On Toll-like receptors and the innate immune response in sepsis caused by Burkholderia pseudomallei (melioidosis)
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Endogenous Interleukin-18 improves the early antimicrobial host response in severe melioidosis


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ABSTRACT

Melioidosis is caused by the soil saprophyte *Burkholderia pseudomallei*, and is endemic in South East Asia. The pathogenesis of melioidosis is still largely unknown, although interferon (IFN)-γ seems to play an obligatory role in host defense. Previously, we have shown that IFN-γ production in melioidosis is controlled in part by interleukin (IL)-18. The aim of the present study was to determine the role of IL-18 in the immune response to *B. pseudomallei*. For this the following investigations were performed: (1) plasma IL-18 and blood monocyte IL-18 mRNA levels were elevated in 34 patients with culture proven melioidosis when compared to 32 local healthy controls; in addition, IL-18 binding protein levels were markedly elevated in patients, strongly correlating with mortality. (2) IL-18 gene-deficient (IL-18 KO) mice showed an accelerated mortality after intranasal infection with a lethal dose of *B. pseudomallei*, which was accompanied by an enhanced bacterial growth in their lungs, liver, spleen, kidneys and blood at 24 and 48 h post infection when compared to WT mice. In addition, IL-18 KO mice displayed evidence of enhanced hepatocellular injury and renal insufficiency. Together these data indicate that the enhanced production of IL-18 in melioidosis is an essential part of a protective immune response to this severe infection.
INTRODUCTION

Melioidosis is caused by the aerobic Gram-negative soil-dwelling bacillus *Burkholderia pseudomallei* and is an important cause of severe sepsis in Southeast Asia and Northern Australia. Humans acquire melioidosis by inoculation through skin abrasions or inhalation. More than half of patients with melioidosis present with pneumonia associated with bacterial dissemination to distant organs. Mortality due to melioidosis is high, varying from 50% in northeast Thailand to 20% in the higher technology setting of Northern-Australia. Interest in disease pathogenesis of melioidosis has increased following its classification as category B disease/agent of bioterrorism by the US Centers for Disease Control and Prevention.

*B. pseudomallei* is an intracellular pathogen that multiplies within macrophages. Although more is becoming known about the pathogenesis of melioidosis, host-pathogen interactions are still ill defined. Several investigations have implicated interferon (IFN)-γ as an important mediator in protective immunity against melioidosis. In mice infected with *B. pseudomallei* intraperitoneally, inhibition of IFN-γ lowered the LD50 from >5 x 10^5 to ~2 colony-forming units (CFUs) and was associated with an 8,500- and 4,400-fold increase in bacterial loads in liver and spleen respectively. Similarly, IFN-γ deficient mice displayed early mortality after intraperitoneal infection with *B. pseudomallei* together with strongly increased bacterial burdens in their spleen. Inhibition of interleukin (IL)-12 or IL-18, the predominant endogenous inducers of IFN-γ production, resulted in increased mortality in the same model.

IL-18 is mainly produced by activated macrophages, and is first synthesized as a precursor protein (pro-IL-18, 24 kD), which requires splicing by caspase-1 to liberate the 18 kD mature active protein. The biological activity of IL-18 is further regulated by IL-18 binding protein (IL-18BP), which binds IL-18 thereby preventing the interaction with its cell-associated receptor. Besides its IFN-γ-inducing effect, IL-18 has many proinflammatory effects on T and natural killer (NK) cells, enhancing proliferation and cytotoxicity, activating nuclear factor-κB and stimulating the production of cytokines, including tumor necrosis factor (TNF)-α, IL-1, IL-2 and IL-6. Thus far only one investigation has directly addressed the role of endogenous IL-18 in experimental melioidosis. In that study administration of a blocking anti-IL-18 receptor antibody increased the early mortality after intraperitoneal injection of *B. pseudomallei*; the impact of IL-18 inhibition on antibacterial defense was not examined. The aim of the present study was to determine the role of IL-18 in the immune response to melioidosis. For this we measured the expression of IL-18 and IL-18BP in 34 Thai patients with culture proven melioidosis and assessed the function of endogenous IL-18 using IL-18 knockout (KO) mice and a model of intranasal infection with *B. pseudomallei* that mimics melioidosis with severe pneumonia and bacterial dissemination to distant body sites.
MATERIALS AND METHODS

Patients study
34 patients with melioidosis (mean age 52 years, range 18-86 years; 50% male) were recruited prospectively at Sapprasithiprasong Hospital, Ubon Ratchathani, northeast Thailand in 2004. Sepsis due to melioidosis was defined as culture positivity for B. pseudomallei from any clinical sample plus systemic inflammatory response syndrome (SIRS). To meet the SIRS criteria patients had to meet at least three of the following four criteria: a core temperature of ≥ 38°C or ≤ 36 ºC; a heart rate of ≥ 90 beats/min; a respiratory rate of ≥ 20 breaths/min or a PaCO₂ of ≥ 32 mmHg or the use of mechanical ventilation for an acute respiratory process; and a white-cell count of ≥ 12 x 10⁹/l or ≤ 4 x 10⁹/l or a differential count showing > 10% immature neutrophils. These definitions have been used in large clinical trials and were modified according to the latest revisions. Exclusion criteria were the use of dialysis and/or immunosuppressive therapy, known disorders of coagulation and concomitant infection with human immunodeficiency virus. Blood samples were drawn within 36 hours of the start of appropriate antimicrobial therapy. 32 healthy blood donors (mean age 41 years, range 21-59 years; 71% male) recruited from the Sapprasithiprasong hospital blood bank served as a control population. The study was approved by both the Ministry of Public Health, Royal Government of Thailand and the Oxford Tropical Research Ethics Committee, University of Oxford, UK and written informed consent was obtained from all study subjects.

IL-18 and IL-18 BP measurements
Human IL-18 and IL-18BPα were measured by ELISA (R&D, Minneapolis, MN). In addition, IL-18 mRNA levels were measured as follows. Heparin blood samples were drawn from the antecubital vein and immediately put on ice. Leukocytes were isolated using erythesis buffer. Monocyte and granulocyte enriched populations were isolated using Polymorphprep (Axis-Shield, Dundee, United Kingdom). Monocyte and granulocyte fractions were > 98% pure as determined by their scatter pattern on flow cytometry. After isolation leukocytes, monocyte and granulocyte were dissolved in Trizol and stored at ~80 ºC until used for RNA isolation. RNA was isolated and analyzed by multiplex ligation-dependent probe amplification (MLPA) as described using a inflammatory-specific kit developed in collaboration with MRC-Holland (Amsterdam, the Netherlands). All samples were tested with the same batch of reagents. The levels of mRNA were expressed as a normalized ratio of the peak area divided by the peak area of the β2 microglobulin (B2M) gene, resulting in the relative abundance of mRNAs of the genes of interest. Transcription of the B2M gene was not affected by B. pseudomallei infection.

Mouse infection
The Animal Care and Use of Committee of the University of Amsterdam approved all experiments. Pathogen-free 8 to 10 week old wild-type (WT) C57BL/6 mice were purchased from Harlan Sprague Dawley Inc. (Horst, The Netherlands). IL-18 KO mice (backcrossed 6 times to a C57BL/6 background) were generated previously as described and generously provided by Dr. Shizuo Akira (Osaka University, Japan). Age and sex-matched animals were used in all experiments. For
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each timepoint one separate experiment was performed. The survival experiment was performed once. For preparation of the inoculum, *B. pseudomallei* strain 1026b (kindly provided by Dr. Don Woods, University of Calgary, Canada) was pipetted from frozen aliquots into 50 ml Luria broth (Difco, Detroit, MI) and placed overnight at 37°C in a shaking incubator. Thereafter, a 1 ml portion was transferred to fresh Luria broth and grown for ± 5h to midlogarithmic phase. Bacteria were harvested by centrifugation at 1500 x g for 15 minutes, washed and resuspended in sterile isotonic saline at a concentration of 5x10^2 CFUs/50 μl, as determined by plating serial 10-fold dilutions on blood agar plates. Pneumonia was induced by intranasal inoculation of 50 μl (5x10^2 CFU) bacterial suspension. For this procedure mice were lightly anesthetized by inhalation of isoflurane (Upjohn, Ede, The Netherlands).

**Determination of bacterial outgrowth**

24 and 48 hrs after infection, mice were anesthetized with Hypnorm (Janssen Pharmaceutica, Beerse, Belgium: active ingredients fentanyl citrate and fluanisone) and midazolam (Roche, Mijdrecht, The Netherlands) and sacrificed by bleeding from the vena cava inferior. The lungs, spleen, liver, kidneys and the brain were harvested and homogenized at 4°C in 4 volumes of sterile saline using a tissue homogenizer (Biospec Products, Bartlesville, OK). CFUs were determined from serial dilutions of organ homogenates and blood, plated on blood agar plates and incubated at 37°C at 5% CO_2 for 16 h before colonies were counted.

**Preparation of lung tissue for cytokine measurements**

For cytokine measurements, lung homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM Tris, 2 mM MgCl_2, 2 mM CaCl_2, 1% Triton X-100, and 8 μg/ml AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride), 100 μg/ml EDTA-NA2, 20 μg/ml Pepstatin and 20 μg/ml Leupeptin (pH 7.4) and incubated at 4°C for 30 min. Homogenates were centrifuged at 1500 x g at 4°C for 15 minutes, and supernatants were stored at -20°C until assays were performed.

**Assays**

Mouse IL-18 was measured by ELISA (MBL International, Woburn, MA). Mouse TNF-α, IFNγ, monocyte chemotactic protein (MCP)-1 and IL-6 were measured by cytometric bead array (CBA) multiplex assay (BDBiosciences, San Jose, CA) in accordance with the manufacturer's recommendations. Aspartate aminotransferase (ASAT), creatinine and urea were determined with commercially available kits (Sigma-Aldrich), using a Hitachi analyzer (Roche) according to the manufacturer’s instructions.

**Pathology**

Lungs, spleen, liver, kidneys and the brain of each mouse were harvested after infection, fixed in 10% formalin and embedded in paraffin. Four μm sections were stained with H&E, and analyzed by a pathologist who was blinded for groups. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following parameters: % surface with pneumonia, necrosis/abscess formation, interstitial inflammation, endothelialitis, bronchitis, edema, thrombus formation and pleuritis. Liver and spleen sections were scored on inflammation, necrosis/
abscess formation and thrombus formation. Each parameter was graded on a scale of 0 to 4, with 0: absent, 1: mild, 2: moderate, 3: severe, 4: very severe.

**Statistical analysis**

Values are expressed as means ± SEM unless indicated otherwise. Differences between groups were analyzed by Mann-Whitney U test. Correlations were calculated using the Spearman’s rho test. For survival analysis, Kaplan-Meier analysis followed by log rank test was performed. These analyses were performed using GraphPad Prism version 4.00, GraphPad Software (San Diego, CA). Values of P<0.05 were considered statistically significant.

**RESULTS**

**Elevated plasma IL-18, IL-18 BP and blood leukocyte IL-18 mRNA levels in patients with culture proven melioidosis**

To obtain a first insight into IL-18 and IL-18BP expression during septic melioidosis, we measured these cytokines in the plasma from patients with septic melioidosis and healthy controls. In addition we quantified IL-18 mRNA in their blood leukocytes (Figure 1). We first confirmed our earlier data on increased serum IL-18 concentrations in patients with melioidosis in the.

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**Fig. 1. Plasma IL-18, IL18 BP and blood leukocyte IL-18 mRNA levels in patients with culture proven melioidosis.** Increased levels of IL-18 plasma (A), IL-18 mRNA in peripheral blood leukocytes (B) and IL-18 mRNA in peripheral blood monocytes (C) of patients (n=34) with septic melioidosis compared to healthy controls (n=32). The strongly increased levels of IL-18 BP (D) correlated with IL-18 levels (E). ** p < 0.01; *** p < 0.001.
current study population: relative to healthy controls (195 ± 13 pg/ml), patients displayed strongly elevated plasma IL-18 levels (4616 ± 601 pg/ml, P < 0.001; Figure 1A). We extended this finding by showing elevated plasma IL-18 mRNA levels in whole blood (unfractionated) leukocytes of patients with septic melioidosis (Figure 1B, P < 0.001 versus controls). Monocytes were at least in part responsible for this enhanced IL-18 mRNA production since patients had significantly elevated IL-18 mRNA levels in monocyte enriched cell fractions (Figure 1C, P < 0.01 versus controls). IL-18 mRNA was not detectable in granulocyte enriched cell fractions (data not shown). Of note, the difference in circulating IL-18 levels between patients and controls was much larger than the difference in IL-18 mRNA expression in peripheral blood leukocytes, suggesting that cell types not present in peripheral blood (e.g. tissue macrophages) contribute to circulating IL-18 levels and/or that more mature IL-18 is generated from pre-IL-18 in patients. Plasma IL-18BP levels were strongly elevated in patients (59 ± 3.7 ng/ml) relative to healthy controls (10 ± 0.5 ng/ml, P < 0.001; Figure 1D). In melioidosis patients, plasma IL-18 and IL-18BP levels showed a strong positive correlation (r = 0.70, P < 0.0001; Figure 1E).

**Plasma IL-18 and IL-18BP correlate with mortality**

High plasma levels of both IL-18 and IL-18BP on admission correlated with an adverse outcome. Patients who died during hospital admission had higher IL-18 (7552 ± 797 pg/ml vs 3014 ± 591 pg/ml) and IL-18BP (73 ± 2.6 vs 48 ± 4.7 ng/ml) concentrations than those who survived to discharge (both P < 0.001 for the differences between groups, Figures 2A and B). Further proof for a correlation between the plasma levels of IL-18 and IL-18BP and disease severity was obtained in 8 patients who survived and from whom a second blood sample was drawn after successful therapy. In these patients a strongly significant decrease in plasma IL-18 and IL-18BP concentrations was detected (both P < 0.001; Figures 2C and D).

![Figure 2. Correlation of IL-18 and IL-18 BP levels with outcome in patients with severe melioidosis.](image_url)

Both IL-18 and IL-18 BP levels strongly correlated with outcome. Both IL-18 (A) and IL-18 BP (B) were strongly upregulated in patients who died in comparison to the patients who survived. Patients (n=8) that survived after two weeks of intensive treatment showed near normalization of IL-18 (C) and IL-18 BP (D) levels. **p < 0.01; *** p < 0.001.
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IL-18 KO mice show accelerated mortality during experimental melioidosis

Having established that IL-18 is upregulated in patients with severe melioidosis and correlates with mortality, we next investigated the involvement of IL-18 in the host response to *B. pseudomallei* infection in a murine model of melioidosis. Wild type (WT) and IL-18 KO mice were intranasally infected with *B. pseudomallei*. Inoculation with *B. pseudomallei* resulted in an increase in IL-18 concentrations in lung homogenates (from 44 ± 27 pg/ml to 269 ± 44 pg/ml; *P = 0.0025*) but not in plasma of WT mice at 48 hours after infection; in IL-18 KO mice IL-18 remained undetectable throughout. As a first experiment, WT and IL-18 KO mice were intranasally infected with a lethal dose of *B. pseudomallei* and followed until death. IL-18 deficiency had a negative effect on survival. Whereas all WT mice were dead after 123 hours (median survival time 90 hours), all IL-18 KO mice had died within 87 hours (median survival time 79 hours; *P = 0.0052* for the difference between groups; Figure 3A).

IL-18 KO mice display an enhanced bacterial load of *B. pseudomallei*

To obtain an insight into the mechanisms underlying the accelerated mortality of IL-18 KO mice during experimental melioidosis, we infected WT and IL-18 KO mice with *B. pseudomallei* and sacrificed them after 24 and 48 hours (i.e. before the first deaths occurred) to determine

Fig. 3. Survival and bacterial outgrowth in IL-18 KO mice infected with *B. pseudomallei* compared to wildtype (WT) mice. Survival of WT (open squares) and IL-18 KO mice (closed squares) after intranasal inoculation with *B. pseudomallei* (A). Mortality was assessed every four hours (*n = 12* per group; *p*-value indicates the difference between groups). IL-18 KO mice display strongly increased bacterial loads at 24 and 48 hours after infection with *B. pseudomallei* in the lungs (B) and liver (C) in addition with higher bacterial outgrowth in the spleen (D) and blood (E). Data are mean ± SEM (*n = 8* per group). WT mice (open bars), IL-18 KO mice (black bars). CFU: colony forming unit; ** *p < 0.01*, *** *p < 0.001*.
bacterial loads in lungs (the primary site of the infection), spleen, kidneys, brain and blood so as to evaluate bacterial loads and dissemination to distant body sites (Figure 3B to E). Relative to WT mice, IL-18 KO mice displayed strongly increased bacterial loads in the lungs and liver at 24 and 48 hours after infection, as well as in blood, kidneys (data not shown) and spleen at 48 hours (Figure 3B to E). There was no bacterial growth in the brains of either WT or IL-18 KO mice (data not shown).

**Lung histology and distant organ injury**

To further evaluate the role of IL-18 in the early antibacterial defense against *B. pseudomallei*, histological samples of lung, spleen, liver, kidney and brain obtained 24 and 48 hours after infection, were semi-quantitatively scored on the extent of inflammation. Pulmonary inflammation was characterized by significant inflammation, pleuritis, peribronchial inflammation, oedema and endothelialitis in both WT and IL-18 KO mice (Figure 4). Lung inflammation scores were similar at both 24 and 48 hours after infection in both mice strains (data not shown). In contrast, livers of IL-18 KO mice showed significantly more inflammation after 48 hours compared to WT mice after inoculation with *B. pseudomallei* (Figure 5A to C). Consistent with these pathology data, the plasma levels of ASAT were higher in IL-18 KO mice 48 hours post infection, reflecting increased hepatocellular injury in these animals (P < 0.05 versus WT mice, Figure 5D). In addition,
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**Fig. 5. Liver inflammation in IL-18 KO mice.** Livers of IL-18 KO mice showed more inflammation after 48 hours compared to WT mice after inoculation with *B. pseudomallei*. Representative hematoxilin and eosin stained liver histology slides (original magnification x 4, insets x 20) are shown of WT (A) and IL-18 KO (B) mice at 48 hours after inoculation with 5x10^2 CFU *B. pseudomallei*. Pathology scores (C) are given for 48 hrs after inoculation (means ± SE) as described in the Methods section. At 48 hrs after inoculation IL-18 KO mice also showed enhanced hepatic injury, as reflected by the plasma concentrations of ASAT (D) and a more renal failure, as reflected by plasma creatinine (E) and BUN (F). Data are expressed as mean ± SEM of 8 WT mice (open bars) and 8 IL-18 KO mice (black bars). U/L: units per liter; * p < 0.05 versus WT control.

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IL-18 and IL-6 were measured in lung homogenates. Data are means ± SEM of 8 mice per group per time point. * p < 0.05; ND: not detectable or below detection limit.
IL-18 KO mice showed evidence of renal failure, as indicated by elevated plasma concentrations of urea (P < 0.05 versus WT mice, Figure 5F), whereas the increase in plasma creatinine did not reach significance (Figure 5H). On pathologic examination, renal histology was unremarkable in both IL-18 KO and WT mice. Spleen histology showed mild inflammation that was equally distributed in both mice strains. Moreover, histological examination of the harvested brains revealed no signs of inflammation.

**Enhanced inflammatory cytokine profile in of IL-18 KO mice infected with *B. pseudomallei***

To further examine the impact of IL-18 deficiency on the host response to melioidosis we measured the concentrations of IFN-γ, TNF-α, IL-6 and MCP-1 in lungs and plasma of IL-18 KO and WT mice 24 and 48 hours after infection with *B. pseudomallei* (Table 1). IFN-γ remained undetectable in lung homogenates of either IL-18 KO or WT mice; in plasma, however, WT but not IL-18 KO mice demonstrated elevated IFN-γ levels in plasma. TNF-α concentrations did not differ between the two mouse strains. IL-6 and MCP-1 levels were higher in IL-18 KO mice at 48 hours but not at 24 hours after infection (not significant for IL-6 in plasma).

**DISCUSSION**

In this study we sought to determine the role of IL-18 in the immune response to melioidosis. We demonstrate that the plasma concentrations of IL-18 and IL-18BP are elevated in patients with severe melioidosis and that high IL-18 and IL-18BP levels on admission are correlated with death. In experimental melioidosis induced by intranasal infection with a lethal dose of *B. pseudomallei*, IL-18 KO mice displayed an enhanced bacterial growth in lungs, blood and distant organs, accompanied by increased hepatocellular injury and renal insufficiency, and associated with an accelerated mortality. Together these data indicate that the enhanced production of IL-18 in melioidosis is an essential part of a protective immune response to this severe infection.

The current study builds on an earlier investigation from our group, reporting elevated circulating levels of IL-18 in patients with melioidosis. We here confirm these findings and in addition show that blood monocytes are a source for IL-18. IL-18 mRNA was also detectable in monocyte enriched cell fractions obtained from healthy controls, albeit at lower levels, which is consistent with a previous study showing constitutive IL-18 production by human monocytes in vitro. Circulating IL-18 levels showed a strong positive correlation with IL-18BP. This naturally occurring secreted protein has a high-affinity for binding to IL-18. IL-18BP is not a soluble receptor for IL-18 and is only distantly related to the cell-associated IL-18 receptor. IL-18BP exists as 4 isoforms (IL-18BPa,b,c,d) of which only IL-18BPa (measured in the present study) and IL-18BPC are able to neutralize IL-18. Both IL-18 and IL-18BP levels were higher in patients who eventually died and decreased in survivors after successful treatment. Our data are in line with other studies on IL-18 and IL-18BP performed in septic patients. In a heterogeneous group of septic patients IL-18 and IL-18BPa levels were both significantly elevated. At these observed high
levels, most IL-18 was bound to IL-18BP; however, the remaining free IL-18 was still higher than in healthy individuals. Furthermore, patients with septic shock who did not survive displayed higher IL-18 levels than patients who survived. In addition, in a small cohort of 13 patients with sepsis IL-18 levels correlated significantly with APACHE II scores.

One previous investigation studied the role of IL-18 in experimental melioidosis. In a recent study by Haque et al. focusing on the role of T cells in the immune response against *B. pseudomallei*, blockade of the IL-18 receptor in a mouse model resulted in a decreased survival after intraperitoneal injection of *B. pseudomallei*; the effect of IL-18 inhibition on bacterial growth and the host inflammatory response was not investigated. Humans usually acquire melioidosis by inoculation through skin abrasions or inhalation. Pneumonia with bacterial dissemination to distant body sites is a common presentation of melioidosis. We therefore used a model of melioidosis in which mice were infected with *B. pseudomallei* via the airways. In this model we found a strong protective role for IL-18: IL-18 KO mice were unable to control the infection, which resulted in increased distant organ injury and early mortality. The bacterial loads in particularly increased in the livers of IL-18 KO mice between 24 and 48 hours after infection, which was accompanied with significant pathology in this organ. The increased hepatocellular injury observed in IL-18 KO mice is in line with an earlier study from our laboratory, showing a similar response of these animals during abdominal sepsis caused by *Escherichia coli*. Of note, IL-18 KO mice had biochemical evidence of renal insufficiency, in particular elevated plasma urea concentrations, without histological changes in their kidneys, suggesting a pre-renal cause of the diminished kidney function.

IFN-γ release into the circulation was completely abrogated in IL-18 KO mice, indicating that IL-18 is an important factor in IFN-γ production induced by *B. pseudomallei*. In accordance, our group previously showed that the addition of anti-IL-18 to whole blood stimulated with heat-killed *B. pseudomallei* reduced IFNγ levels. Moreover, treatment with anti-IL-18 receptor antibody reduced the number of IFN-γ producing T and natural killer cells in mice injected with *B. pseudomallei* intraperitoneally. The protective role of IL-18 can be explained at least in part by reduced IFN-γ production, considering that elimination of IFN-γ rendered mice more susceptible to intraperitoneal infection with *B. pseudomallei*.

The present study adds to several previous investigations addressing the role of IL-18 in host defense against Gram-negative bacterial infection *in vivo*. Administration of anti-IL-18 to mice intravenously infected with *Salmonella typhimurium* resulted in enhanced bacterial growth in liver and spleen, similar to what was found here after infection with *Burkholderia*. In line, anti-IL-18 treatment during *Yersinia enterocolitica* infection was associated with a relatively enhanced growth of bacteria. In addition, IL-18 deficiency facilitated bacterial growth after intranasal infection with *Shigella flexneri* and intraperitoneal infection with *Escherichia coli*. IL-18 also contributed to an effective host defense against Gram-positive infection, including systemic infection with *Listeria monocytogenes* and pneumonia caused by *Streptococcus pneumoniae*. Remarkably, however, IL-18 deficiency was associated with an enhanced clearance of *Pseudomonas aeruginosa* from mouse lungs, and a diminished dissemination of the infection. In this respect it should be...
noted that although *Pseudomonas* and *Burkholderia* share some common features, the diseases induced by intranasal infection of mice with either pathogen markedly differ. Indeed, *Pseudomonas* is cleared by immunocompetent mice \(^{24,25}\), whereas *Burkholderia* exponentially grows. Moreover, whereas experimental *Pseudomonas* pneumonia is associated with acute illness and acute lung inflammation, disease induced by *Burkholderia* develops more gradually, eventually resulting in disseminated abscess formation characteristic of human melioidosis. The differences are further illustrated by the fact that endogenous IFN-\(\gamma\), like IL-18, impaired host defense against experimentally induced *Pseudomonas* pneumonia \(^{25}\). In addition, Remick *et al* demonstrated that inhibition of IL-18 in septic peritonitis induced by cecal ligation and puncture had a variable effect on outcome dependent on the severity of the initial inflammatory response; IL-18 inhibition decreased mortality rates in mice with an increased risk of dying, but increased lethality in those mice with a predicted low mortality rate \(^{26}\). In acute shock produced by administration of LPS, IL-18 KO mice tolerated a 50% higher LPS dose than WT mice \(^{27}\), and treatment with an anti-IL-18 antiserum protected mice against the lethal effects of both *E. coli* and *Salmonella* LPS \(^{28}\). Taken together these findings are consistent with the concept of proinflammatory cytokines, like IL-18, acting as double-edged swords; inhibition of their activity during exaggerated inflammation in acute overwhelming infection or shock is beneficial for the host but their inhibition during more gradual infection facilitates bacterial growth \(^{29}\). The present investigations clearly identify IL-18 as a protective mediator during melioidosis by limiting replication and dissemination of *B. pseudomallei*.

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