Infectious diseases and fibrotic disorders: Potential novel targets
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Citation for published version (APA):

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CCAAT-Enhancer Binding Protein Delta (C/EBPδ) protects against *Klebsiella pneumoniae*–induced pulmonary infection: potential role for macrophage migration

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The Journal of Infectious Diseases
2012;\textbf{206}:1826-1835
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ABSTRACT
Mounting evidence suggests an important role for CCAAT-enhancer binding protein delta (C/EBPδ) in the acute-phase response after bacterial infection. However, whether C/EBPδ limits pneumonia remains elusive and is the aim of this study. Therefore, bacterial outgrowth, inflammatory responses, inflammatory cell influx, and survival were assessed in wildtype and C/EBPδ−/− mice infected with Klebsiella pneumoniae via the airways. We showed that C/EBPδ expression is highly induced in the lung during pulmonary infection and that Klebsiella-induced mortality was significantly increased among C/EBPδ−/− mice. Bacterial loads and inflammatory responses were similar in wildtype and C/EBPδ−/− mice early during infection, whereas bacterial loads were increased in C/EBPδ−/− mice later during infection. Moreover, macrophage numbers were reduced in lungs of C/EBPδ−/− mice. In vitro experiments showed that C/EBPδ only slightly affects macrophage function. Our data thus show that C/EBPδ contributes to host defense against Klebsiella-induced pneumonia and suggests that C/EBPδ-dependent macrophage mobilization is a key mechanism.

INTRODUCTION
Pneumonia is a common and serious disease that is a major cause of morbidity and mortality. The frequently isolated causative pathogen Klebsiella pneumoniae is one of the most common hospital-derived pathogens found in lower respiratory tract infections.1–3 The increasing amount of pathogens resistant to antibiotics urges the need of extending our knowledge on the pathogenesis of pneumonia to develop new therapies.1,4 CCAAT-enhancer binding protein delta (C/EBPδ), also known as NF-IL6β, CRP3, CELF, or Rcc/EBP2, is a member of the C/EBP family of transcription factors, which contains 6 unique members: C/EBPα, C/EBPβ, C/EBPδ, C/EBPγ, C/EBPγ, and C/EBPζ. All these members consist of an N-terminal transactivation domain, a basic DNA binding domain, and a C-terminal leucine zipper domain that allows homo- or hetero-dimerization of the different members.5 Expression of C/EBPδ is typically low in most cell types but is rapidly induced by a variety of extracellular stimuli, such as interleukin (IL)-1, IL-6, lipopolysaccharide (LPS), and tumor necrosis factor (TNF)-α, in myeloid cells and lung tissue (among others).6–8 C/EBPδ seems to be an important regulator of proinflammatory TNF-α, IL-6, and monocyte chemoattractant protein (MCP)-1 production in bone marrow-derived macrophages9,10, and several C/EBP-binding motifs have been identified in the regulatory regions of various inflammatory genes, including those encoding the cytokines IL-6, MCP-1, and TNF-α11–13 and genes encoding proteins important for macrophage function, such as iNOS.14 Moreover, C/EBPδ expression levels are correlated with increased expression levels of proinflammatory cyto-
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kines in LPS-stimulated HUVECs\textsuperscript{15}, and C/EBPδ induces cyclooxygenase-2 (COX-2) expression in chondrocytes and epithelial carcinoma cells.\textsuperscript{16,17} Recently, an important role of C/EBPδ in innate immunity has been established by showing that C/EBPδ\textsuperscript{−/−} mice are highly susceptible to *Escherichia coli*-induced peritonitis. Indeed, Litvak et al\textsuperscript{18} show a clear role for C/EBPδ in a persistent bacterial infection as amplifier of the inflammatory response that is essential for bacterial elimination and survival. On the contrary, C/EBPδ\textsuperscript{−/−} animals had a prolonged survival time, compared with wildtype mice in a double-hit LPS model for disseminated intravascular coagulation, the Shwartzman model.\textsuperscript{19} These on-first-sight contradictive results clearly indicate that the role of C/EBPδ in infectious disease is complex and not fully elucidated yet.

In the current study, we aimed to determine the relevance of C/EBPδ in a murine model of *Klebsiella*-induced pneumonia, mimicking the clinical scenario of a gradually growing bacterial load at the primary site of infection, followed by dissemination and distant organ injury. To this end, we subjected wildtype and C/EBPδ-deficient mice to *Klebsiella*-induced pulmonary infection and we showed that C/EBPδ serves a protective role. C/EBPδ\textsuperscript{−/−} animals died earlier during infection and showed increased bacterial loads in the lung, blood, and peripheral organs 48 hours after inoculation. Moreover, we showed that neutrophil numbers did not differ between the 2 genotypes, whereas macrophage numbers and function were reduced in C/EBPδ\textsuperscript{−/−} mice.

**METHODS**

**Animals.** C/EBPδ\textsuperscript{−/−} mice, generated as described previously\textsuperscript{20}, were backcrossed at least 10 times to a C57BL/6 background. C57BL/6 wildtype mice were purchased from Charles River. Animals were maintained at the animal facility of the Academic Medical Center Amsterdam with free access to food and water. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam.

**Pneumonia Model.** Pneumonia was induced as described earlier.\textsuperscript{21,22} In brief, mice (8 per group) were anesthetized by inhalation of isoflurane (Abbott Laboratories) and inoculated intranasally with 50 μL saline containing 5 × 10\textsuperscript{4} colony-forming units (CFU) *K. pneumoniae* serotype 2 (ATCC 43816). Control animals received 50 μL of sterile, endotoxin-free saline only. At predefined time points after inoculation, lungs, spleens, kidneys, and blood samples were collected for further analysis. For survival studies, mice (12–14 per group) were monitored for a maximum of 6 days after infection.
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**Bacterial Outgrowth.** Bacterial outgrowth was determined using lung homogenates prepared as described elsewhere.²³

**Organ Damage Markers.** Plasma aspartate aminotransferase, alanine aminotransferase, creatinine, and lactate dehydrogenase levels were determined as described elsewhere.²⁴

**Preparation of Lung Homogenates and Cytokine Measurements.** Lung homogenates were diluted 1:2 in lysis buffer containing 300 mM NaCl, 30 mM tris-(hydroxymethyl)-aminomethane (Tris), 2 mM MgCl₂, 2 mM CaCl₂, 2% Triton X-100 and 4-(2-aminoethyl)benzenesulfonyl fluoride, Na₂EDTA, pepstatin, and leupeptin (all 8 μg mL⁻¹; pH, 7.4) and were incubated on ice for 30 minutes. Homogenates were centrifuged at 1500 × g at 4°C for 15 minutes and stored at -20°C until assays were performed. TNF-α, IL-6, and MCP-1 levels were determined using a cytometric beads array multiplex assay (BD Biosciences) according to the manufacturer’s recommendations. Keratinocyte chemoattractant levels were measured using enzyme-linked immunosorbent assay according to the standard protocol (R&D systems).

**Immunohistochemistry.** Lungs for histology were prepared and analyzed, as described previously.²⁵ In brief, lungs were harvested, fixed in 10% formalin, embedded in paraffin, and cut in 4 μm-thick sections for staining procedures. Paraffin lung sections were stained with hematoxylin and eosin according to routine procedures. To score lung inflammation and damage, the lung sample was screened by a pathologist using a well-established and validated scoring system.²⁶,²⁷ Scoring was performed in a blinded fashion for the following parameters: interstitial inflammation, intra-alveolar inflammation, edema, vasculitis, bronchitis, pleuritis, and thrombus formation. Each parameter was graded on a scale of 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; and 4, very severe). The total injury score was expressed as the sum of the score for all parameters, with a maximum of 28. Granulocyte staining was performed using a fluorescein isothiocyanate–labeled anti-mouse Ly-6G mAb (553127;BD Pharmingen), as described by van Zoelen et al.²⁸ Macrophage stainings were performed using a rat anti-F4/80 antibody (MCA497GA; AbD Serotec). Slides were deparaffinized, and endogenous peroxidase was inhibited by 0.3% H₂O₂ for 10 minutes. Antigen retrieval was performed using heat-inducible epitope retrieval for 20 minutes with use of a citrate buffer (pH, 6.0); sections were blocked with Ultra V block for 10 minutes and incubated over night with the primary antibody (1:500) at 4°C. Sections were incubated with a rabbit (FAB2)-anti-rat IgG (#6130-01; ITK Diagnostics; 1:3000) in 20% normal mouse...
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serum and/or phosphate-buffered saline (PBS) for 30 minutes. Next, slides were incubated with Powervision PolyHRP-anti-rabbit IgG (DPVM-55HRP; Immunologic) for 30 minutes at room temperature and stained using 3,3′ diaminobenzidine dihydrochloride (BS04-999; Immunologic). Finally, slides were counterstained with hematoxylin and were dehydrated, and coverslips were mounted with perpex. C/EBPδ staining was performed as described elsewhere.29 Ly-6G and F4/80-stained slides were scanned using the Olympus Slide system (Olympus), and TIF images spanning the full tissue section were generated. Positive areas were analyzed using Image Pro Plus (Media Cybernetics) and were expressed as percentage of the total lung surface area.

Bone Marrow–Derived Macrophages (BMDØs) Isolation and Stimulation. Tibia and femur of naive wildtype and C/EBPδ−/− mice were harvested, and bone marrow was flushed out of the bones with RPMI 1640 supplemented with 10% FCS, L-glutamine, and penicillin-streptomycin. Erythrocytes were lysed with erylysis buffer, and cells were plated in a non-tissue culture-treated petridish in medium containing 30 ng/mL M-CSF to allow macrophage differentiation. After 7 days, cells were harvested using 4 mg/mL lidocaine in 5 mM EDTA/PBS solution and were used for stimulation experiments. For both wildtype and C/EBPδ−/− BMDØs, 100,000 cells were double-stained with F4/80 APC (1:400; MCA497APC; Serotec) and CD11b PE (1:1000; 557 397; BD Pharmingen) antibodies and analyzed using flow cytometry. A total of 96%-99% of the live cells were positive for both macrophage markers (data not shown), indicating that a homogenous macrophage population was used in the experiments using BMDØs. For stimulation experiments, 10,000 cells were seeded in 100 μL in 96-well plates with or without 100 ng/mL LPS (L4268; Sigma) for 4, 8, and 24 hours, after which the supernatant was collected and stored at -20°C for further analysis.

Oxidative Burst. BMDØs were isolated as described above, and 3.5 × 10⁵ cells were seeded in 300 μL HBSS containing MgCl₂ and CaCl₂ (24020-133; Invitrogen). Next, 100 μM diHydroRhodamine123 (DHR; Invitrogen) was added to the cells, and the cells were incubated for 10 minutes at 37°C. Subsequently, cells were stimulated with 5 μg/mL PMA for 1 hour at 37°C. Ice cold HBSS with 5% normal mouse serum (Sanquin) was added to wash the cells before they were resuspended in ice cold FACS buffer, after which flow cytometry analysis was performed using a FACSCalibur (BD Pharmingen). Mean fluorescence signal in Fl-3 was analyzed.
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**Statistical Analysis.** Data are expressed as means ± standard error of the mean. Differences between groups were analyzed using t test (*in vitro* experiments) or Mann-Whitney U test (*in vivo* experiments). For the survival experiment, Kaplan-Meier analysis was performed using the log rank test. Analyses were performed using GraphPad Prism, version 4.0. Statistically significant differences were considered with a P <.05.

**RESULTS**

**C/EBPδ Expression Increases During Klebsiella-Induced Pneumonia.** To reveal whether C/EBPδ might play a role during *Klebsiella* pneumonia, we first determined C/EBPδ expression in the lungs of infected animals. As shown in Figure 1, C/EBPδ levels in the lung increased over time (Figure 1A–C) in epithelial cells, in macrophages, and, to a lesser extent, in neutrophils (for higher magnification, see Figure 1D). Lung sections of C/EBPδ/− mice were negative for the staining (data not shown).

![Figure 1](image)

**Figure 1.** C/EBPδ expression increases during *Klebsiella*-induced pneumonia. (A-D) Immunohistochemical staining of C/EBPδ protein expression in wildtype lung tissue before (A) and 24 (B) or 48 (C and D) hours after the intranasal inoculation with *Klebsiella pneumoniae*. (A-C: 200× magnification; D: 400× magnification).

**C/EBPδ Deficiency Aggravates Klebsiella-Induced Pneumonia and Exaggerates Bacterial Dissemination.** To investigate whether C/EBPδ upregulation during *Klebsiella*-induced pneumonia was important for disease progression, we assessed the effect of C/EBPδ
C/EBPδ deficiency on survival of mice inoculated intranasally with 5 × 10⁴ CFU Klebsiella. As shown in Figure 2A, survival is dramatically affected by C/EBPδ deficiency, leading to a higher and more rapid mortality rate, compared with wildtype controls. Of interest, all C/EBPδ−/− mice died of the infection, whereas 40% of the wildtype mice survived the study period of 6 days. Thus, C/EBPδ plays an essential protective role during Klebsiella pulmonary infection. To get more insight into the role of C/EBPδ during Klebsiella pneumonia, bacterial loads were determined in lung homogenates of wildtype and C/EBPδ−/− mice. At 6 and 24 hours after inoculation, bacterial loads were similar in both mouse strains (2B). Forty-eight hours

Figure 2. C/EBPδ deficiency aggravates Klebsiella-induced pneumonia and exaggerates bacterial dissemination. (A) Survival curves of wildtype and C/EBPδ−/− mice after intranasal inoculation with Klebsiella species (n = 14 for wildtype and 12 for C/EBPδ−/− mice). (B-E) Bacterial outgrowth in lungs (B), blood (C), spleen (D) and kidney (E) samples from wildtype and C/EBPδ−/− mice after intranasal inoculation with Klebsiella species. Data are means ± standard error of the mean (n = 7-8). *P < .05, ***P < .001.
after inoculation, bacterial loads were approximately 10-fold higher in lungs of C/EBPδ−/− mice, although this difference was not statistically significant. To obtain insight into the dissemination of the infection, we determined bacterial loads in blood, spleen, and kidney samples. At 6 hours, bacteria were not detectable yet in these organs, whereas after 24 hours, bacterial loads were similar in the organs from wildtype and C/EBPδ−/− mice (Figure 2C–E). Of interest, bacterial loads were significantly increased in spleen and kidney homogenates of C/EBPδ−/− mice, compared with wildtype animals 48 hours after inoculation (Figure 2D–E). Bacterial loads were also increased in blood samples from C/EBPδ−/− mice, although this difference did not reach statistical significance (Figure 2C). These results thus show that C/EBPδ limits bacterial outgrowth and dissemination during Klebsiella infection.

The widespread dissemination of bacteria suggests that the mice succumb to systemic disease leading to multiple organ failure, and we therefore analyzed organ damage 48 hours after inoculation. As shown in Figure S1, aspartate aminotransferase, alanine aminotransferase, creatinine, and lactate dehydrogenase levels were not increased in wildtype animals infected with Klebsiella, whereas the levels in C/EBPδ−/− mice were slightly (although not significantly) higher. Of interest, organ damage markers were positively correlated with bacterial loads in C/EBPδ−/− mice (data not shown).

**C/EBPδ Dependent Cytokine Production During Klebsiella-Induced Pneumonia.** The success of combating pulmonary infections strongly depends on the efficacy of the local inflammatory response elicited. Consequently, we measured cytokine levels in lung, plasma, and spleen samples. As shown in Figure 3, cytokine levels increased over time, and no significant differences were observed between wildtype and C/EBPδ−/− animals within the first 24 hours after inoculation. At 48 hours after inoculation, however, plasma TNF-α levels showed a trend toward higher levels in C/EBPδ−/− mice (Figure 3C), and plasma IL-6 levels were significantly higher in C/EBPδ−/− mice (Figure 3D), compared with wildtype mice; in lung (Figure 3A and 1B) and spleen (Figure 3E–F) samples, no differences were observed. To further evaluate the role of C/EBPδ in lung inflammation, histological slides prepared from lungs harvested 24 and 48 hours after infection were scored for signs of pulmonary inflammation. Of interest, 48 hours after inoculation, lung weights of C/EBPδ−/− mice were significantly increased, compared with wildtype mice (Figure S2A). Surprisingly, histological examination of the lung tissue did not reveal any difference in edema, bronchitis, interstitial inflammation, vasculitis, and pleuritis between wildtype and C/EBPδ−/− mice (Figure S2B, for total histological score).
C/EBPδ Enhances Macrophage Influx Into the Lungs During Klebsiella-Induced Pneumonia.

Because it was evident from immunohistochemical analysis of Klebsiella-infected lungs that C/EBPδ was highly expressed by macrophages and, to a lesser extent, by neutrophils (Figure 1), we next analyzed macrophage and neutrophil numbers in the lungs of wildtype and C/EBPδ−/− mice after inoculation with Klebsiella species. As shown in Figure 4A–C, macrophages were present in both wildtype and C/EBPδ−/− mice 24 and 48 hours after inoculation. Of interest, macrophage numbers increased over time in wildtype mice, whereas macrophage numbers in the lungs of C/EBPδ−/− mice were similar at both 24 and 48 hours after inoculation. Of most importance, macrophage numbers were significantly reduced in C/EBPδ−/− mice 48 hours after inoculation. As shown in Figure S3A–C, considerable amounts of neutrophils were present 24 and 48 hours after inoculation, but no statistically significant differences were observed between wildtype and C/EBPδ−/− animals.

To determine whether reduced macrophage numbers in C/EBPδ−/− mice are attributable to reduced production of macrophage chemoattractants, we analyzed MCP-1 levels (ie, the most important chemokine for monocyte and macrophage migration). As evident from Figure 4D, MCP-1 was highly expressed in lungs of infected animals, but the levels were simi-
In both wildtype and C/EBPδ−/− mice. Of interest, plasma MCP-1 levels were significantly increased in C/EBPδ−/− mice 24 and 48 hours after inoculation, whereas it was not increased in wildtype animals during this period (Figure 4E). Next, we determined keratinocyte che-moattractant levels (ie, the most important chemokine for neutrophils), and as expected, no differences were observed between wildtype and C/EBPδ−/− mice (Figure S3D–E).

**C/EBPδ Slightly Modifies Macrophage Function.** We studied whether macrophage function was also affected by C/EBPδ. To this end, BMDØs were obtained from wildtype and C/EBPδ−/− mice and stimulated with LPS (100 ng/mL), after which cytokine levels in the supernatant were determined. As shown in Figure 5A, TNF-α, IL-6, and MCP-1 secretion increased over time in both wildtype and C/EBPδ−/− BMDØs, but no differences were observed between the 2 genotypes.
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In addition to cytokine production, we determined macrophage function by assessing the oxidative capacity of wildtype and C/EBPδ−/− BMDØs. As shown in Figure 5B, after PMA stimulation (a well-known inducer of oxidative burst in macrophages), oxidative burst increased by approximately 2-fold in wildtype BMDØs and only 1.5-fold in C/EBPδ−/− BMDØs.

DISCUSSION

We here show that transcription factor C/EBPδ protects mice from Klebsiella-induced mortality. Our data demonstrate that C/EBPδ was highly induced in the lung during Klebsiella pneumonia, and it prolonged survival. Of note, all C/EBPδ−/− mice died of infection with a median survival of 60 hours, whereas 40% of wildtype mice were still alive at the end of the observation period of 144 hours. Of interest, both bacterial loads and inflammatory responses were similar in wildtype and C/EBPδ−/− mice early during the infection (ie, up to 24 hours). At 48 hours after inoculation, bacterial loads tended to be higher locally in the lung and were significantly increased in peripheral organs of C/EBPδ−/− mice. In line with these higher bacterial loads, organ damage markers aspartate aminotransferase, alanine aminotransferase, creatinine, and lactate dehydrogenase levels also tended to be increased in C/EBPδ−/− mice. Of interest, organ damage markers correlated with bacterial loads in C/EBPδ−/− mice. It is thus tempting to speculate that the lack of C/EBPδ leads to higher bacterial loads resulting in multiple organ failure and premature death.

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Several studies suggest that C/EBPδ is an important regulator of proinflammatory cytokine production and that C/EBPδ plays an important role during inflammation in vitro\textsuperscript{9,30} and in vivo.\textsuperscript{18,19} Indeed, LPS stimulation of C/EBPδ\textsuperscript{-/-} macrophages does not lead to increased IL-6 mRNA levels, compared with a large increase in wildtype macrophages.\textsuperscript{18} Moreover, TNF-α and IL-6 levels were reduced in C/EBPδ\textsuperscript{-/-} mice, compared with wildtype controls in a double-hit LPS model.\textsuperscript{19} At first glance, our data showing that the inflammatory response during \textit{Klebsiella}-induced pneumonia was not different in lungs and peripheral organs and even increased in plasma in C/EBPδ\textsuperscript{-/-}, compared with wildtype mice, may thus be surprising. However, seminal papers by Hu et al\textsuperscript{30} and Lu et al\textsuperscript{9} show that the activity of C/EBPδ may be redundant to the activity of C/EBPβ in regard to expression of IL-6 and MCP-1. The role of C/EBPδ in the regulation of the inflammatory response during infection seems to be complex and is not yet fully understood. However, C/EBPδ is of pivotal importance for combating bacterial infections (the current study and reference no. 18\textsuperscript{18}).

Probably the most interesting difference between wildtype and C/EBPδ\textsuperscript{-/-} mice during \textit{Klebsiella} infection was the large increase in macrophage numbers in wildtype mice that was not observed in C/EBPδ\textsuperscript{-/-} mice. Indeed, macrophages are well-known to express C/EBPδ after LPS stimulation\textsuperscript{9,10}, and we show that C/EBPδ is also increased in macrophages during bacterial infection. Because macrophages have been shown to be important during \textit{Klebsiella}-induced pneumonia\textsuperscript{31}, it is tempting to speculate that the lower macrophage number in the lungs of C/EBPδ\textsuperscript{-/-} mice may explain the disadvantage in survival. Indeed, mice depleted from alveolar macrophages showed a dramatically decreased survival during \textit{Klebsiella}-induced pneumonia, compared with non-depleted controls. Of interest, alveolar macrophage-depleted mice had a median survival of approximately 60 hours after inoculation, which is similar to the median survival of C/EBPδ\textsuperscript{-/-} mice in our study.\textsuperscript{31}

The differential influx of macrophages in C/EBPδ\textsuperscript{-/-} mice, compared with wildtype animals, could not be explained by C/EBPδ-driven MCP-1 expression in the lung. Indeed, pulmonary expression levels of the well-known macrophage chemoattractant MCP-1 were similar in wildtype and C/EBPδ\textsuperscript{-/-} mice. Of interest, however, MCP-1 levels were increased in plasma samples from C/EBPδ\textsuperscript{-/-} mice, compared with controls, and it is thus tempting to speculate that monocyte and/or macrophage migration may be reduced in C/EBPδ\textsuperscript{-/-} mice because of a less steep gradient in MCP-1 levels between plasma and the lung. Consequently, monocytes and/or macrophages may reside in the circulation of C/EBPδ\textsuperscript{-/-} mice, instead of migrating into the lung. Although this may be an attractive hypothesis, alternative mechanisms, such as the expression of alternative macrophage attractants in the lung or chemokine receptor expression on monocytes and/or macrophages, cannot be excluded, and
ongoing studies aim at a full understanding of the role of C/EBPδ in macrophage migration. In addition to differential macrophage influx into infected lungs, C/EBPδ may slightly modify macrophage function. Indeed, we showed that C/EBPδ−/− BMDØs produce less reactive oxygen species after PMA stimulation, compared with wildtype BMDØs. The bactericidal properties of the reactive oxygen species are well established, and reduced ROS production may thus (in part) be responsible for diminished bacterial killing and subsequent increased bacterial loads. As opposed to the effect of C/EBPδ on oxidative burst, we did not observe any effect of C/EBPδ on cytokine production by BMDØs. These latter data are in line with studies showing that IL-6 and TNF-α production by BMDØs is not solely dependent on C/EBPδ, because C/EBPβ may compensate for the absence of C/EBPδ. In a recent study by Litvak et al, however, IL-6 mRNA expression in BMDØs was strongly dependent on C/EBPδ expression. To date, we do not have a proper explanation for the discrepancy between the different studies. Potential explanations may be the analysis of mRNA and protein expression, the type and concentration of LPS (Salmonella minnesota, E. coli, and Klebsiella-derived LPS), and the amount of M-CSF used to differentiate bone marrow-derived precursor cells. Overall, these in vitro data suggest that C/EBPδ only plays a minor role in macrophage function and that the observed difference in macrophage numbers could potentially be responsible for reduced survival in C/EBPδ−/− mice.

Considering the unaltered local bacterial clearance in the lungs but the significantly impaired survival in C/EBPδ−/− mice, one might suggest that the resolution process is impaired in mice lacking C/EBPδ. Indeed, macrophages are key players in the resolution phase of the host response. Classically activated M1 macrophages promote inflammation and bactericidal activities, whereas alternatively activated M2 macrophages promote mainly tissue regeneration and inhibit inflammatory responses. It would thus be interesting to determine whether C/EBPδ may influence macrophage polarization toward M2 macrophages, although the importance of the resolution phase during acute pulmonary infection to improve survival remains elusive.

It is important to realize that the used Klebsiella pneumonia model largely resembles human pneumonia and sepsis, because in both situations, pneumonia is associated with invasive respiratory tract infection leading to dissemination of infection and distant organ injury. Data obtained using this model of bacterial pneumonia are considered to be of relevance for understanding the pathogenesis of pneumonia, but extrapolation to human disease should be made with care. In conclusion, our data show that C/EBPδ contributes to host defense against Klebsiella-induced pneumonia, and we suggest that macrophage mobilization into the lung may be a key mechanism by which C/EBPδ limits mortality.
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Acknowledgements: This work was supported by the Dutch Kidney Foundation (C06.2198 to JD); Prof. Dr. Esta Sterneck, for the kind gift of C/EBPδ-/- mice; Marieke ten Brink and Joost Daalhuisen, for their technical assistance during the animal experiments; Regina de Beer, for performing Ly-6G immunohistochemical stainings; Chris van der Loos, for his expertise on the immunohistochemical stainings; and Onno de Boer and Wilfried Meun, for scanning the immunohistochemical slides before analysis.
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SUPPORTING INFORMATION
SUPPLEMENTARY FIGURES

**Figure S1.** Organ damage markers in wildtype and C/EBPδ−/− mice 48 hours after *Klebsiella* inoculation. (A) ASAT, (B) ALAT, (C) creatinine and (D) LDH levels in plasma.

**Figure S2.** C/EBPδ-dependent lung inflammation. (A) Lung weight of wildtype and C/EBPδ−/− mice 24 and 48 hours after *Klebsiella* inoculation. (B) Total histological score of lungs harvested 24 and 48 hours after infection with *Klebsiella*. Data are means ± SEM; n=7-8, ** p<0.01.
Figure S3. C/EBPδ does not influence neutrophil influx into the lungs upon *Klebsiella*-induced pneumonia. (A-B) Immunohistochemical staining of Ly6-G protein expression in wildtype (A) and C/EBPδ−/− (B) lung tissue 24 hours after the intranasal inoculation with *Klebsiella pneumoniae*. (200x magnification). (C) Quantification of the immunohistochemical staining as depicted in panels A and B. (D-E) KC levels in lung homogenates (D) and plasma (E) of wildtype and C/EBPδ−/− mice after inoculation with *Klebsiella*. Data are means ± SEM; n=7-8.