Inhibition of pulmonary nuclear factor kappa-B decreases the severity of acute *Escherichia coli* pneumonia but worsens prolonged pneumonia

James Devaney1,2, Gerard F Curley1,3, Mairead Hayes2, Claire Masterson2, Bilal Ansari2, Timothy O’Brien1, Daniel O’Toole1,2 and John G Laffey1,3*

**Abstract**

**Introduction:** Nuclear factor (NF)-κB is central to the pathogenesis of inflammation in acute lung injury, but also to inflammation resolution and repair. We wished to determine whether overexpression of the NF-κB inhibitor IκBα could modulate the severity of acute and prolonged pneumonia-induced lung injury in a series of prospective randomized animal studies.

**Methods:** Adult male Sprague-Dawley rats were randomized to undergo intratracheal instillation of (a) 5 × 10⁹ adenovirus (AAV) vectors encoding the IκBα transgene (5 × 10⁹ AAV-IκBα); (b) 1 × 10¹⁰ AAV-IκBα; (c) 5 × 10¹⁰ AAV-IκBα; or (d) vehicle alone. After intratracheal inoculation with *Escherichia coli*, the severity of the lung injury was measured in one series over a 4-hour period (acute pneumonia), and in a second series after 72 hours (prolonged pneumonia). Additional experiments examined the effects of IκBα and null-gene overexpression on *E. coli*-induced and sham pneumonia.

**Results:** In acute pneumonia, IκBα dose-dependently decreased lung injury, improving arterial oxygenation and lung static compliance, reducing alveolar protein leak and histologic injury, and decreasing alveolar IL-1β concentrations. Benefit was maximal at the intermediate (1 × 10¹⁰) IκBα vector dose; however, efficacy was diminished at the higher (5 × 10¹⁰) IκBα vector dose. In contrast, IκBα worsened prolonged pneumonia-induced lung injury, increased lung bacterial load, decreased lung compliance, and delayed resolution of the acute inflammatory response.

**Conclusions:** Inhibition of pulmonary NF-κB activity reduces early pneumonia-induced injury, but worsens injury and bacterial load during prolonged pneumonia.

**Keywords:** Acute lung injury, inhibitory kappa-B alpha, rat, acute respiratory distress syndrome, bacteria, pneumonia, gene therapy

**Introduction**

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening disorders, for which no specific therapy is known. When ARDS occurs in the setting of multisystem organ failure, mortality rates more than 60% have been reported, with significant morbidity in 50% of survivors [1]. ALI and ARDS develop most commonly in the context of severe sepsis [2], particularly infection with gram-negative bacilli such as *Escherichia coli* (*E. coli*) [3], and sepsis-induced ARDS has the worst outcome [4].

Nuclear factor kappa B (NF-κB) is a key transcriptional regulator in the setting of inflammation and injury and plays a role in diverse inflammatory disorders, including acute lung injury [5]. Activation of NF-κB occurs in response to diverse stimuli, such as endotoxin, which binds to cell-surface receptors that in turn activate the canonical and/or noncanonical signaling pathway. This signaling cascade ultimately results in the phosphorylation and inactivation of the cytosolic inhibitor IκB complex, which then
dissociates, allowing NF-κB to translocate to the nucleus to initiate gene transcription [5]. Inhibition of NF-κB reduces injury in preclinical models of ALI, including ischemia-reperfusion [6], endotoxemia [7], and cecal ligation and puncture-induced sepsis [8]. However, NF-κB also exerts important cytoprotective effects, promoting cell survival, resolution of inflammation, and wound repair [9]. Of interest, NF-κB signaling plays a central role in the host response to lung bacterial infection [10]. Consequently, inhibition of NF-κB may constitute a double-edged sword, particularly in pneumonia-induced ALI/ARDS, in which immune competence is essential to eradication of the infectious agent [11].

We wished to determine the potential for inhibition of pulmonary NF-κB activity to modulate the severity of pneumonia-induced lung injury. We used a gene-based therapy approach, via intrapulmonary delivery of three different doses of adenovasecreted viral vector encoding the NF-κB inhibitor Inh-Ba gene (AAV-Inh-Ba), to modulate the NF-κB signaling pathway in the lung. We hypothesized that pulmonary overexpression of the NF-κB inhibitor Inh-Ba would (a) attenuate the severity of the lung injury induced by acute *E. coli* pneumonia; but would (b) worsen the severity of prolonged *E. coli* pneumonia-induced lung injury; and (c) a dose-response relation would exist, with higher AAV-Inh-Ba doses having the greatest effect.

Materials and methods
Specific-pathogen-free adult male Sprague-Dawley rats (350 to 450 g) were used in all studies. The experimental model was based on those previously reported [12-14]. All work was approved by the National University of Ireland Galway Research Ethics Committee and conducted under license from the Department of Health, Ireland.

Preparation of AAV vectors
AAV-vector production was carried out as previously described, with several modifications [15]. The Inh-Ba-SuperRepressor (Inh-Ba-SR) gene (1,566 bp) was ligated into the pAAV-MCS vector (Agilent Technologies Inc., Santa Clara, CA, USA), and plasmid size confirmed by gel electrophoresis and validated by sequencing (Eurofins MWG Operon, Ebersberg, Germany). The Inh-Ba-FLAG plasmid DNA and AAV serotype 6 envelope were generated and sent to Virapur for AAV production (VIRAPUR, 6160 Lusk Blvd., Suite C-101, San Diego, CA, USA). AAV serotype 6 was chosen because AAV has reduced immunogenicity, the virus plasmid size is sufficient for the Inh-Ba-SuperRepressor (Inh-Ba-SR) gene, and serotype 6 has demonstrated tropism for lung epithelial cells.

Viral vector particle titers were determined with quantitative real-time polymerase chain reaction (qRT-PCR) and aliquoted and stored at -80°C. As required, an aliquot was thawed and added to 75 μl of the porcine surfactant Curosurf (120 mg/ml) (Trinity-Chiesi Pharmaceuticals Limited, Cheadle, UK), and a final instillate volume of 300 μl was made up with PBS. For those animals receiving vehicle only, the instillate was 75 μl of Curosurf mixed with 225 μl of phosphate-buffered saline (PBS). Curosurf surfactant was added to each instillate, as it was demonstrated in prior studies to enhance spread and improve transgene expression [16].

Acute and prolonged *E. coli*-induced lung injury
Experimental design
Animals were randomized to intratracheal instillation of the following: (a) vehicle alone; (b) 5 × 10⁹ drp AAV Inh-Ba-SR; (c) 1 × 10¹⁰ drp AAV Inh-Ba-SR; or (d) 5 × 10¹⁰ drp AAV Inh-Ba-SR, and to undergo subsequent acute (Series 1) or prolonged (Series 2) pneumonia-induced ALI. Additional experimental series examined the effects of 1 × 10¹⁰ AAV-Inh-Ba versus 1 × 10¹⁰ AAV-Null in both acute and prolonged *E. coli* and sham pneumonia.

Vector instillation
Animals were anesthetized by inhalational induction with isoflurane and an intraperitoneal injection of 40 mg/kg ketamine (Pfizer, Kent, UK). After confirmation of depth of anesthesia, laryngoscopy was performed (Welch Allyn Otoscope; Buckinghamshire, UK), and the trachea intubated with a size 16 intravenous catheter (BD Insyte; Becton Dickinson Ltd., Oxford, UK). After instillation of vector or vehicle, depending on the specific series, animals were extubated and allowed to recover from anesthesia.

*E. coli*/vehicle instillation
The *E. coli* used in these experiments is E5162 (serotype: O9 K30 H10) and was supplied by the National Collection of Type Cultures, Central Public Health Laboratory, London, England. The *E. coli* were stored on preservative beads (Protect, Lancashire, England) at -80°C. Beads were placed in 3-ml vials of peptone water (Cruinn Diagnostics, Dublin, Ireland) and incubated at 37°C for 18 to 24 hours to allow bacterial concentrations to reach a plateau. The bacterial suspension was then centrifuged and washed in phosphate-buffered saline to produce the inoculum. The bacterial load in each inoculum was determined by plating serial dilutions on agar plates. Preliminary experiments were performed to determine the bacterial load of intratracheal *E. coli* required to produce a lung injury over a 4-hour and over a 72-hour period.

Acute pneumonia protocol
Ninety-six hours after virus instillation, animals were anesthetized with intraperitoneal 80 mg/kg ketamine and 8 mg/kg xylazine, and anesthesia maintained with Alfaxalone (Alfaxadone 0.9% and alfadadolone acetate 0.3%). A tracheostomy tube was inserted, and intratraenicular access was sited in the carotid artery. Muscle relaxation was induced with cisatracurium besylate, and the lungs were mechanically ventilated with 30% O₂ in 70% N₂. After
20 minutes, arterial blood gas measurement was performed and $1 \times 10^{11}$ *E. coli* in a 300-μl PBS suspension (or vehicle alone) instilled via the tracheostomy [12,13]. Animals were ventilated for 4 hours, with systemic arterial blood pressure, peak airway pressure, and body temperature continually measured. Lung compliance and arterial blood pressure, peak airway pressure, and body temperature was maintained at 36°C to 37.5°C.

**Prolonged pneumonia protocol**

Animals were anesthetized by inhalational induction with isoﬂurane and intraperitoneal 40-mg/kg ketamine (Pﬁzer, Kent, UK). After conﬁrmation of anesthesia depth, $5 \times 10^9$ *E. coli* in a 300-μl PBS suspension was instilled into the trachea under direct vision, and the animals allowed to recover [17]. Animals were monitored closely for 72 hours after *E. coli* instillation, and then reanesthetized, tracheostomized, and mechanical ventilation was instituted, and injury severity assessed, as described [18].

**Postmortem analyses**

At the end of the protocols, the animals were killed by exsanguination under anesthesia, and the plasma snap-frozen for later analysis. The heart-lung block was dissected from the thorax, bronchoalveolar lavage (BAL) was performed, and BAL fluid differential leukocyte counts and lung bacterial colony counts were completed. BAL fluid was centrifuged, and the supernatant was snap-frozen and stored at -80°C. BAL concentrations of IL-1β, TNF-α, IL-6, CINC-1, IL-10, and KGF were determined by using ELISA (R&D Systems, Abingdon, UK), and BAL protein concentrations were measured (Micro BCA Protein assay kit; Pierce, Rockford, IL, USA) [16]. The left lung was isolated and ﬁxed, and the extent of histologic lung damage determined by using quantitative stereologic techniques [18].

**Assessment of transgene expression and efﬁcacy**

*InBα-FLAG* transgene expression was determined in lung homogenates with real-time PCR and Western blotting, as previously described [16,19]. In brief, RNA was extracted from lung tissue, cDNA was synthesized, and quantitative PCR was performed for *InBα-FLAG*, normalized against a GAPDH control product. For Western blot *InBα-FLAG* analysis, total cell protein was extracted, protein concentration was determined, and samples were electrophoresed on an SDS-PAGE gel and transferred to nitrocellulose [20]. Primary anti-human *InBα-FLAG* monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) was used, with secondary antibody conjugated to horseradish peroxidase (Cell Signaling Technology, Danvers, MA, USA), and the membrane incubated with a chemiluminescent substrate (SuperSignal West Pico; Pierce).

The effect of *InBα* overexpression on the activation of the NF-κB pathway was assessed by measurement of nuclear accumulation of the activated P65 subunit of NF-κB [19]. Nuclear extracts were performed on homogenized rat lung tissue by using a NE-PER Nuclear and Cytoplasmic Extraction Kit (Fisher Scientiﬁc Ireland, Dublin, Ireland), and NF-κB (p65) measured by using an NF-κB Transcription Factor Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA).

**Data presentation and analysis**

Continuous responsive variables are summarized by using mean (SD) and median (interquartile range, IQR) as necessary. The proportion of animals surviving was analyzed by using the $\chi^2$ test. All other data were analyzed with one-way ANOVA, followed by the Dunnett test or by Kruskal-Wallis, followed by the Dunn test, with the vehicle group used as the reference group for all comparisons. The assumptions underlying all models were checked by using suitable residual plots. A P value of <0.05 was considered statistically signiﬁcant.

**Results**

**Acute pneumonia-induced ALI**

Sixty-eight animals were entered into these experimental series. In the ﬁrst set of experiments, 48 animals were randomized to receive (a) vehicle alone ($n = 12$); (b) $5 \times 10^9$ AAV-*InBα* ($n = 12$); (c) $1 \times 10^{10}$ AAV-*InBα* ($n = 12$); (d) $5 \times 10^{10}$ AAV-*InBα* ($n = 12$), and all animals subsequently underwent intratracheal *E. coli* instillation. No differences were noted between the groups at baseline (Table 1).

**Survival and bacterial load**

*InBα* expression and function Pulmonary instillation of AAV-*InBα* produced a dose-dependent increase in lung *InBα* gene transcription (Figure 1A) and protein production (Figure 1B, C). Densitometry of *InBα-FLAG* Western blot ($n = 3$ per group) conﬁrmed this dose-dependent increase in *InBα* protein (Figure 1C). *InBα* overexpression decreased *E. coli*-induced lung bacterial loads, as measured by nuclear accumulation of the activated P65 subunit of NF-κB (Figure 1D).

**Survival and bacterial load**

*InBα* overexpression enhanced animal survival, with survival rates of 33% in the Vehicle group, 58% with $5 \times 10^9$ *InBα*, and 92% with $1 \times 10^{10}$ *InBα* Of concern, the highest *InBα* ($5 \times 10^{10}$) dose abolished the survival beneﬁt (Table 1). *InBα* overexpression also enhanced the duration of animal survival, which followed this same pattern, with the effect greatest with the $1 \times 10^{10}$ *InBα* dose (Table 1). Importantly, *InBα* did not alter lung bacterial loads (Table 1).

**InBα modulated lung-injury severity**

The *E. coli* instillation induced a severe acute lung injury (Figure 2). *InBα* overexpression reduced the
Table 1 Data regarding animals subjected to acute pneumonia-induced ALI

| Variable               | Vehicle   | 5 × 10⁹ I-Bα | 1 × 10¹⁰ I-Bα | 5 × 10¹⁰ I-Bα |
|------------------------|-----------|--------------|--------------|--------------|
| Number of animals      | 12        | 12           | 12           | 12           |
| Animal weight (g)      | 456 ± 55  | 486 ± 54     | 469 ± 52     | 438 ± 86     |
| Bacteria instilled (CFU × 10¹⁰) | 1.1 ± 0.2 | 2.1 ± 0.2    | 2.6 ± 2.0    | 2.1 ± 2.2    |
| Baseline arterial PO₂ (kPa) | 18.7 ± 0.5 | 18.7 ± 0.8   | 18.8 ± 0.8   | 18.7 ± 0.8   |
| Baseline static compliance (ml/mm Hg) | 0.93 ± 0.1 | 0.95 ± 0.1   | 0.99 ± 0.2   | 0.87 ± 0.2   |
| Animal survival (number, %) | 4 (33)    | 7 (58)       | 11 (92)*     | 5 (42)       |
| Duration of animal survival (hours) | 2.75 (2, 4) | 4 (1.5, 4)   | 3 (1.75, 4)  |
| Lung homogenate bacterial counts (CFU × 10⁹) | 0.8 (0.4, 1.7) | 1.4 (0.2, 2.6) | 0.6 (0.4, 0.9) | 0.7 (0.4, 0.9) |
| BAL neutrophils (× 10⁷/ml) | 5.9 (1.5, 8.4) | 3.7 (1.5, 8.9) | 6.2 (1.8, 6.7) | 8.5 (1.1, 9.1) |
| BAL mononuclear cells (× 10⁷/ml) | 2.2 (1.4, 9.1) | 0.57 (2.7, 7.0) | 1.5 (1.2, 9.3) | 1.8 (1.1, 9.6) |

Note: Data are expressed as mean ± SD. *Significantly different from Vehicle group.

Figure 1 Overexpression of IκBα-reduced NF-κB activity. Delivery of the adeno-associated viral vector encoding IκBα resulted in a dose-dependent increase in lung homogenate IκBα FLAG mRNA expression (A) and IκBα protein concentrations, as demonstrated by the representative Western blot (B), and by densitometry of Western blots (n = 3 per group) (C). IκBα decreased Escherichia coli-induced nuclear accumulation of the activated P65 subunit of NF-κB (D). Vehicle, animals that received intratracheal vehicle alone; IκBα 5 × 10⁹, animals that received 5 × 10⁹ AAV6 particles encoding IκBα; IκBα 1 × 10¹⁰, animals that received 1 × 10¹⁰ AAV6 particles encoding IκBα; IκBα 5 × 10¹⁰, animals that received 5 × 10¹⁰ AAV6 particles encoding IκBα. *Significantly different from vehicle group (P < 0.05, ANOVA).
E. coli-induced decrement in arterial oxygenation, with benefit greatest at intermediate (1 × 10^{10}) IkBα vector dose, but abolished at the higher (5 × 10^{10}) IkBα dose (Figure 2A and Table 1). A similar pattern was seen with regard to the decrement in static compliance (Figure 2B), and pulmonary permeability, as assessed by protein leak into the BAL fluid (Figure 2C), but no effect was seen in regard to BAL neutrophil infiltration (Figure 2D). The gray bars represent sham (that is, uninfected) animals. Vehicle, animals that received intratracheal vehicle alone; IkBα 5 × 10^9, animals that received 5 × 10^9 AAV6 particles encoding IkBα; IkBα 1 × 10^{10}, animals that received 1 × 10^{10} AAV6 particles encoding IkBα; IkBα 5 × 10^{10}, animals that received 5 × 10^{10} AAV6 particles encoding IkBα. Significantly different from vehicle group (P < 0.05, ANOVA).

**Figure 2** IkBα modulated severity of acute pneumonia-induced ALI. Overexpression of IkBα attenuated the decrement in arterial oxygenation (A), and in static lung compliance (B), and decreased BAL protein concentrations (C), after *Escherichia coli*-induced ALI. Beneficial effects were greatest with the intermediate IkBα (1 × 10^{10} particles) dose but were abolished by the higher IkBα (5 × 10^{10} particles) dose. No effect of IkBα was noted on BAL neutrophil infiltration (D). The gray bars represent sham (that is, uninfected) animals. Vehicle, animals that received intratracheal vehicle alone; IkBα 5 × 10^9, animals that received 5 × 10^9 AAV6 particles encoding IkBα; IkBα 1 × 10^{10}, animals that received 1 × 10^{10} AAV6 particles encoding IkBα; IkBα 5 × 10^{10}, animals that received 5 × 10^{10} AAV6 particles encoding IkBα. Significantly different from vehicle group (P < 0.05, ANOVA).

Additional experiments were performed to determine the effect of overexpression of a null transgene (versus vehicle; n = 4 per group) on the severity of *E. coli* pneumonia, and to determine the effect of IkBα and null-gene overexpression in sham pneumonia (versus vehicle; n = 4 per group). In these studies, the null transgene did not alter the severity of *E. coli*-induced lung injury (Table 2). In addition, overexpression the null or IkBα transgenes in sham-injured animals did not alter lung function (Table 3).
Figure 3 IkBα modulated structural lung injury induced by acute pneumonia. Overexpression of IkBα attenuated the decrement in alveolar airspace and the increase in alveolar tissue (A) after pneumonia-induced lung injury. (B) Image from a pneumonia-injured lung that received intratracheal vehicle, demonstrating increased wall thickness and inflammatory cell infiltrate. (C through E) Images from pneumonia-injured lungs that received increasing doses of intratracheal vector encoding the IkBα transgene. The animals that received the intermediate (5 × 10⁹) dose demonstrated reduced injury and inflammatory cell infiltration. Vehicle, animals that received intratracheal vehicle alone; IkBα 5 × 10⁹, animals that received 5 × 10⁹ AAV6 particles encoding IkBα; IkBα 1 × 10¹⁰, animals that received 1 × 10¹⁰ AAV6 particles encoding IkBα; IkBα 5 × 10¹⁰, animals that received 5 × 10¹⁰ AAV6 particles encoding IkBα. Significantly different from vehicle group (P < 0.05, ANOVA). Scale bar, 200 μm.
**Figure 4** IκBα dose-dependently modulated the inflammatory response to acute pneumonia-induced ALI. Overexpression of IκBα attenuated the increase in BAL interleukin-1β (A), but did not alter BAL TNF-α (B), or interleukin-6 concentrations (C), after *Escherichia coli*-induced ALI. IκBα dose dependently decreased BAL CINC-1 (D), did not alter BAL IL-10 concentrations (E), and dose dependently decreased BAL KGF concentrations (F). These effects were greatest with the highest IκBα (5 × 10¹⁰ particles) dose. The gray bars represent sham (uninfected) animals. Vehicle, animals that received intratracheal vehicle alone; IκBα 5 × 10⁹, animals that received 5 × 10⁹ AAV6 particles encoding IκBα; IκBα 1 × 10¹⁰, animals that received 1 × 10¹⁰ AAV6 particles encoding IκBα; IκBα 5 × 10¹⁰, animals that received 5 × 10¹⁰ AAV6 particles encoding IκBα, BAL, bronchoalveolar lavage; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; CINC-1, cytokine-induced neutrophil chemoattractant-1; KGF, keratinocyte growth factor. Significantly different from vehicle group (P < 0.05, ANOVA).
Table 2 Overexpression of Null transgene does not modulate the severity of acute pneumonia-induced ALI

| Variable                          | E. coli + vehicle | E. coli + null transgene |
|----------------------------------|------------------|-------------------------|
| Number of animals                | 4                | 4                       |
| Animal weight (g)                | 443 ± 25         | 462 ± 33                |
| Bacteria instilled (CFU × 10^10) | 1.10 (0.9, 1.2)  | 1.20 (0.5, 3.6)         |
| Animal survival (n, %)           | 2 (50%)          | 2 (50%)                 |
| Duration of animal survival (hours) | 3.5 (2, 4)   | 3.75 (2.5, 3.7)         |
| Lung homogenate bacterial counts (CFU × 10^9) | 1.7 (1.4, 3.8) | 2.2 (1.2, 3.7)         |
| Final arterial PO2 (kPa)         | 10.8 ± 0.8       | 9.3 ± 1.2               |
| Final static compliance (ml/mm Hg) | 0.39 ± 0.1     | 0.39 ± 0.1              |
| BAL protein (mg/L)               | 7.203 (5.3, 19, 7.646) | 5.542 (2.994, 8.554) |
| BAL neutrophils (× 10^7/ml)      | 3.8 (2.1, 5.2)   | 8.1 (4.6, 11.2)         |
| BAL mononuclear cells (× 10^7/ml)| 0.9 (0.13, 0.72)| 1.2 (0.18, 2.0)        |

Data are expressed as mean ± SD or median (interquartile range). Final data are data collected on completion of the experimental protocol. *Significantly different from Vehicle group. BAL, bronchoalveolar lavage; CFU, colony-forming units.

Prolonged pneumonia-induced ALI

Fifty-six animals were entered into these experimental series. In the first set of experiments, 44 animals were randomized to receive: (a) vehicle (n = 11); (b) 5 × 10^9 AAV-1κBα (n = 11); (c) 1 × 10^10 AAV-1κBα (n = 11); (d) 5 × 10^10 AAV-1κBα (n = 11), and underwent prolonged E. coli-induced lung injury. No differences were found between the groups at baseline (Table 4).

Animal Survival and Bacterial Load

1κBα did not alter the number or duration of animal survival (Table 4). However, 1κBα substantially increased lung E. coli bacterial loads at all three 1κBα doses (Table 4).

1κBα worsens lung injury

E. coli instillation induced a severe lung injury compared with sham pneumonia (Figure 5). 1κBα overexpression did not alter the decrement in arterial oxygenation (Figure 5A). 1κBα overexpression dose dependently worsened static lung compliance (Figure 5B). 1κBα did not alter alveolar protein leak (Figure 5C), but did increase the proportion of neutrophils in the alveolar infiltrate (Figure 5D), while decreasing alveolar mononuclear cells (Figure 5D), while decreasing alveolar mononuclear cells (Table 4). 1κBα worsened E. coli-induced histologic injury, with the intermediate (1 × 10^10) and higher (5 × 10^10) dose of 1κBα, resulting in significantly greater injury (Figure 6A through E).

1κBα modulates pulmonary inflammation

Prolonged E. coli pneumonia activated the inflammatory response compared with sham pneumonia (Figure 7). 1κBα overexpression dose-dependently increased alveolar IL-β (Figure 7A) and TNF-α (Figure 7B), with concentrations highest after 5 × 10^10 1κBα. In contrast, no effect was seen on alveolar IL-6 (Figure 7C) or CINC-1 (Figure 7D) concentrations. 1κBα increased alveolar IL-10 (Figure 7E) and KGF concentrations, with a step-wise dose effect seen (Figure 7F).

Sham Prolonged Pneumonia

In a subsequent experiment, 12 animals were randomized to receive: (a) Vehicle (n = 4); (b) 1 × 10^10 AAV-Null (n = 4); and (c) 1 × 10^10 AAV-1κBα and to undergo subsequent

Table 3 Overexpression of Null or 1κBα transgenes does not alter lung function in animals subjected to sham pneumonia

| Variable                          | Vehicle | 1κBα transgene | Null transgene |
|----------------------------------|---------|----------------|---------------|
| Number of animals                | 4       | 4              | 4             |
| Animal weight (g)                | 413 ± 30| 426 ± 17       | 427 ± 19      |
| Animal survival (n, %)           | 4 (100%)| 4 (100%)       | 4 (100%)      |
| Duration of animal survival (hours) | 4 (4,4) | 4 [4,4]       | 4 [4,4]       |
| Final arterial PO2 (kPa)         | 18.6 ± 0.5 | 18.4 ± 1.1     | 19.9 ± 0.7    |
| Final static compliance (ml/mm Hg) | 0.65 ± 0.1 | 0.71 ± 0.17    | 0.58 ± 0.16   |
| BAL protein (mg/L)               | 1197 [1061, 1296] | 1353 [991, 2167] | 1337 [950, 1860] |
| BAL neutrophils (× 10^7/ml)      | 0.2 [0.0, 1.4] | 1.3 [0.5, 1.8] | 1.1 [0.2, 2.0] |
| BAL mononuclear cells (× 10^7/ml)| 21.0 [11, 22] | 23 [10, 29]    | 14 [12, 18]   |

Data are expressed as mean ± SD or median (interquartile range). Final data are data collected on completion of the experimental protocol. *Significantly different from Vehicle group. BAL, bronchoalveolar lavage; CFU, colony-forming units.
Table 4 Data regarding animals subjected to prolonged pneumonia-induced ALI

| Variable                                | Vehicle          | $5 \times 10^9$ IκBα | $1 \times 10^{10}$ IκBα | $5 \times 10^{10}$ IκBα |
|-----------------------------------------|------------------|---------------------|------------------------|------------------------|
| Number of animals                       | 11               | 11                  | 11                     | 11                     |
| Animal weight (g)                       | 383 ± 13         | 378 ± 19            | 413 ± 35               | 401 ± 47               |
| Animal survival (number, %)             | 8 (73)           | 9 (82)              | 9 (82)                 | 8 (73)                 |
| Duration of animal survival (hours)     | 65.5 ± 11.2      | 64.8 ± 16.2         | 63.3 ± 19.4            | 63.3 ± 16.1            |
| Instilled E. coli bacterial load (CFU × 10^6) | 5.0 ± 1.0        | 5.0 ± 1.0           | 5.0 ± 1.0              | 5.0 ± 1.0              |
| Peak airway pressure (mm Hg)            | 8.1 ± 1.4        | 6.5 ± 1.0           | 7.4 ± 1.0              | 8.0 ± 1.3              |
| Lung homogenate bacterial counts (CFU × 10^4) | 1.1 (0.7, 3.1)  | 51.3 (3.1, 96)^*    | 20.7 (15.9, 36.2)^*    | 19.3 (16.5, 34.6)^*    |
| BAL neutrophils (× 10^9/ml)             | 3.7 ± 3.3        | 2.3 ± 0.8           | 3.8 ± 2.2              | 1.7 ± 0.9              |
| BAL mononuclear cells (× 10^9/ml)       | 23 ± 18          | 0.4 ± 0.2^*         | 0.9 ± 0.6^*            | 0.4 ± 0.2^*            |

Data are expressed as mean ± SD or median (interquartile range). Final data are data collected on completion of the experimental protocol. *Significantly different from Vehicle group. BAL, bronchoalveolar lavage; CFU, colony-forming units.

Figure 5 IκBα worsened ALI induced by prolonged pneumonia. Overexpression of IκBα did not alter the decrement in arterial oxygenation (A). IκBα worsened the Escherichia coli-induced decrement in static lung compliance, with effect greatest with $5 \times 10^{10}$ IκBα particles (B). IκBα did not alter BAL protein concentrations (C), but did significantly increase the percentage of neutrophils in the alveolar infiltrate (D). The gray bars represent sham (uninfected) animals. Vehicle, animals that received intratracheal vehicle alone; IκBα $5 \times 10^9$, animals that received $5 \times 10^9$ AAV6 particles encoding IκBα; IκBα $1 \times 10^{10}$, animals that received $1 \times 10^{10}$ AAV6 particles encoding IκBα; IκBα $5 \times 10^{10}$, animals that received $5 \times 10^{10}$ AAV6 particles encoding IκBα. Significantly different from vehicle group ($P < 0.05$, ANOVA).
Figure 6 IκBα worsened structural lung injury induced by prolonged pneumonia. Overexpression of IκBα worsened the decrement in alveolar airspace and the increase in alveolar tissue (A) after pneumonia-induced lung injury. (B) Image from a prolonged pneumonia-injured lung that received intratracheal vehicle, demonstrating increased wall thickness and inflammatory cell infiltrate. (C through E) Images from a pneumonia-injured lung that received increasing doses of intratracheal vector encoding the IκBα transgene. The animals that received 1 to 5 × 10^{10} doses demonstrated greater injury. Vehicle, animals that received intratracheal vehicle alone; IκBα 5 × 10^9, animals that received 5 × 10^9 AAV6 particles encoding IκBα; IκBα 1 × 10^{10}, animals that received 1 × 10^{10} AAV6 particles encoding IκBα; IκBα 5 × 10^{10}, animals that received 5 × 10^{10} AAV6 particles encoding IκBα. *Significantly different from vehicle group (P < 0.05, ANOVA). Scale bar, 200 μm.
Figure 7 IκBα dose-dependently modulated the inflammatory response to prolonged pneumonia. Overexpression of IκBα dose-dependently increased BAL interleukin-1β (A) and TNF-α (B) concentrations compared with vehicle. No effect of IκBα was seen on interleukin-6 (C) or CINC-1 (D) concentrations. IκBα dose-dependently increased BAL IL-10 (E), and KGF concentrations (F). These effects were greatest with the highest IκBα (5 × 10^10 particles) dose. The gray bars represent sham (uninfected) animals. Vehicle, animals that received intratracheal vehicle alone; IκBα 5 × 10^9, animals that received 5 × 10^9 AAV6 particles encoding IκBα; IκBα 1 × 10^10, animals that received 1 × 10^10 AAV6 particles encoding IκBα; IκBα 5 × 10^10, animals that received 5 × 10^10 AAV6 particles encoding IκBα. *Significantly different from vehicle group (P < 0.05, ANOVA).
sham (vehicle) instillation. The null or IκBα transgenes did not alter lung function in sham infected animals (Table 5).

Discussion
Pulmonary overexpression of IκBα attenuated acute pneumonia-induced lung injury, decreasing the severity of the decrement in lung function, whereas also decreasing the inflammatory response. In contrast, IκBα worsened lung injury and inflammation induced by prolonged pneumonia, increasing lung bacterial load and delaying resolution of the inflammatory response. These findings provide novel insights into the effects of NF-κB in pneumonia-induced lung injury, and raise concerns regarding therapeutic potential of inhibiting NF-κB, particularly in prolonged untreated pneumonia.

E. coli acute and prolonged pneumonia
Infection with gram-negative bacilli such as E. coli is the commonest cause of ARDS [21,22] and is also a very common complication of ARDS due to other causes [23]. We studied E. coli-induced pneumonia, a well-characterized animal model that mimics the clinical development of ARDS very closely [23-28]. Intratracheal instillation of 1 × 10¹¹ E. coli resulted in physiological and pathologic changes consistent with a severe lung injury over a 6-hour period, similar to that previously reported [12,13]. Instillation of a lower dose of 5 × 10⁹ E. coli produced a more gradually evolving injury over a 72-hour period, as previously described [14,17].

NF-κB: role in lung inflammation
The role of the NF-κB signaling in the host immune response to lung injury is increasingly well understood [19]. NF-κB activation pathway gene polymorphisms alter the susceptibility to [29] and severity [30] of clinical ARDS. NF-κB is a dimer of a number of related proteins, including RelA (also known as P65), p50, p52, RelB, and cRel, with the RelA and p50 heterodimer, the most common form. On cell activation, by stimuli such as gram-negative bacterial endotoxin, diverse signaling pathways are activated, which converge to phosphorylate and activate the IκB kinase complex proteins (IKK), which then phosphorylate and inactivate the IκB proteins, which dissociates from NF-κB. The active NF-κB translocates to the nucleus and binds to specific cognate-binding sequences in the promoter or enhancer regions of different target genes to initiate transcription [5].

The therapeutic potential of strategies to inhibit NF-κB is evident from the demonstration that NF-κB inhibition decreases injury in nonseptic ALI models, including pulmonary [6] and systemic reperfusion, and endotoxemia [7]. Pulmonary overexpression of the RelB member of the NF-κB family decreases cigarette smoke-induced lung injury [31].

Table 5 Overexpression of Null or IκBα transgenes does not alter lung function or activate the immune response in animals subjected to sham prolonged pneumonia-induced ALI

| Variable                          | Vehicle | IκBα transgene | Null transgene |
|-----------------------------------|---------|----------------|---------------|
| Number of animals                 | 4       | 4              | 4             |
| Animal weight (g)                 | 411 ± 24| 409 ± 14       | 395 ± 11      |
| Animal survival (n, %)            | 4 (100%)| 4 (100%)       | 4 (100%)      |
| Duration of animal survival (hours)| 72 (72, 72)| 72 (72, 72)  | 72 (72, 72)  |
| Final arterial PO₂ (kPa)          | 18.3 ± 0.3| 17.6 ± 2.5     | 18.3 ± 0.2    |
| Final static compliance (ml/mm Hg)| 0.71 ± 0.07| 0.78 ± 0.09    | 0.75 ± 0.12   |
| BAL protein (mg/L)                | 961 ± 221| 829 ± 226      | 826 ± 180     |
| BAL neutrophils (x 10⁹/ml)        | 1.1 ± 0.7| 2.2 ± 1.7      | 1.4 ± 1.2     |
| BAL mononuclear cells (x 10⁹/ml)  | 5.5 ± 3.6| 5.2 ± 3.9      | 3.8 ± 2.4     |

Data are expressed as mean ± SD or median [interquartile range]. Final data are data collected on completion of the experimental protocol. *Significantly different from Vehicle group. BAL, bronchoalveolar lavage; CFU, colony-forming units.

ARDS, pneumonia, and NF-κB
The effects of modulation of the NF-κB in the setting of lung bacterial infection are less well understood [19]. NF-κB decoy oligodeoxynucleotides reduce ALI in mice in the early phases of cecal ligation and puncture-induced sepsis [8]. Selective inhibition of vascular endothelialNF-κB activity in endotoxemic transgenic mice reduced lung inflammation and increased survival [32]. Of interest, this approach improved survival and systemic organ function, but did not alter bacterial clearance, in septic mice [32]. However, others have found inhibition of NF-κB signaling to exert detrimental effects in the setting of infection. Inhibition of hepatocyte NF-κB activity reduced Listeria monocytogenes clearance, decreasing murine survival [11]. In contrast, clearance of pseudomonas bacteria from the mouse lung was enhanced by pulmonary overexpression of the RelA subunit of NF-κB [33], suggesting that the NF-κB pathway plays a pivotal role in maintaining immune competence and is essential to eradication of the infectious agent [11].
NF-κB: role in lung inflammation, injury, and repair

NF-κB also promotes cell survival, resolution of inflammation, and repair after injury. Inhibition of NF-κB signaling retards pulmonary [20] and intestinal [34] epithelial wound healing. Maturational differences in lung NF-κB activation profiles exist, with NF-κB activation protecting the lung against hyperoxia [35] and endotoxemia [36] in neonatal rats. Consequently strategies to inhibit NF-κB, particularly if not spatially or temporally targeted, may have detrimental effects.

Targeting pulmonary NF-κB

We wished to determine whether inhibition of pulmonary NF-κB activity could modulate the severity of pneumonia-induced lung injury. We found that pulmonary overexpression of the IκBα gene did reduce acute pneumonia-induced injury. IκBα decreased the decrement in arterial oxygenation and lung static compliance, decreased alveolar protein leak, and decreased histologic injury, compared with vehicle. IκBα modulated the cytokine response to E. coli instillation, decreasing alveolar IL-1β but increasing CINC-1 concentrations. Of importance, these effects were dose dependent, with benefit maximal at the intermediate (1 × 10^10) IκBα dose, and a loss of efficacy at the higher (1 × 10^11) IκBα concentration.

In contrast, in prolonged untreated E. coli pneumonia, pulmonary IκBα gene overexpression worsened the lung injury, and increased lung bacterial load. IκBα delayed the resolution of the acute inflammatory response, increasing the proportion of alveolar neutrophils while decreasing alveolar mononuclear cells, which comprise lymphocytes and macrophages and are considered important to repair. IκBα increased alveolar concentrations of TNF-α and IL-1β, cytokines implicated in the early phase of the response to septic insult. IκBα increased alveolar IL-10 concentrations, which may partly explain the ineffective response to the bacterial insult.

NF-κB and sepsis: an integrated paradigm

The contrasting effects of NF-κB inhibition in acute versus prolonged pneumonia generate a number of important insights. First, the finding that NF-κB inhibition reduced the severity of acute pneumonia and decreased the host response induced ALI suggests that any benefit in this setting is mediated via a decreased host response to the instillation of E. coli [37]. This is consistent with the concept that in the early phases of pneumonia, secreted toxins, and the host immune response may be the predominant source of injury [37,38]. This is supported by the fact that NF-κB inhibition is protective in nonseptic inflammatory ALI models. Second, this effect was dose dependent, with lower and intermediate IκBα doses protective and the beneficial effects ablated at higher doses. This suggests a U-shaped dose-effect curve, reducing the therapeutic utility of approaches to inhibit NF-κB in the setting of acute pneumonia. Third, IκBα worsened the severity of prolonged pneumonia, substantially increasing lung bacterial loads, and resulting in a persisting pulmonary inflammatory response. IκBα, by inhibiting the early host response to E. coli instillation, may have led to a persistence of infection, ultimately worsening lung injury. Finally, the beneficial effects of NF-κB inhibition in acute pneumonia were lost at the highest dose. The reasons for this is unclear, but may include NF-κB inhibition mediated reduction in lung epithelial repair [20], apoptosis of airway epithelial cells [16] and effects on lung macrophage function [39].

Conclusions

IκBα overexpression reduces injury severity and inflammation in early pneumonia, but slows resolution of inflammation and bacterial clearance and worsens injury during prolonged pneumonia. These findings raise serious questions regarding the utility of strategies that inhibit the innate immune response, such as NF-κB, in live bacterial pneumonia.

Key Message: Inhibition of pulmonary NF-κB activity decreases the severity of early pneumonia-induced lung injury, but worsens injury severity and bacterial load during prolonged pneumonia.

Abbreviations

AAV: adenoassociated virus; ALI: acute lung injury; ANOVA: analysis of variance; ARDS: acute respiratory distress syndrome; BAL: bronchoalveolar lavage; CINC-1: cytokine-induced neutrophil chemoattractant-1; DNA: deoxyribonucleic acid; E. coli: Escherichia coli; IκBα: inhibitory factor kappa B alpha; IL: interleukin; IQR: interquartile range; KGF: keratinocyte growth factor; qRT-PCR: quantitative real-time polymerase chain reaction; NF-κB: nuclear factor kappa B; PBS: phosphate-buffered saline; RNA: ribonucleic acid; TNF-α: tumor necrosis factor-α

Authors’ contributions

JD performed the animal experiments, assays, and histologic analyses, analyzed the data, drafted the manuscript, and agreed to the final submitted version. GFC and MH assisted with animal experiments, contributed to drafting the manuscript, and agreed to the final submitted version. CM, BA, and DOT performed assays and histologic analyses, contributed to drafting the manuscript, and agreed to the final submitted version. TOB conceived and designed the experiments, provided viral vector expertise, drafted the manuscript, and agreed to the final submitted version. JLS conceived and designed the experiments, provided viral vector expertise, drafted the manuscript, and agreed to the final submitted version. CM, BA, and DOT performed assays and histologic analyses, contributed to drafting the manuscript, and agreed to the final submitted version. GFC and MH assisted with animal experiments, contributed to drafting the manuscript, and agreed to the final submitted version. CM, BA, and DOT performed assays and histologic analyses, contributed to drafting the manuscript, and agreed to the final submitted version. TOB conceived and designed the experiments, provided viral vector expertise, drafted the manuscript, and agreed to the final submitted version. JLS conceived and designed the experiments, provided viral vector expertise, drafted the manuscript, and agreed to the final submitted version.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors acknowledge the help of Dr. Shirley Hanley in regard to the FACs analyses, and of Dr. Jeremy A. Scott for his critical review of the manuscript. Data from this manuscript were presented at the American Thoracic Society Annual Scientific Meeting, Denver, Colorado, May 2011. This study was funded by the Health Research Board, Ireland and by European Research Council. Dr Devaney is a postdoctoral researcher with the Health Research Board, Ireland (grant RP/2008/193). Dr OToole is a Research Fellow with the European Research Council (grant ERC-2007-StG
References

1. Rubenfeld GD: Epidemiology of acute lung injury. Crit Care Med 2003, 31: S276-284.

2. Zilberberg MD, Epstein SK: Acute lung injury in the medical ICU: comorbid conditions, age, etiology, and hospital outcome. Am J Respir Crit Care Med 1998, 157:1159-1164.

3. Markowicz P, Wolff M, Djedaini K, Cohen Y, Chastre J, Delclaux C, Merrer J, Herman B, Veber B, Fontaine A, et al: Multicenter prospective study of ventilator-associated pneumonia during acute respiratory distress syndrome: incidence, prognosis, and risk factors: ARDS Study Group. Am J Respir Crit Care Med 2000, 161:1942-1948.

4. TenHoor T, Mannino DM, Moss M: Risk factors for ARDS in the United States: analysis of the 1993 National Mortality Followback Study. Chest 2001, 119:1179-1178.

5. Park GT, Christian JW: Nuclear factor kappa B is a promising therapeutic target in inflammatory lung disease. Drug Targets 2006, 7:661-668.

6. Ishiyama T, Dharmarajan S, Hayama M, Moriya H, Grapperhaus K, Patterson GA: Inhibition of nuclear factor kappaB by IkappaB superrepressor gene transfer ameliorates ischemia-reperfusion injury after experimental lung transplantation. J Thorac Cardiovasc Surg 2005, 130:104-201.

7. Matsuda N, Hatton Y, Takahachi Y, Nishihira J, Jesmin S, Kobayashi M, Gando S: Therapeutic effect of in vivo transfection of transcription factor decoy to NF-kappaB on septic lung in mice. Am J Physiol 2004, 287:L1248-1255.

8. Matsuda N, Hatton Y, Jesmin S, Gando S: Nuclear factor-kappaB decoy oligodeoxynucleotides prevent acute lung injury in mice with cecal ligation and puncture-induced sepsis. Mol Pharmacol 2005, 67:1018-1025.

9. Rahman A, Faaz F: Blocking NF-kappaB: an inflammatory issue. Proc Am Thorac Soc 2011, 8:B97-503.

10. Batra S, Balamayooran G, Sahoo MK: Nuclear factor-kappaB: a key regulator in health and disease of lungs. Arch Immunol Ther Exp 2011, 59:335-351.

11. Lavon I, Goldberg J, Amit S, Landsman L, Jung S, Tsuberi BZ, Barshack I, Kopolovic J, Galun E, Bujard H, et al: High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF-kappaB activation. Nat Med 2000, 6:573-577.

12. O’Cinoin DF, Hopkins NO, Moore MM, Boylan JF, McGoughlin P, Laffey JG: Hypercapnic acidosis does not modulate the severity of bacterial pneumonia-induced lung injury. Crit Care Med 2005, 33:2606-2612.

13. Ni Chonghaile M, Higgins BD, Costello JF, Laffey JG: Hypercapnic acidosis attenuates severe acute bacterial pneumonia-induced lung injury by a neutrophil-independent mechanism. Crit Care Med 2008, 36:3135-3144.

14. Chonghaile MN, Higgins BD, Costello JF, Laffey JG: Hypercapnic acidosis attenuates lung injury induced by established bacterial pneumonia. Anesthesiology 2008, 109:837-848.

15. Sen S, Coutts S, Hydes SO, McMahon J, O’Doherty A, Barrant JS, Akhtar Y, Adegboyela T, Connolly CE, Sultan S, et al: Gene delivery to the vasculature mediated by low-titre adenovirus-associated virus serotypes 1 and 5. J Gene Med 2008, 10:143-151.

16. Hassett P, Curley GF, Contrasera M, Masterson C, Higgins BD, O’Brien T, Devaney J, O’Toole D, Laffey JG: Overexpression of pulmonary extracellular superoxide dismutase attenuates endotoxin-induced acute lung injury. Intensive Care Med 2011, 37:1680-1687.

17. O’Cinoin DF, Nichol AD, Hopkins N, Boylan J, O’Brien S, O’Conner C, Laffey JG, McGoughlin P: Sustained hypercapnic acidosis during pulmonary infection increases bacterial load and worsens lung injury. Crit Care Med 2008, 36:2128-2135.

18. Laffey JG, Honan D, Hopkins N, Hyvelin JM, Boylan JF, McGoughlin P: Hypercapnic acidosis attenuates endotoxin-induced acute lung injury. Am J Respir Crit Care Med 2004, 169:46-56.

19. Contrasera M, Ansari B, Curley G, Higgins BD, Hassett P, O’Toole D, Laffey JG: Hypercapnic acidosis attenuates ventilation-induced lung injury by a nuclear factor-kappaB-dependent mechanism. Crit Care Med 2012, 40:2622-2630.

20. O’Toole D, Hassett P, Contrasera M, Higgins B, McKeown S, McAuley D, O’Brien T, Laffey J: Hypercapnic acidosis attenuates pulmonary epithelial wound repair by an NF-κB dependent mechanism. Thorax 2009, 64:976-982.

21. Fein AM, Lippmann M, Holtzman H, Eliaza A, Goldberg SK: The risk factors, incidence, and prognosis of ARDS following sepsis. Chest 1983, 83:40-42.

22. Martin MA, Silverman HU: Gram-negative sepsis and the adult respiratory distress syndrome. Clin Infect Dis 1992, 14:1123-1128.

23. Markowicz P, Wolff M, Djedaini K, Cohen Y, Chastre J, Delclaux C, Merrer J, Herman B, Veber B, Fontaine A, et al: Multicenter prospective study of ventilator-associated pneumonia during acute respiratory distress syndrome: incidence, prognosis, and risk factors: ARDS Study Group. Am J Respir Crit Care Med 2000, 161:1942-1948.

24. Freeman BD, Correa R, Karzai W, Natanson C, Patterson M, Banks S, Fitz Y, Danner RL, Wilson L, Eichacker PQ: Controlled trials of rG-CSF and CD11b-directed MAb during hyperoxia and E. coli pneumonia in rats. J Appl Physiol 1998, 80:2066-2076.

25. Karzai W, von Specht BU, Parent C, Haberstroh J, Wollersen K, Natanson C, Banks SM, Eichacker PQ: G-CSF during Escherichia coli versus Staphylococcus aureus pneumonia in rats has fundamentally different and opposite effects. Am J Respir Crit Care Med 1999, 159:1377-1382.

26. Russo TA, Bartholomew LA, Davidson BA, Helinski JD, Carloin UB, Knight PR, Beers MF, Atchochina EN, Notter RH, Holm BA: Total extracellular surfactant is increased but abnormal in a rat model of gram-negative bacterial pneumonia. Am J Physiol Lung Cell Mol Physiol 2002, 283:L655-663.

27. Song GW, Robertson B, Cundt B, Gan XZ, Huang WX: Surfactant treatment in experimental Escherichia coli pneumonia. Acta Anesthesiol Scand 1996, 40:1154-1160.

28. Zeni F, Parent C, Correa R, Natanson C, Freeman B, Fontana J, Quezado M, Danner RL, Fitz Y, Richmond S, et al: ICAM-1 and CD11b inhibition worsen outcome in rats with E. coli pneumonia. J Appl Physiol 1999, 87:299-307.

29. Zhai R, Zhou W, Gong MN, Thompson BT, Su L, Yu C, Kraft P, Christiansen DC: Inhibitor kappaB-alpha-haplotyping in human B cells is associated with susceptibility to acute respiratory distress syndrome in Caucasians. Crit Care Med 2007, 35:893-898.

30. Adamaki M, Frey UH, Rieaman K, Sitt B, Beiderlingen M, Siffert W, Peters J: Insertion/deletion polymorphism in the promoter of NFκB1 influences severity but not mortality of acute respiratory distress syndrome. Intensive Care Med 2007, 33:1199-1203.

31. McMillan DH, Baglole CJ, Thatcher TH, Maggirwar S, Sime PJ, Phipps RP: Lung-targeted expression of the NF-kappaB member RelB inhibits cigarette smoke-induced inflammation. Am J Pathol 2011, 179:125-133.

32. Ye X, Ding J, Zhou X, Chen G, Liu SF: Divergent roles of endothelial NF-kappaB in multiple organ injury and bacterial clearance in mouse models of sepsis. J Exp Med 2008, 205:1303-1315.

33. Sadikot RT, Zeng H, Joo M, Everhart MB, Sherrill TP, Li B, Cheng DS, Yull FE, Christman JW, Blackwell TS: Targeted immunomodulation of the NF-kappaB pathway in airway epithelium impacts host defense against Pseudomonas aeruginosa. J Immunol 2006, 176:4923-4930.

34. Egan LJ, Eckermann L, Greten FR, Choe S, Li ZW, Myhre GM, Robine S, Karin M, Kappooff MF: IkappaB-kinaselbeta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. Proc Natl Acad Sci USA 2004, 101:2452-2457.

35. Yang G, Abate A, George AG, Weng YH, Denney PA: Maturational differences in lung NF-kappaB activation and their role in tolerance to hyperoxia. J Clin Invest 2004, 114:649-658.

36. Alvira CM, Abate A, Yang G, Denney PA, Rabonvitch M: Nuclear factor-kappaB activation in neonatal lung protects against lipopolysaccharide-induced inflammation. Am J Respir Crit Care Med 2007, 175:805-815.
37. Curley G, Contreras MM, Nichol AD, Higgins BD, Laffey JG: Hypercapnia and acidosis in sepsis: a double-edged sword? Anesthesiology 2010, 112:462-472.

38. Laffey JG, O’Croinin D, McLoughlin P, Kavanagh BP: Permissive hypercapnia: role in protective lung ventilatory strategies. Intensive Care Med 2004, 30:347-356.

39. Groesdonk HV, Schlottmann S, Richter F, Georgieff M, Senftleben U: Escherichia coli prevents phagocytosis-induced death of macrophages via classical NF-κB signaling, a link to T-cell activation. Infect Immun 2006, 74:5989-6000.

doi:10.1186/cc12696
Cite this article as: Devaney et al: Inhibition of pulmonary nuclear factor kappa-B decreases the severity of acute Escherichia coli pneumonia but worsens prolonged pneumonia. Critical Care 2013 17:R82.