Modulation of *Pseudomonas aeruginosa* lipopolysaccharide-induced lung inflammation by chronic iron overload in rat

Bà Vuong Lê, Hafida Khorsi-Cauet, Véronique Bach & Jérôme Gay-Quéhéillard

Peritox Laboratory EA4285-UMI 01, Faculty of Medicine, Picardy Jules Verne University, Amiens, France

**Abstract**

Iron constitutes a critical nutrient source for bacterial growth, so iron overload is a risk factor for bacterial infections. This study aimed at investigating the role of iron overload in modulating bacterial endotoxin-induced lung inflammation. Weaning male Wistar rats were intraperitoneally injected with saline or iron sucrose [15 mg kg\(^{-1}\) body weight (bw), 3 times per week, 4 weeks]. They were then intratracheally injected with *Pseudomonas aeruginosa* lipopolysaccharide (LPS) (5 \(\mu\)g kg\(^{-1}\) bw) or saline. Inflammatory indices were evaluated 4 or 18 h post-LPS/saline injection. At 4 h, LPS-treated groups revealed significant increases in the majority of inflammatory parameters (LPS-binding protein (LBP), immune cell recruitment, inflammatory cytokine synthesis, myeloperoxidase activity, and alteration of alveolar-capillary permeability), as compared with control groups. At 18 h, these parameters reduced strongly with the exception for LBP content and interleukin (IL)-10. In parallel, iron acted as a modulator of immune cell recruitment; LBP, tumor necrosis factor-\(\alpha\), cytokine-induced neutrophil chemoattractant 3, and IL-10 synthesis; and alveolar-capillary permeability. Therefore, *P. aeruginosa* LPS may only act as an acute lung inflammatory molecule, and iron overload may modulate lung inflammation by enhancing different inflammatory parameters. Thus, therapy for iron overload may be a novel and efficacious approach for the prevention and treatment of bacterial lung inflammations.

**Introduction**

Iron is an essential mineral required for life but excess iron can be toxic, causing oxidative stress-dependent tissue and cell damage. Furthermore, it plays a critical role as a nutrient source for bacterial growth, leading to a competition between host and microorganisms (Marx, 2002; Doherty, 2007). It has already been demonstrated that iron was a risk factor for microbial infection in patients with acquired iron overload (Waterlot et al., 1985; Sunder-Plassmann et al., 1999). Indeed, this metal has been identified as an immunomodulatory agent that could affect macrophages, neutrophils, and T-cell function and production of cytokines such as tumor necrosis factor (TNF)-\(\alpha\), interleukin (IL)-6, IL-12, and interferon-\(\gamma\) (IFN-\(\gamma\)) (Olynyk & Clarke, 2001; Ward et al., 2002; Purohit et al., 2003; Wang et al., 2009). Then, manipulation of body iron homeostasis has been suggested as a new therapeutic approach for inflammation control (Wang et al., 2009).

*Pseudomonas aeruginosa* is a ubiquitous gram-negative bacterium, which plays an important role in the pathogenesis of the cystic fibrosis lung disease in humans, especially in young children who have been demonstrated to be at risk of early *P. aeruginosa* colonization (McKay et al., 2009). A study has reported a high prevalence (83%) of *P. aeruginosa* in Brazilian pediatric patients with cystic fibrosis under 2 years of age (Santana et al., 2003). However, only one case report revealed the ability of iron to facilitate *P. aeruginosa* lung infection in adult patients (Berlutti et al., 2005). Thus, the impact of iron overload on *P. aeruginosa*-induced lung inflammation requires further studies in young subjects. As high frequencies of resistance of *P. aeruginosa* to antibiotic therapy have been reported (Rosenthal et al., 2008), therapies directed against its virulence factors has been suggested as
potential novel approaches to improve outcomes in P. aeruginosa infections (Veesenmeyer et al., 2009). Moreover, Ramphal and coworkers have suggested that the control of P. aeruginosa in the lung required the recognition of its lipopolysaccharide (LPS) (Ramphal et al., 2008). Therefore, in our study, we decided to use P. aeruginosa LPS to induce lung inflammation in healthy and iron sucrose-loaded rats instead of living P. aeruginosa. In a preliminary study, we have evaluated two different lung inflammatory models using two different doses of P. aeruginosa LPS (5 vs. 20 μg kg⁻¹ bw). The first dose has been applied in a study of the effect of LPS on lung epithelial permeability (Eutamène et al., 2005). We confirmed by different parameters its efficacy to induce lung inflammation and used it in this work. The fourfold higher dose that caused death of animals in all experimental groups was determined as a lethal dose and could not be used.

Iron sucrose is widely used in anemic patients with chronic kidney disease, especially in children whose oral iron therapy has failed (Pinsk et al., 2008). Nevertheless, a study of hemodialysis patients under intravenous iron sucrose therapy showed that risk of infection was dependent mainly on the amount of administered iron and pulmonary infection was the most frequent event observed (Canziani et al., 2001). Furthermore, several studies have demonstrated the ferric iron (Fe³⁺) accumulation in different organs of rats given intraperitoneal injections of iron sucrose (Legssyer et al., 2003; Brbroowicz et al., 2006). In a previous study, we have established four models of iron overload in weaning male Wistar rats using iron sucrose (Lê et al., 2011). We demonstrated that chronic intraperitoneal administrations of iron sucrose at two different doses of 15 and 75 mg kg⁻¹ bw (3 times per week, 4 weeks) could induce iron overload. The dose of 15 mg kg⁻¹ bw in rat corresponding to 3.6 mg kg⁻¹ bw in child was calculated and obtained using the formula for dose translation based on body surface area (Reagan-Shaw et al., 2008). It is slightly higher than that of 3 mg kg⁻¹ bw recommended for the prevention of iron deficiency in children (Schröder, 2003). So we decided to use this dose of iron for this study to reflect the possible risks of iron overload associated with bacterial infections.

The overall objective of this work was to investigate the effects of iron sucrose-induced chronic iron overload on P. aeruginosa LPS-induced lung inflammation in weaning male Wistar rats. To examine this question, we evaluated the role of iron as a modulator of recruitment of immune cells, cytokine production, and lung injury during inflammatory process. We further assessed the evolution of LPS-mediated lung inflammatory response in a context of iron overload.

**Material and methods**

**Animals**

Male Wistar rats (Janvier, Le Genest St Isle, France) were housed in the animal care facility (four rats per cage), randomly assigned in each experimental group (6–8 animals per group):

1. S/S groups: saline.
2. I/S groups: iron.
3. S/L group: saline + LPS.
4. I/L group: iron + LPS.

Animals were allowed to acclimatize for 5 days prior to the start of the experimental procedure and were 4 weeks old at the beginning of this study. They were provided a 12:12-h light/dark cycle and given ad libitum pelleted chow and tap water. All animal experimental procedures were approved by the Animal Care and Use Committee of the Picardy Jules Verne University (Amiens, France).

**Iron overload induction**

Half of the animals (I/S and I/L groups) were given intraperitoneal injections of iron sucrose (Venofer®) (Vifor France SA, Levallois Perret, France) (15 mg kg⁻¹ bw, 3 times per week) for 4 consecutive weeks. The others (S/S and S/L groups) were given injections of equivalent volumes of sterile saline (0.9% NaCl) (Aguettant SAS, Lyon, France).

**Lung inflammation induction**

At the beginning of the fifth week of experiments, animals in S/S, S/L, I/S, and I/L (two groups of each) were anesthetized with ketamine/xylazine (80 : 20 mg kg⁻¹ bw, i.p) (Sigma Aldrich, St-Quentin Fallavier, France), and then, the tracheotomy was carried out. Animals in S/L and I/L groups (two groups of each) were exposed to intratracheal instillation of LPS from P. aeruginosa (5 μg kg⁻¹ bw) (Sigma Aldrich) at a constant rate of 10 μL per min for 10 min. The incision was sutured, and the animals were returned to their cages. Animals in S/S and I/S groups (two groups of each) were given saline in place of LPS and were used as controls. Euthanasia was carried out 4 h (four groups: S/S, S/L, I/S, and I/L) and 18 h (four remaining groups) after LPS instillation, and blood was drawn in heparin-coated tubes (Laboratoires Terumo France SA, Guyancourt, France). Plasma was separated by centrifugation (3640 g, 10 min, 4 °C) and stored at −70 °C for LPS-binding protein (LBP) and cytokine measurements. Lung tissues were harvested and stored at −70 °C until use.
Iron overload modulates LPS-induced lung inflammation

**Bronchoalveolar lavage**

Bronchoalveolar lavage was performed by instillation of 15 mL of PBS (Sigma Aldrich). The first 2 mL instilled into the lungs were collected and centrifuged at 1560 g for 10 min. The supernatant was kept and frozen at −70 °C for cytokine measurements. The last volume was used for bronchoalveolar lavage fluid (BALF) cell counts. For differential BALF cell counts, cytospin preparations were stained with Hemacolor Rapid staining set (Merck KGaA, Darmstadt, Germany). Cells were counted and classified as polymorphonuclear neutrophils (PMNs) or macrophages based on morphological criteria using oil immersion microscopy (Olympus France SAS, Rungis, France).

**Enzyme immunoassay assay**

The concentrations of TNF-α, cytokine-induced neutrophil chemoattractant (CINC-3), IFN-γ, IL-1β, IL-6, and IL-10 (R&D Systems, Inc., Minneapolis, MN) in the BALF and plasma, the concentration of IL-23 (Cusabio Biotech Co., Ltd, Newark, DE) in the BALF as well as the concentration of LBP (Biometec GmbH, Greifswald, Germany) in the plasma were determined by ELISA using commercially available kits according to the manufacturer’s instructions. The results were calculated and expressed as picogram of cytokine per milliliter of BALF (pg mL−1), microgram of LBP per milliliter of serum (μg mL−1).

**RNA isolation, cDNA synthesis, and real-time quantitative (RT-PCR) analysis**

Total RNA was extracted from lung tissues (≈ 30 mg) using Qiagen RNeasy mini kit (Qiagen SA, Courtaboeuf, France) according to the manufacturer’s instructions. The amount of mRNA was determined by spectrophotometry using an Eppendorf Biophotometer (Eppendorf AG, Le Pecq, France). cDNA was generated by reverse transcription of 2 μg RNA using the high-capacity cDNA reverse transcription kit containing RNase inhibitor (Applied Biosystems, Inc., Courtaboeuf, France) according to the manufacturer’s instructions.

The RT-PCR was run on 96-well PCR microplates (Applied Biosystems, Inc.) containing 9 μL diluted cDNA, 1 μL Taqman gene expression assay primers (Table 1) (Applied Biosystems, Inc.), and 10 μL Taqman gene master mix (Applied Biosystems, Inc.) for a total of 20 μL per sample per well. PCR was run on an Applied Biosystems 7900 thermal cycler following the instructions protocol.

The cycle number at the linear amplification threshold (Ct) of the endogenous control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (Table 1) (Applied Biosystems, Inc.), and the target gene were recorded. Relative gene expression (the amount of target, normalized to the endogenous control gene) was calculated and given as 2−ΔΔCt (Livak & Schmittgen, 2001).

**Lung MPO activity**

The activity of the enzyme myeloperoxidase (MPO), a marker of polymorphonuclear primary granules, was determined in a lung fragment after pulmonary lavage according to Gay and coworkers (Gay et al., 2000). Lung protein concentration was determined using a DC protein assay kit (Bio-Rad Laboratories, Inc., Marne-la-Coquette, France). MPO activity was expressed as units (μ) of MPO activity per gram of protein (μ g−1), whereby one unit was defined as the quantity of enzyme able to convert 1 μmol of H2O2 to water in 1 min at room temperature.

**BALF iron content**

The BALF iron content was determined by a method based on the use of ferrozine. This method involves the use of an iron-releasing reagent (1.4 M HCl and 4.5% w/v KMnO4), which releases iron from proteins in biologic samples (Riemer et al., 2004). The iron content was calculated by comparing its absorbance to that of a range of standard concentrations as previously described (Rebouche et al., 2004) and expressed as microgram of iron content per milligram of protein (μg mg−1 protein).

**Alveolar-capillary permeability**

Eight remaining groups (S/S, S/L, I/S, and I/L, two groups of each) were assigned to the study of alveolar-capillary permeability. As previously described, the animals were instilled with LPS or sterile saline in the same conditions. Animals were subjected to intravenous injections of Evans blue dye (EBD) (Sigma Aldrich) at a dose of 30 mg kg−1 bw 1 h before euthanasia. After sacrifice, the lungs were incised and infused with 20 mL of sterile saline via the main pulmonary artery and lavaged to wash out residual blood and EBD (Tsuji et al., 1998). The

---

**Table 1. Taqman gene expression assay primers**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene symbol</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>Tnf</td>
<td>Rn99999017_m1</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Il1b</td>
<td>Rn99999009_m1</td>
</tr>
<tr>
<td>CINC-3</td>
<td>Cxcl2</td>
<td>Rn01414104_g1</td>
</tr>
<tr>
<td>IL-6</td>
<td>Il6</td>
<td>Rn99999011_m1</td>
</tr>
<tr>
<td>IL-10</td>
<td>Il10</td>
<td>Rn00563409_m1</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Gapdh</td>
<td>Rn9999916_s1</td>
</tr>
</tbody>
</table>
lungs and heart were then removed en bloc, and the left lung was used for EBD assay. Details of the EBD assay have been described previously (Narita et al., 2004). The EBD content was calculated and expressed as μg of EBD per g of wet tissue.

**Data analysis**

Data corresponding to the time 4 h post-LPS instillation were analyzed separately to those corresponding to the time 18 h. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Fisher LCD post hoc test using SIGMASTAT version 3.5 (Systat Software Inc., Chicago, IL). Data were expressed as mean ± SEM, and probability (P) < 0.05 was considered statistically significant.

**Results**

**Effect of iron on LBP synthesis**

We observed a significant increase in serum LBP content in I/S compared with S/S group at both 4 and 18 h. LBP content was also significantly higher in S/L compared with S/S group at both 4 and 18 h. However, I/L group only revealed significant increase in LBP content, as compared with I/S group 18 h post-LPS instillation (Fig. 1).

**LPS-induced PMN and macrophage recruitment**

Significantly higher number of BALF PMNs was observed in S/L group compared with S/S group and in I/L group compared with I/S group both 4 and 18 h post-LPS instillation although S/L and I/L groups revealed strongly decreases in BALF PMN number at 18 h. No significant difference in BALF PMN number between S/L and I/L group was observed at 4 h. However, we found that BALF PMN number was significantly higher in S/L group than I/L group at 18 h (Fig. 2a).

Absence of macrophages in the BALF was observed in all experimental groups at 4 h. However, cell counts revealed significant increases in BALF macrophage number in S/L group compared with S/S group and I/L group compared with I/S group at 18 h. (Fig. 2b).

**LPS-induced inflammatory cytokine synthesis**

All cytokine concentrations were not detectable in the plasma samples of all experimental groups. IFN-γ activity...
revealed no significant difference between experimental groups during LPS treatment. All measured cytokines revealed significantly higher levels in S/L group compared with S/S group and I/L group compared with I/S group at 4 h. Only IL-10 showed significantly higher levels in S/L group compared with S/S group and I/L group compared with I/S group (Fig. 3f), and TNF-α revealed significantly higher level in S/L compared with S/S group at 18 h (Fig. 3a). In parallel, we observed a significant reduction of TNF-α level in I/L group compared with S/L group at 4 h.

**Effect of iron on TNF-α, CINC-3, and IL-10 mRNA expressions in the lung tissue**

Significant increases in TNF-α, CINC-3, IL-1β, IL-6, and IL-10 mRNA expressions were observed in S/L group compared with S/S group and I/L group compared with I/S group at 4 h (Fig. 4a–d). At 18 h, TNF-α, IL-1β, and IL-10 revealed significantly higher upregulations in S/L compared with S/S group (Fig. 4a, c and e), whereas CINC-3 and IL-1β revealed significantly higher upregulations in I/L compared with I/S group (Fig. 4b and c). Furthermore, we found a significant decrease in TNF-α mRNA expression in I/L compared with S/L group at 18 h (Fig. 4a).

**Lung MPO activity**

We observed significant increases in MPO activity in S/L group compared with S/S group and I/L group compared with I/S group at 4 h. By contrast, no significant difference between all experimental groups was found at 18 h (Fig. 5).

---

**Fig. 3.** Concentrations of (a) TNF-α, (b) CINC-3, (c) IL-23, (d) IL-1β, (e) IL-6, and (f) IL-10 in the BALF. BALF and plasma cytokine levels were determined by ELISA. S/S: control animals; I/S: iron-loaded animals; S/L: inflamed animals; I/S: inflamed iron-loaded animals. Values are mean ± SEM for 6–8 animals in each group (*P < 0.05; **P < 0.01 and ***P < 0.001).
BALF iron content was significantly increased in S/L group compared with S/S group and I/L group compared with I/S group at both 4 and 18 h. At 4 h, BALF iron content in S/L group is significantly higher than in I/L group. However, no significant difference in BALF iron content between S/L and I/L groups was found at 18 h (Fig. 6).

**Effect of iron on alveolar-capillary permeability**

The lung EBD content, marker for alveolar-capillary permeability, was significantly increased in S/L group compared with S/S group and I/L group compared with I/S group at both 4 and 18 h. At 4 h, BALF iron content in S/L group is significantly higher than in I/L group. However, no significant difference in BALF iron content between S/L and I/L groups was found at 18 h (Fig. 6).

---

**Fig. 4.** Expressions of (a) TNF-α, (b) CINC-3, (c) IL-1β, (d) IL-6, and (e) IL-10 mRNA in the lung homogenates. Lung tissues were collected 4 or 18 h post-LPS instillation. RT-PCR was carried out to evaluate expression of cytokines and chemokine genes. Results were calculated and expressed as $2^{-\Delta\Delta C_T}$. S/S: control animals; I/S: iron-loaded animals; S/L: inflamed animals; I/S: inflamed iron-loaded animals. Values are mean ± SEM for 6–8 animals in each group (*P < 0.05; **P < 0.01 and ***P < 0.001).

**Fig. 5.** Lung MPO activity. The MPO activity was determined in the homogenates of lung tissues. S/S: control animals; I/S: iron-loaded animals; S/L: inflamed animals; I/S: inflamed iron-loaded animals. Values are mean ± SEM for 6–8 animals in each group (**P < 0.01 and ***P < 0.001).
groups at 4 h. Significantly higher level of EBD was observed in I/L compared with I/S group, whereas no significant difference between S/L and S/S groups was found at 18 h (Fig. 7).

Discussion

*P. aeruginosa* LPS is not widely used to induce experimental lung inflammation in animals. Moreover, inflammatory status was always examined after a short incubation period, which was not able to determine whether *P. aeruginosa* LPS-induced lung inflammation could persist for a long time. In this study, we have evaluated inflammatory status 4 and 18 h post-LPS instillation to confirm the ability of this LPS to induce acute lung inflammation and determine whether it could induce chronic lung inflammation. Most inflammatory parameters revealed a significant increase 4 h after LPS instillation (S/L vs. S/S groups), with exception for macrophage recruitment and IL-10 gene expression. Our results are consolidated by a previous study, which showed significant increases in immune cell recruitment and MPO activity as well as alveolar-capillary hyperpermeability resulting from LPS treatment at 4 h (Eutamène et al., 2005). We therefore confirm the ability of *P. aeruginosa* LPS to induce acute lung inflammation in a rat model.

At 18 h, we observed significant increases in PMN recruitment; BALF iron content; LBP, TNF-α, IL-1β, and IL-10 synthesis in S/L group compared with S/S group, whereas other inflammatory parameters did not reveal significant difference between S/L and S/S groups. Although BALF PMN number was still significantly higher in S/L group than S/S group at 18 h, it decreased strongly as compared with that at 4 h. LBP is well recognized as a transporter protein of LPS, which permits the induction of inflammatory reaction. Therefore, significant increase in LBP content because of LPS treatment was accompanied by significant increases in inflammatory parameters at 4 h. Paradoxically, LBP content was still significantly higher in S/L group compared with S/S group at 18 h, whereas the majority of inflammatory parameters revealed no significant difference between S/L and S/S groups. Thus, LBP might play its second anti-inflammatory role at 18 h. Indeed, it has been demonstrated that LBP could neutralize LPS by transferring it to other plasma lipoproteins or by inhibiting monocyte response to LPS (Thompson et al., 2003). TNF-α is a potent proinflammatory cytokine implicated in the initiation and progression of inflammation, but its persistence is not maintained for a long time. A study carried out in mice demonstrated that significant increase in TNF-α production was only observed at 1.5–6 h post-LPS instillation (Snyder et al., 2010). In this study, TNF-α concentration was still significantly higher in S/L group compared with S/S group at 18 h. We therefore suggest that TNF-α might act as an anti-inflammatory cytokine rather than proinflammatory cytokine at 18 h. This hypothesis is consolidated by two studies that reported the novel anti-inflammatory role of TNF-α (Zakharova & Ziegler, 2005; Masli & Turpie, 2009). Furthermore, IL-10, a main anti-inflammatory cytokine, revealed a significantly higher concentration in S/L group than S/S group. In brief, S/L group did not reveal a significant difference in most inflammatory parameters compared with S/S group at 18 h. In parallel, three parameters including TNF-α, LBP, and IL-10 acted as anti-inflammatory factors, indicating a healing phase. In other words, *P. aeruginosa* LPS might only induce an acute lung inflammation, at least in a rat model.
In this study, we showed for the first time an elevation of LBP synthesis associated with iron overload (I/S vs. S/S groups). Other inflammatory parameters did not reveal significant difference between I/S and S/S groups, indicating the absence of inflammatory reaction. These data confirm the result of our previous study, which has demonstrated that iron sucrose-induced chronic iron overload could not induce inflammation (Lê et al., 2011). LBP has been accepted to be synthesized in the liver, lung, and small intestine (Dentener et al., 2006; Hansen et al., 2009). Moreover, our previous study has reported iron accumulation because of chronic iron sucrose supply in the liver and small intestine (Lê et al., 2011). We therefore hypothesize that iron accumulation in the liver and small intestine alters and enhances the production of LBP. As presented earlier, LBP mainly acts as a specific transporter of LPS, and this bacterial endotoxin must primarily stimulate LBP production to launch inflammation reactions. Therefore, if iron-stimulated LBP synthesis is sufficient for the transfer of LPS, inflammatory reactions could be launched earlier. In other words, iron overload might accelerate LPS-induced inflammatory responses.

PMNs are sources of lung cytokines during inflammatory processes because the majority of BALF immune cells were PMNs (65–85%) (Nelson et al., 1989; Eutamène et al., 2005). However, no coherence between BALF PMN number, BALF iron content, and lung MPO activity was observed. At 4 h, I/L group revealed a significantly lower BALF iron content as compared with S/L group, whereas no significant difference in BALF PMN number between I/L and S/L group was found, and the presence of macrophages was not detectable. We therefore suggest that iron overload might exert an inhibitory effect on the recruitment of other cell type rather than PMNs or macrophages at 4 h. At 18 h, we observed a significantly lower BALF PMN number in I/L group compared with S/L group, indicating the inhibitory effect of iron overload on PMN recruitment at 18 h. However, no significant difference in MPO activity between I/L and S/L groups was found at 18 h although MPO is generally considered as a marker of PMN activation (Faith et al., 2008). We suggest that not only PMNs but other cell types such as macrophages (Paquet et al., 2010) may also be involved in the production of MPO in the healing phase of inflammation.

We also detected that chronic iron overload could modulate the synthesis of several inflammatory cytokines and enhance alveolar-capillary permeability. As presented earlier, TNF-α acted as a proinflammatory cytokine 4 h post-LPS instillation but exerted an anti-inflammatory role at 18 h. At 4 h, we observed a significantly lower concentration of BALF TNF-α in I/L group compared with S/L group, indicating that iron overload might inhibit proinflammatory effect of TNF-α in the acute phase of inflammation. At 18 h, BALF TNF-α concentration and TNF-α mRNA expression did not reveal significant difference between I/L and I/S groups, whereas a significant difference in these two parameters between S/L and S/S groups was observed. Moreover, TNF-α mRNA expression was significantly lower in I/L group compared with S/L group at 18 h. These findings permit us to suggest that iron overload might inhibit anti-inflammatory effect of TNF-α in the healing phase of inflammation. We also detected that iron overload could inhibit anti-inflammatory effect of IL-10 in the healing phase. Indeed, we did not observe significant difference in IL-10 mRNA expression between I/L and I/S groups at 18 h, whereas S/L group revealed a significant increase in IL-10 mRNA expression in S/L group compared with S/S group. At 18 h, no significant difference in CINC-3 mRNA expression and lung EBD content between S/L and S/S groups was found. By contrast, a significant increase in CINC-3 mRNA expression and lung EBD content was observed in I/L group as compared with I/S group. CINC-3 is well recognized as a chemokine required for attraction of PMNs (Wolpe et al., 1988) and as a consequent, for starting inflammatory responses. Thus, iron overload might potentize proinflammatory effect of CINC-3 and slow down the restoration of alveolar-capillary permeability in the healing phase of inflammation.

In summary, we show for the first time the ability of P. aeruginosa LPS to induce only acute lung inflammation in a rat model. We confirm the role of iron as an immunomodulatory factor that affects TNF-α synthesis. We also demonstrate that iron overload, in the healing phase of inflammation, can slow down the restoration of altered alveolar-capillary permeability, inhibit PMN recruitment and anti-inflammatory effect of TNF-α and IL-10, and potentize proinflammatory effect of CINC-3. In parallel, we suggest for the first time other function of iron as an accelerator of LPS-dependent immune response by increasing LBP production. Thus, iron sucrose-induced iron overload might aggravate P. aeruginosa LPS-induced lung inflammation. Moreover, LPS is required for the control of P. aeruginosa in the lung (Ramphal et al., 2008). Thus, anemic patients under iron sucrose therapy are perhaps populations at high risk of P. aeruginosa infections because they also reveal high risk of iron overload. Recently, therapies directed against P. aeruginosa virulence factors (e.g., LPS, flagellin, exotoxin A) have been suggested to be potential novel approaches to improve outcomes of P. aeruginosa infections because of high frequencies of resistance of P. aeruginosa to antibiotic therapy (Veessenmeyer et al., 2009). These findings permit us to suggest that therapy for iron overload and maintenance of body iron homeostasis may be
novel and efficacious approaches for the prevention and treatment of *P. aeruginosa* infections.

**Competing and financial interests**
The authors declare that they have no competing and financial interests.

**Authors’ contributions**
B.V.L. participated in the design of the study, collected the study material, carried out ELISA, spectrophotometric, histological, and statistical analyses, and drafted the manuscript. H.K.C. helped to draft the manuscript. V.B. helped to perform the statistical analysis and draft the manuscript. J.G.Q. is the head of the project, participated in the design of the study, collected the study material, carried out ELISA, RT-PCR, and histological analyses, and helped to draft the manuscript. All authors have read and approved the final manuscript.

**Acknowledgements**
The authors thank Picardy Regional Council (France) for financial support in this work and a doctoral award for B.V.L.

**References**


