Respiratory Syncytial Virus infection facilitates acute colonization of *Pseudomonas aeruginosa* in mice

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Respiratory Syncytial Virus infection facilitates acute colonization of *Pseudomonas aeruginosa* in mice

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Keywords:	viral-bacterial synergism, inflammation, pneumonia, cystic fibrosis

Bacteriology of lung homogenates (n=10 in each group, results of two experiments, each showing a significantly higher median number of CFU for co-infected mice), representing the number of CFU per ml of lung homogenate, 24 hours after inoculation with 0.5×10^5 CFU per mouse. Bars represent median number of CFU per group.
27x18mm (600 x 600 DPI)

Enhanced pause (A), minute ventilation (B) and breathing frequency (C) measured at baseline and one day after simultaneous exposure to either RSV, *P. aeruginosa*, saline or both. Boxes represent mean values per group of mice (n=15 in each group), with bars showing SD. * $p < 0.05$

59x89mm (600 x 600 DPI)

	saline	RSV	<i>P. aeruginosa</i>	RSV + <i>P. aeruginosa</i>
epithelial damage	-/+	+++	++	+
inflammation	-/+	-/+	++	++

Table 1 Histopathology scores of mouse lungs (n=5 mice in each group)

Histopathology of mouse lungs 24 hours after inoculation with saline (A), RSV (B), *P. aeruginosa* (C) or RSV and *P. aeruginosa* (D). RSV-infected control mice display extensive damage to cells of the bronchial epithelium. Mice infected with *P. aeruginosa* alone or RSV and *P. aeruginosa* show inflammatory changes in the lung parenchyma. Magnification 320 x (c,d), 500 x (b) and 800 x (a). 169x127mm (300 x 300 DPI)

Title:

Respiratory Syncytial Virus infection facilitates acute colonization of *Pseudomonas aeruginosa* in mice

Running title:

RSV facilitates *P. aeruginosa* colonization

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Abstract

Pseudomonas aeruginosa causes opportunistic infections in immunocompromised individuals and patients ventilated mechanically and is the major pathogen in patients with cystic fibrosis, in which it causes chronic infections. Epidemiological, *in vitro* and animal data suggest a role for respiratory virus infections in facilitating colonization and infection with *P. aeruginosa*. A study was undertaken to determine whether respiratory syncytial virus (RSV) infection could facilitate the initiation of an acute infection with *P. aeruginosa* *in vivo*. Balb/c mice were infected intranasally with *P. aeruginosa*, with and without simultaneous inoculation with RSV. Lung function measurements were undertaken using Whole Body Plethysmography and lungs were harvested 24 hours after inoculation. Mice exposed to RSV and *P. aeruginosa* showed 2000 times higher colony forming unit (CFU) counts of *P. aeruginosa* in the lung homogenates when compared to mice which were only infected with *P. aeruginosa* and lung function changes were more severe in co-infected mice. Control mice receiving RSV alone showed no significant changes in lung function or cytokine production, and no inflammatory changes in the lung parenchyma. These results suggest that RSV can facilitate the initiation of acute *P. aeruginosa* infection without the RSV infection being clinically apparent. This could have implications for treatment strategies to prevent opportunistic *P. aeruginosa* lung infection.

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20 **Introduction**

21

22 *Pseudomonas aeruginosa* occurs ubiquitously throughout the environment but rarely causes
23 disease in immunocompetent individuals, even though it is present in water and the
24 gastrointestinal or upper respiratory tract [Sordelli et al., 1985]. In immunocompromised
25 individuals or patients ventilated mechanically *P. aeruginosa* causes opportunistic infections
26 like pneumonias, medical device-related infections and super infection of burn wounds
27 [Sutterwala et al., 2007]. *P. aeruginosa* is the principal infectious pathogen in cystic fibrosis,
28 a hereditary disease characterized mainly by chronic infections of the respiratory tract. In
29 these patients up to 80% develop a chronic infection which is accompanied by significant
30 morbidity and eventually mortality [Pressler et al., 2006].

31

32 Factors resulting in a predisposition to infection with *P. aeruginosa* are poorly understood.
33 Decrease of innate or adaptive immunity in the immunocompromised host and decrease of
34 local defence mechanisms in patients with burn wounds or mechanical ventilation might play
35 a role. In patients with cystic fibrosis, dehydrated airway secretions and mucus plugging,
36 altered composition of airway surface liquid and impaired host defence against *P. aeruginosa*
37 are suggested [Sadikot et al., 2005]. Additionally, ceramide accumulation has been reported
38 to facilitate *P. aeruginosa* infection in a cystic fibrosis mouse model [Teichgraber et al.,
39 2008]. Furthermore, several bacterial factors of *P. aeruginosa* are thought to contribute to the
40 establishment and persistence of chronic infection, such as the ability to form biofilms,
41 conversion to a mucoid phenotype and the accumulation of multiple mutations over time,
42 including some which may promote persistence and chronic infection [Smith et al., 2006].

43

44 It has been suggested that viruses can play a role in the initiation or facilitation of *P.*
45 *aeruginosa* respiratory infections in previously healthy subjects. In a 25 year retrospective
46 study in Danish patients with cystic fibrosis, the onset of initial colonization and chronic

infection with *P. aeruginosa* was observed to have a seasonal pattern that paralleled the respiratory virus season [Johansen and Hoiby, 1992]. In another study on viral respiratory infections in cystic fibrosis, viral infections like respiratory syncytial virus (RSV) were shown to be more common in patients who developed a chronic infection with *P. aeruginosa* during the study period than in patients who did not develop a chronic infection, and these infections were frequently associated with a rise in *P. aeruginosa* antibodies in patients who were chronically infected with this pathogen [Petersen et al., 1981].

Additionally, several *in vitro* studies have investigated the role of viruses in *P. aeruginosa* adherence to and infection of epithelial cells. In a study on adherence of bacteria to murine tracheas *P. aeruginosa* adhered to the desquamating cells of tracheas infected with influenza but not to normal mucosa [Ramphal et al., 1980], suggesting that the influenza virus might play a facilitating role in the pathogenesis of *P. aeruginosa* infection. In a mouse model of RSV and *Streptococcus pneumoniae*, the clearance of *S. pneumoniae* was found to be decreased following RSV infection [Stark et al., 2006]. Furthermore, *in vitro* studies in human airway epithelial cells showed an increase in adherence of *P. aeruginosa* to cells pre-infected with RSV. Even simultaneous infection of virus and bacteria resulted in enhanced adherence of *P. aeruginosa* [Van Ewijk et al., 2007].

The present study builds on previous epidemiological and *in vitro* observations and investigates whether simultaneous RSV infection can facilitate the initiation of an acute infection with *P. aeruginosa* in otherwise healthy mice. To this end an acute lung infection mouse model was used, with intranasal inoculation of the pathogens. The primary goal was to investigate the effect of RSV on the pulmonary bacterial load after acute infection with *P. aeruginosa*. Secondly, the functional effects were studied by measuring associated changes in lung function and the inflammatory response by evaluating lung histopathology and cytokine production in bronchoalveolar lavage fluid. The data reported below demonstrate that RSV

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74 infection can facilitate the initiation of an acute infection with *P. aeruginosa* in otherwise
75 healthy mice.

76

77 ***Materials and methods***

78 **Mice**

79 Female Balb/c mice aged 12-15 weeks were obtained from Taconic (Tornbjerg, Denmark).
80 After transportation the mice were allowed to recover for at least one week, before being used
81 in experiments. The mice were housed in cages of 5, in a clean experimental unit (specific
82 pathogen-free) with barrier provisions and systematic health monitoring, at the Panum
83 Institute, Copenhagen, Denmark. The mice received commercial food and water *ad libitum*.
84 After inoculation with RSV, the mice were housed in an isolated cabinet. All cages were
85 placed on a heat pad for several hours after infection (Scanbur, Karlslunde, Denmark). The
86 local animal care committee approved the procedures performed on the mice in this study.

87 **Bacteria**

88 A clinical *P. aeruginosa* isolate was obtained from a patient with cystic fibrosis who attended
89 the cystic fibrosis centre at the Rigshospitalet, Copenhagen, Denmark. It was the first isolate
90 obtained in this patient, and has been described [Jelsbak et al., 2007]. Bacteria were
91 inoculated in Luria-Bertani (LB) medium and grown shaking overnight at 37°C. Serial
92 dilutions were made with sterile 0.9 % saline, which were plated on blue agar plates (a
93 modified Conradi Drigalski's medium selective for Gram-negative rods; State Serum
94 Institute, Copenhagen, Denmark) for determination of colony-forming units (CFU) after
95 overnight incubation at 37°C. The dilution factor that yielded around 1 x 10⁶ CFU/ml was
96 determined and used for diluting the overnight culture with sterile saline. Inocula were
97 confirmed by plating the serial dilutions.

98 Virus

99 RSV serotype A was used in all experiments [Hament et al., 2005]. Stock aliquots contained
100 1.26×10^7 plaque-forming units (PFU)/ml and were stored at -80°C .

101 Intranasal inoculation

102 Mice were anaesthetized by intraperitoneal injection of a freshly prepared mixture of
103 ketamine (65 mg/kg), xylazine (13 mg/kg) (both Intervet, Skovlunde, Denmark) and sterile
104 isotonic saline. Mice were held in an upright position and while pushing their mandible to
105 close the mouth, 50 μl of RSV inoculum (activity 0.63×10^6 PFU) was applied drop wise to
106 the nostrils with a pipette, followed by 50 μl ($\sim 0.5 \times 10^5$ CFU) of bacterial inoculum. Mice
107 were held upright until the inoculum was inhaled completely. A period of 60 minutes was
108 allowed between the viral inoculation and the bacterial challenge, to prevent the mice from
109 inhaling large amounts of fluid at once. Control mice received bacteria, RSV or sterile saline
110 only.

111 Validation of the nasal inoculation model

112 Nasal application of *P. aeruginosa* or RSV was described previously [Allewelt et al., 2000;
113 Hament et al., 2005]. In order to investigate whether this would be a practical method of
114 administering a sufficient amount of inoculum to the lungs after nasal inoculation, a radio-
115 labelled peptide ((125)I-TFF2) was used [Poulsen et al., 2003]. Mice were anaesthetized as
116 described above. The mice were then held in an upright position and while pushing the
117 mandible to close the mouth, 50 μl of the peptide solution was placed over the nostrils. Mice
118 were held in an upright position until the solution was inhaled completely. The mice were
119 then killed using pentobarbital and the lungs and stomach were removed. Radioactivity was
120 measured in the lungs, stomach and a control amount of 50 μl of the peptide solution using a
121 gamma counter.

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Measurement of pulmonary function

To quantify changes in respiratory physiology induced by infection Whole Body Plethysmography (WBP) was used in unrestrained mice (Buxco, Troy, NY). Before each measurement, each chamber was calibrated to ambient pressure and temperature by rapidly introducing 1 ml of air into the chamber. Each mouse was placed inside a separate chamber and was provided with fresh air through a bias flow inlet. Readings were taken for 5 minutes, in which pressure differences between the main chamber (containing the mouse) and the reference chamber were measured. Several respiratory variables including enhanced pause (PenH; a non-dimensional measurement that correlates with airway resistance [Hamelmann et al., 1997]) were calculated using software supplied by the manufacturer. Lung function was measured at baseline in all mice (15 mice per treatment group), and again 24 hours after inoculation, right before termination of the experiment.

Lung bacteriology evaluation

After completion of lung function measurements 24 hours after inoculation, mice were killed using intraperitoneal pentobarbital (200mg/ml)/lidocaine (20 mg/ml) injection (10 mice per group in each experiment). Lungs were removed and homogenized on ice at 13,500 rpm in 3 ml sterile saline, using a Heidolph diax 600 homogenizer (Struers, Denmark). The homogenates were serially diluted in sterile saline. 100 µl of the homogenate and 100 µl of every dilution were plated on blue agar plates and incubated overnight at 37°C for counting of colony-forming units (CFU). Colonies were assessed by appearance and development of a deep blue colour within 10 seconds when stained with oxydase reagents (State Serum Institute, Copenhagen, Denmark) to detect *P. aeruginosa* (presence of the enzyme complex cytochrome c).

Bronchoalveolar lavage fluid studies and histopathology

In each group 5 mice were randomly selected to undergo bronco alveolar lavage after having been killed as described above. The trachea was exposed and a venflon catheter (22G) was

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3 148 inserted into the trachea. Lavage was done by flushing the lungs once with 0.7 ml of sterile
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5 149 0.9 % saline. Lavage fluid was immediately frozen on dry ice and later stored at -80°C.
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8 150 Antibody bead kit assays (mouse 5-plex and 10-plex) were performed to measure cytokines
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10 151 (Biosource, Camarillo, CA). Concentrations were measured of pro-inflammatory cytokines:
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12 152 IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, GM-CSF (which promotes granulocyte and
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14 153 monocyte production), IFN- γ , and TNF- α . Additionally 5 chemokines were included in the
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16 154 assay: KC (an IL-8 homologue), MCP-1, MIG (monokine induced by IFN- γ), IP10 and MIP-
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18 155 1 α . Measurements were done using a Luminex 100 IS (Luminex Corporation). STarStation
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20 156 software (Applied Cytometry Systems, Sheffield, UK) was used for analysis and acquisition
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22 157 of data. For histopathology studies, lungs were fixed in situ by intratracheal injection of 4%
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24 158 buffered formaldehyde, removed and put into more fixative. After fixation the lungs were
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26 159 embedded in paraffin wax and cut into 5 μ m sections, followed by staining with hematoxylin
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28 160 and periodic-acid Schiff (PAS). The samples were evaluated blind by two pathologists.
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33 161 **Statistical analysis**

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35 162 Non-Gaussian distributed results were compared using Mann Whitney U-test. Lung function
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37 163 measurements were compared by unpaired Student's t-tests. Level of significance: $p < 0.05$.
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39 164 SPSS version 15 for Windows was used.
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Results

Nasal inoculation

Effectiveness of nasal application was investigated by administration of 50µl of radio-labelled peptide solution followed by excision of the lungs and stomach for radio-active particle counting. The counts detected in the lungs and stomachs of the mice were compared to an equivalent sample of the original peptide solution. The mean percentage of radioactive particles detected in the lungs (relative to the control fluid) was 71% (range 61-76%). The stomachs of the mice did not contain any detectable radio-active particles indicating that the fluid was delivered to the lungs without also being swallowed.

Effect of simultaneous RSV infection

Lung bacterial load

Mice were infected with RSV and *P. aeruginosa* simultaneously, or only with *P. aeruginosa* (approximately 0.5×10^5 CFU/mouse). Lungs were harvested 24 hours after infection and quantitative cultures of lung homogenates were performed. Mice exposed to both RSV and *P. aeruginosa* showed about 2000 times higher CFU counts of *P. aeruginosa* in the lung homogenates when compared to mice infected only with *P. aeruginosa* (median 3.05×10^1 CFU/ml of lung homogenate for mice infected with bacteria only and 6.0×10^4 CFU/ml of lung homogenate for co-infected mice ($p<0.01$) (Figure 1)).

Lung function

To evaluate the respiratory physiology changes, lung function measurements were performed in unrestrained mice at baseline and 24 hours after inoculation. Measurements were also performed in control mice inoculated with only saline or RSV. Baseline values of Penh (enhanced pause, a measure of airway resistance), minute ventilation and breathing frequency were similar in all groups of mice (Figure 2). After 24 hours, co-infected mice showed a

190 lower breathing frequency when compared to the mice infected only with bacteria (332/min
191 vs. 381/min respectively, $p<0.05$). The breathing frequency in the RSV control mice was no
192 different from the saline control group (mean 494/min vs. 464/min respectively, $p>0.05$). A
193 similar effect was observed for minute ventilation. The co-infected mice had significantly
194 lower minute ventilation when compared to the group infected only with *P. aeruginosa* (65.6
195 and 85.6 ml respectively, $p<0.05$). RSV control mice showed no significant difference
196 compared to the saline group (103.6 ml and 95.7 ml respectively, $p>0.05$). Enhanced pause
197 (Penh) was elevated in both groups of mice infected with bacteria (1.74 for co-infected mice
198 and 1.37 for mice infected with *P. aeruginosa*, $p>0.05$) and was not different in the RSV
199 group compared to the saline group (0.63 and 0.57, $p>0.05$).

200 *Bronchoalveolar lavage fluid*

201 Cytokine analysis of the lavage fluid was performed to evaluate the production of pro-
202 inflammatory cytokines and chemokines in the lungs. IFN- γ was undetectable in all groups
203 and IL-2, IL-4 and IL-5 gave unreliable standard curves, and therefore these results were
204 omitted. For all cytokines, mice infected with RSV alone showed no significant production of
205 cytokines when compared to control mice that received saline. Production of KC (IL-8
206 homologue) was observed but this was minimal (mean 22.3 pg/ml (SD 19.32) for RSV
207 infected mice and 0.0 pg/ml (SD 0.0) for control mice, $p=0.06$). There was increased
208 production of the measured cytokines in mice infected with *P. aeruginosa* only and co-
209 infected mice. However, comparison of these two groups of mice showed no significant
210 differences in cytokine production, except for a minimal difference in MIG (a T-cell
211 chemoattractant) production (54.8 pg/ml (SD 30.3) for *P. aeruginosa* infected and 22.1 pg/ml
212 (SD 8.5) for co-infected mice, $p=0.03$).

213 *Histopathology*

214 Histopathology slides were evaluated and scored for severity of inflammatory changes of lung
215 tissue and bronchial epithelial changes. Scoring was done on a 5-point scale, and the average

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was calculated for results from the two observers (Table 1). Examination of the lungs of mice infected with *P. aeruginosa* and with *P. aeruginosa* and RSV showed a similar severity of inflammation in the lung parenchyma. The inflammation was characterized by perivascular and alveolar infiltration with inflammatory cells (Figure 3 c and d). The lungs of control mice receiving saline or RSV did not show substantial inflammatory changes in the lung parenchyma. Epithelial damage was observed in all groups of mice to some degree, but was most severe in the mice infected with RSV only. Bronchial epithelial cells of these mice showed a high degree of vacuolization and sloughing of nonciliated epithelial cells (Clara cells) and also damage to ciliated epithelial cells. This was more pronounced distally i.e. in the bronchioles (Figure 3 b).

226 Discussion

227

228 Findings from *in vitro* experiments suggest that viral infections can have a facilitating role in
229 *P. aeruginosa* airway infection [Van Ewijk et al., 2007]. Clinical observations provide
230 circumstantial evidence for this enhancing effect in cystic fibrosis [Johansen and Hoiby,
231 1992; Petersen et al., 1981]. The aim of the experiments presented here was to study the *in*
232 *vitro* findings in an *in vivo* model. Lung bacterial load and associated lung function changes
233 were compared between mice infected with RSV and *P. aeruginosa* and mice infected only
234 with *P. aeruginosa*. This showed that co-infected mice had a higher bacterial load and more
235 severe lung function changes, suggesting that *P. aeruginosa* was better able to establish an
236 infection in the presence of an RSV infection. Evaluation of the associated immunological
237 response by histopathology and cytokine analysis showed inflammatory changes in both
238 groups of mice infected with bacteria, but no parenchymal inflammation in control mice
239 infected only with RSV. This suggests that in this early infection model the facilitating effect
240 of RSV occurred independent of an immunological response to the virus.

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242 A facilitating role in bacterial infections by viruses has been reported widely for various
243 pathogenic agents [Hament et al., 1999]. Several mechanisms are suggested that could explain
244 an enhancing effect of viral infections like RSV on acute pulmonary infection with *P.*
245 *aeruginosa*. The first is that respiratory viral infections disrupt normal tissue architecture of
246 the respiratory epithelium and cause loss of cilia, which might allow bacteria to adhere more
247 easily [Patel et al., 1992]. This effect has been observed in a mouse model of influenza, where
248 *P. aeruginosa* was able to adhere only to the desquamating cells of influenza-infected
249 tracheas [Ramphal et al., 1980]. The second is that immunological changes caused by viruses
250 can lead to less efficient clearance of bacteria. In a chinchilla model, influenza infection has
251 been observed to reduce neutrophil functions including respiratory burst and bacterial killing
252 [Abramson et al., 1982]. In another study, clearance of *S. pneumoniae* after RSV infection in

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3 253 mice was significantly reduced 1-7 days following RSV exposure [Stark et al., 2006]. This
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5 254 effect of RSV on bacterial clearance was not specific to *S. pneumoniae*: similar decreases in
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7 255 clearance were observed for *Staphylococcus aureus*. The effect of RSV infection on *P.*
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9 256 *aeruginosa* (PA01) clearance after RSV infection was studied and in common with the
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11 257 present observations significantly higher colony counts were found in mice with RSV
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13 258 infection. These results were obtained after bacterial challenge 7 days following RSV
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15 259 exposure as opposed to the present study in which the bacterial challenge was preformed
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17 260 simultaneously with viral inoculation. In the *S. pneumoniae* experiments, analysis of
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19 261 bronchoalveolar lavage fluid demonstrated a greater rise in macrophages and
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21 262 polymorphonuclear neutrophils in RSV-infected mice with *S. pneumoniae* when compared to
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23 263 controls. Neutrophil cellular myeloperoxidase levels were decreased in RSV infected mice
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25 264 which is suggestive of an alteration in neutrophil function being the possible cause of
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27 265 decreased bacterial clearance [Stark et al., 2006]. In another study, viral infection of mice
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29 266 with Lymphocytic Choriomeningitis Virus (LCMV), through induction of type I IFN, led to
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31 267 apoptosis of bone-marrow granulocytes and impaired granulocyte migration to sites of
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33 268 bacterial infection, suggesting that increased susceptibility to bacterial superinfection in viral
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35 269 disease may occur as a consequence of the innate antiviral response [Navarini et al., 2006].
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37 270 The third mechanism is increased adherence of pathogenic bacteria as a result of virus-
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39 271 induced upregulation of cellular molecules that act as receptors for pathogenic bacteria.
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41 272 Bacteria can bind to virus-induced glycoproteins expressed on the surface of infected cells,
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43 273 e.g. glycoprotein G and F [Hament et al., 1999]. Additionally, *in vitro* studies showed direct
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45 274 binding between RSV and *S. pneumoniae* and RSV and *P. aeruginosa*, suggesting that RSV
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47 275 acts as a coupling agent between these bacteria and respiratory cells [Hament et al., 2004;
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49 276 Van Ewijk et al., 2007].
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53 278 The exact mechanisms responsible for the enhancing effect of RSV on *P. aeruginosa* airway
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55 279 infection in the studies presented require further study. Virus-induced changes in the
56
57 280 immunologic response may have a role. However, control mice receiving only RSV did not
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show significant changes in lung function and minimal upregulation (although not significant) of cytokine production. Histopathological evaluation of the lungs showed damage to the airway epithelium but no alveolar tissue inflammation. This suggests that the inflammatory response to RSV is not substantial 24 hours after inoculation. This is in accordance with studies of primary RSV infections in Balb/c mice, where viral titres peak after 4-6 days, around which time pathological changes are observed in lung tissue [Graham et al., 1988; Jafri et al., 2004]. Others have observed that RSV induced profound effects on ciliated cells of the respiratory epithelium, including loss of cilia and sloughing of cells [Tristram et al., 1998]. In the present model damage to the respiratory epithelium was also observed, especially in non-ciliated epithelial cells (Clara cells). Virus-induced damage to airway epithelium could have been a contributing factor to the facilitating effect of RSV. Promotion of bacterial adhesion to the respiratory epithelium by viruses has also been observed for other viruses and bacteria [Avadhanula et al., 2006]. These findings are important since adherence of bacteria has been shown to be an important first step in the establishment of bacterial pneumonia in a mouse model [Mitsushima et al., 2002]. Additionally, virus-induced loss of ciliary function could have impaired the mucociliary clearance mechanism, leading to an inability of virus-infected mice to successfully clear the bacteria. Furthermore, since the virus and bacteria were added simultaneously, it is a possibility that direct binding between *P. aeruginosa* and RSV, as observed *in vitro* [Van Ewijk et al., 2007], played a role in the study presented here. Hament et al. demonstrated a similar effect, when studying the effect of RSV on *S. pneumoniae* infection [Hament et al., 2005]. In their *in vitro* studies, RSV was shown to increase adherence of pneumococci and they also observed direct binding between the virus and the bacteria. In a mouse model, they observed an enhancing effect on bacteraemia in the presence of RSV, which was strongest when RSV and *S. pneumoniae* were added simultaneously. Similarly, in a mouse model of pneumococci and influenza virus, the virus increased mortality in pneumococcal infection in mice, and an additional effect in mortality rates was observed when mice were inoculated simultaneously with virus and bacteria [McCullers and Rehg, 2002].

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The clinical importance of the present findings is that they provide some insight into the role of respiratory viral infections in *P. aeruginosa* airway infections. The results confirm observations from *in vitro* experiments. Furthermore, these data support circumstantial clinical evidence for a facilitating role in colonization with *P. aeruginosa* as observed in patients with cystic fibrosis. In contrast to other studies on virus-bacterial interaction, an early infection model was used, in which the RSV infection did not last beyond 24 hours, showing that the facilitating effect of RSV occurs within the initial phase of infection, without the presence of a clinical effect of RSV or a substantial inflammatory response. This could have implications for possible strategies to prevent the opportunistic colonization with *P. aeruginosa*, suggesting that the enhancing effect can take place during simultaneous infection. For this purpose, prevention of respiratory viruses by immunization or prophylactic use of antibiotics during the respiratory virus season could be possible treatment options, for instance in patients with cystic fibrosis. For RSV no vaccine is available at present [Malfroot et al., 2005], but the use of passive prophylaxis with palivizumab has been suggested to be of possible benefit in reducing hospitalizations in a recent retrospective study in young CF patients [Giebels et al., 2008]. Further prospective studies are needed to investigate the effect of RSV infection prevention on reduction of bacterial infections.

In conclusion the present study shows that in a mouse model, acute airway colonization with *P. aeruginosa* can be facilitated by viral infections like RSV: The presence of an RSV infection led to increased lung bacterial loads, and more severe lung function changes. This effect occurred during simultaneous infection with RSV, without the occurrence of a significant immune response, suggesting that even without the virus being apparent clinically, its facilitating effect can take place. This could be important for understanding and prevention of opportunistic airway infection with *P. aeruginosa*.

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For Peer Review

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