) and Sigirr⁻/⁻ mice (white boxes) were inoculated with S. pneumoniae intravenously. Sigirr⁻/⁻ mice displayed decreased bacterial loads after 24 and 48 hours in the lung (A), and after 48 hours in the blood (B), liver (C) and spleen (D). Data are expressed as box and whisker plots showing the smallest observation, lower quartile, median, upper quartile and largest observation; n=8 mice per group at each time point. * p < 0.05, ** p < 0.01
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Discussion

*S. pneumoniae* is a common human pathogen that can reside as a commensal in the nasopharynx, from where it is able to enter the lower respiratory tract, causing pneumonia and sepsis (1, 2). The family of TLRs forms an important part of innate defense against invading pneumococci. SIGIRR is a negative regulator of TLR and ILR signaling that is abundantly expressed in the lungs. We here investigated the potential role of SIGIRR in pneumococcal pneumonia and sepsis. SIGIRR was found to impair antibacterial host defense during both pneumonia and sepsis caused by *S. pneumoniae*, as reflected by a reduced survival accompanied by increased bacterial growth and dissemination in WT mice when compared with *Sigirr*−/− animals.

Upon recognition of *S. pneumoniae* by in particular TLR2 (7, 8), TLR4 (10, 11) and TLR9 (13) NF-κB is activated and pro-inflammatory cytokines are produced with the distinct purpose of eradicating the pathogen (5). Uncontrolled stimulation of TLRs leads to disproportionate inflammation and tissue injury. Excessive TLR activation is prevented by negative regulators of TLR signaling of which several have been identified (17), including SIGIRR (18, 28). Specifically, inhibitory activity of SIGIRR has been demonstrated on signaling by TLR4, TLR7, TLR9, IL-1R type I (IL-1RI), IL-18R and ST2 (18). The current study does not elucidate via which receptor SIGIRR exerts its detrimental effect during pneumococcal pneumonia. Potential candidates are TLR4, TLR9, IL-1RI and IL-18R, considering that mice deficient for either one of these signaling pathways were reported to have an impaired antibacterial defense response during infection with *S. pneumoniae*. Indeed, mice with a mutant non-active form of TLR4 showed enhanced pneumococcal growth and dissemination in models of nasopharyngeal colonization and lower respiratory tract infection, accompanied by increased lethality (10-12). Similarly, *TLR9*−/− mice displayed accelerated growth of pneumococci upon infection of the lower airways together with enhanced mortality (13), while *IL-1rl*−/− (29) and *IL-18*−/− (30) mice showed a more modestly impaired immune response only reflected by higher bacterial loads. To our knowledge, the role of TLR7 has not been studied in the context of pneumococcal infections, while our laboratory recently showed that ST2 does not contribute to host defense during *S. pneumoniae* pneumonia (26). Together these data suggest that SIGIRR may impair host defense during pneumococcal pneumonia by inhibition of TLR4, TLR9, IL-1RI and/or IL-18R.

In contrast to the results presented here, previous studies revealed a protective role of SIGIRR in host defense against pulmonary infections. In a high dose lung infection model with *P. aeruginosa* associated with acute inflammation, *Sigirr*−/− mice showed a reduced survival and diminished bacterial clearance relative to WT mice (19). *Sigirr*−/− mice had markedly elevated concentrations of proinflammatory cytokines in whole lung homogenates, including IL-1β, and elimination of IL-1RI signaling in *Sigirr*−/− mice partially reversed their worse outcome (19). Similarly, *Sigirr*−/− mice displayed exaggerated pulmonary inflammation and strongly elevated plasma levels of TNFα and IL-1β during experimental lung tuberculosis, and combined treatment with anti-TNFα and anti-IL-1β antibodies improved their
survival in this model (20). Hence, the protective role of SIGIRR in these previous investigations on lung infection most likely was related to its inhibitory effect on the production of proinflammatory cytokines (19, 20). Our current data, indicating a detrimental role for SIGIRR, suggest another underlying mechanism, considering that SIGIRR deficiency did not alter the local or systemic cytokine response during *S. pneumoniae* pneumonia or sepsis. Nonetheless, studies in which IL-1 signaling is inhibited are warranted to address this issue. Of note, our model of gram-positive pneumonia differs considerably from the previously reported model of gram-negative pneumonia caused by *P. aeruginosa* (19). Indeed, while *S. pneumoniae* (infectious dose $5 \times 10^4$ CFU) gradually grows in lungs of normal mice resulting in a slowly building inflammatory response, airway infection by *P. aeruginosa* (infectious inoculum $10^6$ CFU) is associated with a brisk inflammatory reaction while bacteria are cleared from the airways. The difference between *S. pneumoniae* and *P. aeruginosa* pneumonia models is further illustrated by the different roles of proinflammatory cytokines, which play a protective role in pneumococcal pneumonia (29-31), while they hamper bacterial clearance during *Pseudomonas* pneumonia (32-34).

Earlier studies have indicated that the role for SIGIRR in the immune response to infection varies depending on the infecting organism and the infected site. In accordance with the enhanced susceptibility of *Sigirr*−/− mice during *P. aeruginosa* pneumonia (19), an anti-SIGIRR antibody caused increased bacterial loads in corneas during experimental *Pseudomonas* keratitis (35). In addition, *Sigirr*−/− mice were more susceptible to infection by mucosal and systemic infection by *Candida albicans* and to lung infection by *Aspergillus fumigatus* (36). In contrast, however, *Sigirr*−/− mice demonstrated transiently reduced bacterial burdens in kidneys during *Escherichia coli* pyelonephritis, possibly caused by a faster recruitment of neutrophils to the primary site of infection (37). Although clearly host defense against gram-positive and gram-negative bacteria is regulated in partially different ways (5), the present results, obtained after infection with a gram-positive bacterium, taken together with previous reports using gram-negative organisms (19, 35, 37), cannot be used to establish a differential role of SIGIRR in infections caused by either one of these very broad groups of pathogens. Indeed, the receptors known to be influenced by SIGIRR (see above) are not exclusively activated by either gram-positive or gram-negative bacteria. We consider it likely that the primary site of infection, the initial bacterial load and whether the pathogen multiplies or is cleared, together with differential expression of PAMPs, determines the eventual role of SIGIRR in host defense.

We provided evidence that SIGIRR attenuates *S. pneumoniae* phagocytosis by neutrophils and alveolar macrophages, which at least in part may explain the lower bacterial loads in *Sigirr*−/− mice. TLR stimulation induced expression of phagocytic genes (Fc and complement receptor genes, scavenger receptor and scavenger receptor pathway genes) in bone marrow derived macrophages (38), and resulted in enhanced uptake of both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria by RAW cells in a MyD88 and p38 mitogen activated kinase (MAPK) dependent manner (38). p38 MAPK signaling dependency
was established in LPS enhanced microglial Fc receptor mediated phagocytosis as well (39). In addition, IL-1β and IL-18 have been shown to enhance phagocytosis (39, 40). Thus, in theory, SIGIRR deficiency could impact phagocytosis by either inhibiting TLR effects on phagocytic gene expression and/or by attenuating IL-1β and/or IL-18 production and/or signaling. To the best of our knowledge this is the first report implicating SIGIRR in phagocytosis. The fact that this highly virulent S. pneumoniae strain cannot be killed by macrophages or neutrophils ex vivo (24) and data not shown) precludes studies on the effect of SIGIRR on bacterial killing.

Contrary to E. coli pyelonephritis (37), SIGIRR deficiency did not influence neutrophil influx into the lungs during pneumonia caused by either P. aeruginosa (19) or S. pneumoniae (reported here). SIGIRR is expressed by both hematopoietic and parenchymal cells (19). Bone marrow transfers, creating chimeric mice expressing SIGIRR only in the hematopoietic or parenchymal compartment, can provide insight into which cells drive the phenotype of Sigirr −/− mice in pneumococcal infection.

The pneumococcus is a highly relevant human pathogen, especially in the context of community-acquired pneumonia. The immune system rapidly responds to pneumococci that try to invade the lower airways. TLRs are of paramount importance for the recognition of S. pneumoniae and for the activation of inflammatory pathways among which ILRs. We here report on the role of SIGIRR, a negative regulator of TLRs and ILRs, in S. pneumoniae pneumonia and sepsis. In contrast to earlier investigations that studied the function of SIGIRR during lung infections caused by Aspergillus fumigatus (36), Mycobacterium tuberculosis (20) or P. aeruginosa (19), in which SIGIRR improved outcome by inhibition of excessive inflammation, our results indicate that SIGIRR impairs host defense during pneumonia and sepsis caused by S. pneumoniae.

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