Pseudomonas aeruginosa induced acute emphysema through bacterial secretion in mice

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Authors' Contributions

Yajie Zhu contributed to the original findings and the majority of the study conception, data analysis, and manuscript preparation. Shuming Pan contributed partly to the study conception, data analysis, and manuscript preparation. Shuming Pan was responsible for the study supervision. Both authors critically reviewed the manuscript for important intellectual content and read and approved the final manuscript.

Declaration of interests

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Abstract

Pulmonary emphysema is the major pathological feature of chronic obstructive pulmonary disease (COPD). It is a slowly progressive pathological condition and acute lung infection is not considered to play a role in emphysema initiation. Recently, we found that the secretion from *Pseudomonas aeruginosa* could cause severe lung emphysema in mice rapidly. Since this bacterium is ubiquitous and secrets proteases, we hypothesized that direct *P. aeruginosa* airway infection would have a similar effect. To address this issue, we applied a unilateral lung injury model. *P. aeruginosa* secretion was extracted and instilled intratracheally into the left lung of C57BL/6 and C3H/HeJ mice, while the right lung was saved as self-control. Alveolar diameter and lung compliance were measured. Later, we tested the effect of *P. aeruginosa* inoculation in C57BL/6 mice, immunosuppressed C57BL/6 mice, and C3H/HeJ (TLR4 deficient) mice. *P. aeruginosa* secretion extract caused acute panacinar emphysema and decreased dynamic lung compliance. Different types of emphysema are transformable. However, the *P. aeruginosa* infection could only elicit emphysema in C3H/HeJ mice and immunosuppressed C57BL/6 mice, indicating normal immunity is essential to protect the hosts from emphysema. *P. aeruginosa* could be an important pathogen in COPD initiation. Our finding filled a major gap in COPD pathogenesis.

Key words: Emphysema, COPD, pathology, pathogenesis, unilateral lung injury, *Pseudomonas aeruginosa*

Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death globally, characterized by irreversible obstruction of the small airways (bronchiolitis) and destruction of lung parenchyma (emphysema), which lead to air trapping, dyspnea, and even respiratory failure[1]. The pathogenesis of COPD is largely attributed to cigarette smoking induced chronic lung inflammation; however, it could not explain the fact that many COPD patients did not smoke [2], and only 40% of heavy smokers developed emphysema[3]. Current animal models established by passive tobacco smoking or elastase instillation poorly reproduce the main features of human COPD[4]. Accumulating evidence suggest a better understanding of the disease mechanism is needed.

Pseudomonas aeruginosa is a gram-negative, aerobic, opportunistic bacterium with a wide distribution. In the lung, *P. aeruginosa* is known to prevalent among immunocompromised hosts, causing cystic fibrosis, bronchiectasis and nosocomial pneumonia [5]. *P. aeruginosa* would colonize after COPD formation and contribute to the acute exacerbation [6, 7]. It can be isolated from 4-15% of adults with COPD, a number probably greatly underestimated due to this organism's unique ability to form biofilms, making it difficult to detect[8]. *P. aeruginosa* expresses a variety of virulence factors including endotoxin, exotoxin, elastase, alkaline proteases and hemolysins [9]. The proteases can disrupt epithelial cell tight junctions, cleave collagen, IgG, IgA, complement, and degrade surfactant proteins, causing harm to the lung[10]. However, the manifestation of *P. aeruginosa* pneumonia reported is nonspecific compared with other bacteria[11].

Recently, we discovered accidentlly that distinguished from Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli and Proteus mirabilis, the secretion of *P. aeruginosa* could induce severe pulmonary emphysema in murine rapidly. Surprised by this finding, for bacterial factors were never reported to be an initiator in emphysema

development [4, 12], we hypothesized that this phenomenon was caused by the proteases secreted by *P. aeruginosa*. Thus, theoretically, *P. aeruginosa* infection shall lead to acute emphysema too. To address the issue, a unilateral lung instillation model of normal C57BL/6, immunosuppressed C57BL/6 and C3H/HeJ mice were used. We observed the dynamic pathology evolution and physiology changes in this study.

Materials and Methods

Ethical statement

All experiments referring to the use of animals in this study were approved by the Institutional Animal Care and Use Committee of Shanghai Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine (XHEC-F-2018-047).

Mice

C57BL/6 mice (10 weeks old) were purchased from Shanghai Sippr-BK laboratory animal Co. Ltd; C3H/HeJ mice (10weeks old) were purchased from Shanghai SLAC laboratory animal Co. Ltd. All mice were accommodated at the Model Animal Research Center of Xinhua Hospital in a specific pathogen-free animal facility under constant temperature and humidity, with sufficient qualified food and water for 1 to 2 weeks before use.

Isolation and identification of *P. aeruginosa*

P. aeruginosa strain was originally separated from sputum, confirmed through mass spectrometry and the strain kept at the bacteriology lab of Xinhua Hospital. To observe the morphology and culture characteristics, the purified strain was streaked on Sheep blood agar plates (Yi Hua, Shanghai) and nutrient agar plates (Comagal, Shanghai) and incubated at 37°C for 24-36 hours.

Bacterial secretion extraction and identification

90 1×10^6 CFU *P. aeruginosa* were streaked on sheep blood agar plate and incubated at
91 37°C for 24–48 hours. To harvest the *P. aeruginosa* secretion, all colonies on the blood
92 agar plate were scratched down, collected, and dissolved in 5ml PBS then centrifuged
93 at 3000 rcm for 10 minutes. The supernatant was collected, filtered through a 0.22μm
94 PES membrane filter unit (Millex, Merck), and stored at -20°C for later use. The
95 quantification of the supernatant protein concentration was carried through BCA
96 protein assay (Beyotime, China). Protein types in the secretion were identified by
97 protein mass spectrometry. Briefly, the secretion extract went through enzymatic
98 hydrolysis and was analyzed by a Q-Exactive mass spectrometer (Thermo, USA).
99 Proteome Discoverer (v.2.4) was used to search all of the raw data thoroughly against
100 the protein database (UniProt_ *Pseudomonas aeruginosa*). Names and abundances of
101 proteins were acquired as the result.

102 **Live bacteria preparation**

103 *P. aeruginosa* was streaked on sheep blood agar plate and incubated at 37°C for 8 hours.
104 A proper number of colonies were inoculated in sterile PBS and the turbidity was
105 adjusted to 2 McFarland standards (MCF), which equals 6×10^8 CFU/ml, by the
106 standard procedure for later use.

107 **Immunosuppression model**

108 The immunosuppressed model of C57BL/6 mice was established by intraperitoneal
109 administration of dexamethasone sodium phosphate (DEX) 25mg/kg per day for a
110 consecutive 5 days referring to the method described by Rasmussen KR and
111 colleges[13]. Control mice received the same dosage of normal saline intraperitoneally.
112 The immunosuppression status was confirmed on the sixth day by evaluating the
113 organ-to-body weight ratios of the spleen (relative spleen weight)[14]. C3H/HeJ mice
114 were used directly as immunodeficient mice for their lack of Toll-like receptor 4.

115 **Unilateral lung instillation**

116 In the unilateral lung injury mice model, the left lung was set as the instillation side
117 and the right lung was set as the control side. Mice were anesthetized through
118 injecting 1% pentobarbital sodium (50mg/Kg) intraperitoneally and put to a proper
119 supine position. The anterior cervical hair was removed and the skin disinfected with
120 75% ethanol. To expose the trachea, a small longitudinal incision was made and
121 endotracheal intubation performed between the second to fourth cricoid cartilage
122 using a 24G intravenous indwelling catheter (Introcan, BRAUN). The 19mm long
123 24G cannula was then inserted gently into the left main bronchus. A volume of 30ul *P.*
124 *aeruginosa* secretion extract was delivered into the left lung of each mouse using
125 microliter syringes (Gaoge, Shanghai). Different dosage of live bacteria was
126 inoculated through the same method. After the intratracheal instillation, mice were put
127 back to the mouse cage with a thermal pad underneath, resting in the left recumbent
128 position until full recovery from anesthesia.

129 **Measurement of lung compliance**

130 Dynamic lung compliances were measured 7 days after unilateral lung instillation of
131 bacterial secretion. The main surgery procedures were the same as above. A 22G
132 intravenous indwelling catheter (Introcan, BRAUN) was used for endotracheal
133 intubation. After exposure of the main trachea, 5mm of the catheter tip was inserted
134 into the main trachea and a ventilator for rodents (Vent Jr, Kent) was connected to the
135 catheter. Target tidal volume range between 0.2-0.5ml was set and the corresponding
136 peak pressure was recorded each (RR=100bpm). Lung dynamic compliance (C_{dyn}) was
137 calculated through linear regression by GraphPad Prism 8 software.

138 **Lung histopathology**

139 Mice were sacrificed at different time points set. The lung was inflated with 4%
140 paraformaldehyde fixative under constant pressure of 15 to 25 cm H_2O via the trachea
141 and the trachea then tied off. Lung and heart were harvested en bloc and submerged in

142 fixative for approximately 24 hours before the heart was removed and lung was
143 embedded in paraffin blocks for tissue sections (5μm). Hematoxylin and eosin staining
144 were performed subsequently. Slides were scanned (PANNORAMIC, 3DHISTECH),
145 reviewed and evaluated.

146 **Alveolar mean linear intercept (Lm)**

147 Air space enlargement was measured using the Short & Hennig method, 1967.
148 Briefly, HE stained sections were scanned and reviewed in Caseviewer software. 10
149 random fields of each right lung and left lung were randomly selected. Equally
150 crossed two lines (both 1000um) were drawn on the scope. The mean linear intercept
151 Lm was then calculated: Lm=N*L/m [15]. In this case, Lm=$2*10^4$/m (μm). To better
152 measure how much the left lung air space has enlarged compare to the right lung, Lm
153 Ratio was further calculated, Lm Ratio=Lm (left lung)/ Lm (right lung).

154 **Statistics**

155 The data were expressed as the mean ± standard error of mean. Statistical analysis
156 was performed in the GraphPad Prism 8 software. Pair-wise comparisons were made
157 using t-tests, whereas multiple comparisons were made using one-way analysis of
158 variance (ANOVA) test. The confidence interval was set at 95% for all tests. Groups
159 of three or four animals were used in each experiment.

160 **Results**

161 **Identification of *P. aeruginosa***

162 *P. aeruginosa* was originally isolated and purified from a clinical sputum sample and
163 confirmed through mass spectrometry. The strain exhibited typical *P. aeruginosa*
164 characteristics including strong ginger odor, green metallic sheen, and beta-hemolytic
165 on blood agar with blue-green pigment producing on nutrient agar (**Figure 1**). The *P.*
166 *aeruginosa* secretion extract was translucid, light green colored with a strong ginger

167 odor. The total protein concentration was 0.35mg/ml.

168 ***P. aeruginosa* secretion causes emphysema in different mice**

169 Two days after unilateral pulmonary instillation of *P. aeruginosa* secretion, prominent
170 panacinar emphysema with acute inflammation appeared in the left lung of both
171 C57BL/6 mice and C3H/HeJ mice while the right lung remained mostly unharmed
172 (**Figure 2**). The inflammation is characterized by neutrophil infiltration and erythrocyte
173 diapedesis. Lm Ratio indicates the alveolar of the left lung had enlarged more than three
174 times in C57BL/6 mice, and two times in C3H/HeJ mice.

175 Seven days after the instillation, inflammation faded and panacinar emphysema was
176 well developed and remained in C3H/HeJ mice without fibrosis. While interestingly in
177 C57BL/6 mice, the panacinar emphysema had changed to distal emphysema with most
178 of the left lung consolidated. Fibrosis is obvious, inflammation cells can be seen
179 excreting from alveolars and bronchus. To decide if the panacinar emphysema would
180 recur after inflammation resolution, we observed C57BL/6 mice models to three weeks
181 after instillation. Surprisingly, Large apical and subpleural bullae appeared.
182 Histopathology shows all four types of emphysema (centriacinar, panacinar, distal
183 acinar and irregular) coexisting in the left lung and compensatory emphysema
184 developed in the right lung after 21 days (**Figure 2**).

185 During the above modeling process, C57BL/6 mice underwent dyspnea, huddle, weight
186 loss and scaphoid abdomen while C3H/HeJ mice showed no obvious symptoms or
187 weight loss (**Figure 3**).

188 ***P. aeruginosa* secrets heat-labile proteases**

189 After heated for 80°C, 30mins, the bacterial secretion lost the ability to cause
190 emphysema but still induced neutrophil infiltration (data not shown), indicating
191 different components are responsible for alveolar destruction and inflammation.

192 Protein mass spectrometry confirmed the protein types in the secretion include a high
193 abundance of metal-dependent hydrolase, urocanate hydratase and other enzymes
194 (supplementary material), suggesting the emphysema was caused by heat-labile
195 proteases.

196 **Dynamic lung compliance decreased after instillation**

197 Seven days after unilateral lung instillation of bacterial secretion, C_{dyn} of C57BL/6
198 mice decreased from 0.041 to 0.031 ml/cmH_2O while C_{dyn} of C3H/HeJ mice
199 decreased from 0.045 to 0.036 ml/cmH_2O. Peak pressure and targeted tidal volume
200 were not well linearly correlated as they used to (**Figure 3, D)**.

201 ***P. aeruginosa* infection induces emphysema in immunocompromised but not**
202 **immunocompetent mice**

203 Different bacteria dosages, infection time and other conditions in normal C57BL/6 mice
204 were tested, but none of the mice showed noteworthy emphysema (n≥10). A
205 representative case is the mouse was attacked with 15μl of 5 MCF *P. aeruginosa*, which
206 formed a prominent abscess full of *P. aeruginosa* bacteria at the apex of the left lung
207 after 24 hours, but it only caused bronchiectasis-like pathology change in a few areas
208 (**Figure 4)**. Whereas, all C3H/HeJ mice developed homogenous emphysema after live
209 bacteria instillation (n=7). 15μl of 0.5 MCF *P. aeruginosa*—1/10 of the dosage used in
210 C57BL/6 mice—was able to cause obvious centriacinar emphysema in C3H/HeJ. This
211 result reveals that emphysema induced by *P. aeruginosa* is not dosage-dependent.

212 In order to decide if the emphysema induced is related to species or immunity
213 differences, groups of immunosuppressed C57BL/6 mice, C3H/HeJ mice, and normal
214 C57BL/6 mice were infected with 30μl of 2 MCF *P. aeruginosa* for 9 hours (**Figure 5**).
215 Centriacinar pulmonary emphysema occurred in both immunosuppressed C57BL/6
216 mice and C3H/HeJ mice. A comparison of the Lm Ratios between different groups
217 reveals that *P. aeruginosa* infection had caused airspace enlargement in two

218 immunocompromised mice groups but alveolar collapse and lung compression in
219 normal C57BL/6 mice group.

220 **Discussion**

221 In this study, we applied a unilateral lung injury model and found the secretion of *P.*
222 *aeruginosa* could cause acute emphysema in mice. Utilizing live *P. aeruginosa* bacteria,
223 we successfully induced emphysema in both immunosuppressed C57BL/6 and
224 C3H/HeJ mice, but not in any C57BL/6 mice with normal immunity. Our findings show
225 that the pathogenesis of COPD can be naturally acute and cigarette smoking irrelevant.
226 Emphysema induced by *P. aeruginosa* is not dependent on dosage but more related to
227 the immunity status of the hosts. Chronic inhalation of noxious particles could have
228 impaired immune function and contributed to COPD formation by creating a
229 circumstance that favors *P. aeruginosa* to survive and thrive [16, 17].

230 *P. aeruginosa* is a nonfastidious bacterium that can proliferate in distilled water. The
231 secretion of *P. aeruginosa* is a mixture of metal-dependent hydrolase, enzymes, and
232 bacterial endo/exotoxins which elicited strong inflammation in normal mice.
233 Interestingly, it seems the neutrophils played a positive role in preventing emphysema
234 formation. Immunosuppressed C57BL/6 as well as TLR4 deficient C3H/HeJ mice who
235 had lighter inflammatory response and neutrophils infiltration than C57BL/6 mice,
236 exhibited more severe airspace dilation.

237 Protease–antiprotease imbalance as a result of cigarette smoking induced chronic
238 inflammation is the potential mechanism of COPD, based on the observation that
239 patients with a genetic deficiency of α1-anti-trypsin have a predisposition to develop
240 emphysema[3]. Emphysema caused by lung instillation of porcine pancreatic elastase
241 as a widely used disease model supported this theory[18]. Many studies found elevated
242 matrix metalloproteinases (MMPs) concentration and increased elastin degradation in
243 COPD patients [19-21]. Neutrophil or macrophage elastases were held culprit[22]. But

244 it seems that the proteolytic enzymes secreted by various bacteria had not been taken
245 into account. Proteases secretion is a common feature in *P. aeruginosa* and other
246 bacteria[23], however, it didn't occur to us before that the excessive elastase may come
247 from a pathogen rather than our own neutrophils.

248 The difference of air space volume is hard to detect since lung tissue has a nature of
249 instability. In our study, even lungs were carefully inflated with fixative under constant
250 pressure, the many steps in the histopathology preparation process still impacted the
251 final pressure, which can be seen from the variation of alveolar diameters of control
252 lung between different mouse. We solved this difficulty by utilizing the unilateral
253 instillation model. Unilateral lung injury animals were readily used in a few studies[24,
254 25]. A mouse model of unilateral injury can provide valuable self-control, eliminate
255 systemic influence, and reduce the use of lab animals. By ensuring the pressure is equal
256 within the lung, minor changes could be detected and compared. The Lm Ratio of left
257 to right lung further revealed clearly how much the airspace has enlarged or compressed.

258 However, a unilateral lung injury model is not perfect. Sometimes contamination of the
259 control lung caused by the sneeze or cough of mice is not totally avoidable, especially
260 in bacterial infection models. Thus, the histology changes in the main bronchus of the
261 control lung should be interpreted carefully. We tried to do more quantitative
262 assessment of the lung structure according to standard recommendation[26], but found
263 it is impractical when a heavy inflammation occurs (Figure 2h). Due to this reason, we
264 had to sacrifice bacteria inoculated mice at hour 9, before the inflammatory response
265 makes it impossible to measure Lm.

266 There are multiple pieces of research studied the correlation between P. aeruginosa and
267 COPD[27, 28]. Pulmonary P. aeruginosa infection is not found of any differences than
268 other bacteria[29]. Whereas, most researches conducted used animals with normal
269 immunity. α1-anti-trypsin secreted by local macrophages and liver is a major inhibitor
270 of proteases normally presented in serum and tissue fluids. If the immunity is

271 unimpaired, proper inflammation response can increase α1-anti-trypsin level to
272 eliminate the destructive effects caused by bacteria proteases[12]. Our research
273 supported this concept by demonstrating that P. aeruginosa could only cause limited
274 bronchiectasis in normal mice.

275 Centriacinar and panacinar forms of emphysema are most commonly found in COPD
276 patients. The two types are distinct entities. Centriacinar emphysema is the type most
277 closely associated with tobacco smoking while panacinar emphysema is usually
278 associated with α1-anti-trypsin deficiency[30, 31]. However, we found the forms of
279 emphysema are related and transformable to each other. In C3H/HeJ mice, the acute
280 emphysema transited to stable irreversible panacinar emphysema without fibrosis while
281 in normal C57BL/6 mice, it went through dynamic change interweaved with
282 inflammation and developed to other types of emphysema as well as bullae.

283 On the contrary to the current paradigm taking lung infection as no participant in
284 emphysema initiation, we discovered for the first time that *P. aeruginosa* and its
285 secretion can lead to acute emphysema in immunodeficient animals, which is in comply
286 with its predilection to infect immunocompromised patients. Emphysema induced by P.
287 aeruginosa in mice recapitulates the main features of human emphysema and COPD.
288 The role of *P. aeruginosa* in COPD needs more exploration. In the future, strategies
289 aimed at developing a vaccine or specialized antibiotics targeting *P. aeruginosa* may be
290 useful for COPD prevention and treatment.

291 **Conclusion**

292 *P. aeruginosa* infection caused acute emphysema in immunocompromised mice by
293 bacterial secretion. Different types of emphysema are transformable. *P. aeruginosa*
294 could be an important pathogen in COPD initiation.

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300 **References**

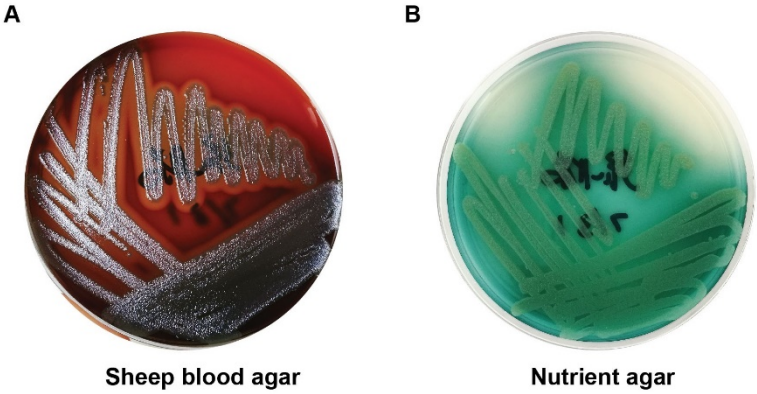
301 1. Rabe KF, Watz H. Chronic obstructive pulmonary disease. *Lancet (London, England)* 2017; 389:
302 1931-1940.
303 2. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet*
304 *(London, England)* 2004; 364: 709-721.
305 3. MacNee W. Pathogenesis of chronic obstructive pulmonary disease. *Proceedings of the*
306 *American Thoracic Society* 2005; 2: 258-266; discussion 290-251.
307 4. Liang GB, He ZH. Animal models of emphysema. *Chinese medical journal* 2019; 132: 2465-2475.
308 5. Restrepo MI, Babu BL, Reyes LF, Chalmers JD, Soni NJ, Sibila O, Faverio P, Cilloniz C, Rodriguez-
309 Cintron W, Aliberti S. Burden and risk factors for Pseudomonas aeruginosa community-
310 acquired pneumonia: a multinational point prevalence study of hospitalised patients. *Eur*
311 *Respir J* 2018; 52.
312 6. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary
313 disease. *N Engl J Med* 2008; 359: 2355-2365.
314 7. Eklöf J, Sørensen R, Ingebrigtsen TS, Sivapalan P, Achir I, Boel JB, Bangsborg J, Ostergaard C,
315 Dessau RB, Jensen US, Browatzki A, Lapperre TS, Janner J, Weinreich UM, Armbruster K,
316 Wilcke T, Seersholm N, Jensen JUS. Pseudomonas aeruginosa and risk of death and
317 exacerbations in patients with chronic obstructive pulmonary disease: an observational
318 cohort study of 22 053 patients. *Clinical microbiology and infection : the official*
319 *publication of the European Society of Clinical Microbiology and Infectious Diseases* 2020;
320 26: 227-234.
321 8. Murphy TF, Brauer AL, Eschberger K, Lobbins P, Grove L, Cai X, Sethi S. Pseudomonas aeruginosa
322 in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008; 177: 853-860.
323 9. Li J, Ramezanpour M, Fong SA, Cooksley C, Murphy J, Suzuki M, Psaltis AJ, Wormald PJ, Vreugde
324 S. Pseudomonas aeruginosa Exoprotein-Induced Barrier Disruption Correlates With
325 Elastase Activity and Marks Chronic Rhinosinusitis Severity. *Frontiers in cellular and*
326 *infection microbiology* 2019; 9: 38.
327 10. Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen-host interactions in Pseudomonas
328 aeruginosa pneumonia. *Am J Respir Crit Care Med* 2005; 171: 1209-1223.
329 11. Fujitani S, Sun HY, Yu VL, Weingarten JA. Pneumonia due to Pseudomonas aeruginosa: part I:
330 epidemiology, clinical diagnosis, and source. *Chest* 2011; 139: 909-919.
331 12. Aster VKAAJ. Robbins Basic Pathology. 2017.
332 13. Rasmussen KR, Healey MC. Experimental Cryptosporidium parvum infections in
333 immunosuppressed adult mice. *Infect Immun* 1992; 60: 1648-1652.
334 14. Manente FA, Quinello C, Ferreira LS, de Andrade CR, Jellmayer JA, Portuondo DL, Batista-
335 Duharte A, Carlos IZ. Experimental sporotrichosis in a cyclophosphamide-induced
336 immunosuppressed mice model. *Medical mycology* 2018; 56: 711-722.
337 15. Dunnill MS. Quantitative methods in the study of pulmonary pathology. *Thorax* 1962; 17: 320-
338 328.
339 16. Faure K, Sawa T, Ajayi T, Fujimoto J, Moriyama K, Shime N, Wiener-Kronish JP. TLR4 signaling

340 is essential for survival in acute lung injury induced by virulent Pseudomonas aeruginosa
341 secreting type III secretory toxins. *Respiratory research* 2004; 5: 1.
342 17. Drannik AG, Pouladi MA, Robbins CS, Goncharova SI, Kianpour S, Stämpfli MR. Impact of
343 cigarette smoke on clearance and inflammation after Pseudomonas aeruginosa infection.
344 *Am J Respir Crit Care Med* 2004; 170: 1164-1171.
345 18. Antunes MA, Rocco PR. Elastase-induced pulmonary emphysema: insights from experimental
346 models. *Anais da Academia Brasileira de Ciencias* 2011; 83: 1385-1396.
347 19. Chaudhuri R, McSharry C, Brady J, Donnelly I, Grierson C, McGuinness S, Jolly L, Weir CJ,
348 Messow CM, Spears M, Miele G, Nocka K, Crowther D, Thompson J, Brannigan M, Lafferty
349 J, Sproule M, Macnee W, Connell M, Murchison JT, Shepherd MC, Feuerstein G, Miller DK,
350 Thomson NC. Sputum matrix metalloproteinase-12 in patients with chronic obstructive
351 pulmonary disease and asthma: relationship to disease severity. *J Allergy Clin Immunol*
352 2012; 129: 655-663.e658.
353 20. Maclay JD, McAllister DA, Rabinovich R, Haq I, Maxwell S, Hartland S, Connell M, Murchison JT,
354 van Beek EJ, Gray RD, Mills NL, Macnee W. Systemic elastin degradation in chronic
355 obstructive pulmonary disease. *Thorax* 2012; 67: 606-612.
356 21. Wells JM, Parker MM, Oster RA, Bowler RP, Dransfield MT, Bhatt SP, Cho MH, Kim V, Curtis JL,
357 Martinez FJ, Paine R, 3rd, O'Neal W, Labaki WW, Kaner RJ, Barjaktarevic I, Han MK,
358 Silverman EK, Crapo JD, Barr RG, Woodruff P, Castaldi PJ, Gaggar A. Elevated circulating
359 MMP-9 is linked to increased COPD exacerbation risk in SPIROMICS and COPDGene. *JCI*
360 *Insight* 2018; 3.
361 22. Tanner L, Single AB. Animal Models Reflecting Chronic Obstructive Pulmonary Disease and
362 Related Respiratory Disorders: Translating Pre-Clinical Data into Clinical Relevance.
363 *Journal of innate immunity* 2020; 12: 203-225.
364 23. Galloway DR. Pseudomonas aeruginosa elastase and elastolysis revisited: recent developments.
365 *Molecular microbiology* 1991; 5: 2315-2321.
366 24. Dames C, Akyüz L, Reppe K, Tabeling C, Dietert K, Kershaw O, Gruber AD, Meisel C, Meisel A,
367 Witzenrath M, Engel O. Miniaturized bronchoscopy enables unilateral investigation,
368 application, and sampling in mice. *Am J Respir Cell Mol Biol* 2014; 51: 730-737.
369 25. Singer T, Brand V, Moehrlen U, Fehrenbach H, Purkabiri K, Ott SR, Stammberger U, Ochs M,
370 Hamacher J. Left-sided mouse intubation: description and evaluation. *Experimental lung*
371 *research* 2010; 36: 25-30.
372 26. Hsia CC, Hyde DM, Ochs M, Weibel ER. An official research policy statement of the American
373 Thoracic Society/European Respiratory Society: standards for quantitative assessment of
374 lung structure. *Am J Respir Crit Care Med* 2010; 181: 394-418.
375 27. Martínez-Solano L, Macia MD, Fajardo A, Oliver A, Martinez JL. Chronic Pseudomonas
376 aeruginosa infection in chronic obstructive pulmonary disease. *Clin Infect Dis* 2008; 47:
377 1526-1533.
378 28. Boutou AK, Raste Y, Reid J, Alshafi K, Polkey MI, Hopkinson NS. Does a single Pseudomonas
379 aeruginosa isolation predict COPD mortality? *Eur Respir J* 2014; 44: 794-797.
380 29. Curran CS, Bolig T, Torabi-Parizi P. Mechanisms and Targeted Therapies for Pseudomonas
381 aeruginosa Lung Infection. *Am J Respir Crit Care Med* 2018; 197: 708-727.

30. Finkelstein R, Ma HD, Ghezzo H, Whittaker K, Fraser RS, Cosio MG. Morphometry of small airways in smokers and its relationship to emphysema type and hyperresponsiveness. *Am J Respir Crit Care Med* 1995; 152: 267-276.

31. Kim WD, Eidelman DH, Izquierdo JL, Ghezzo H, Saetta MP, Cosio MG. Centrilobular and panlobular emphysema in smokers. Two distinct morphologic and functional entities. *Am Rev Respir Dis* 1991; 144: 1385-1390.

389 **Figures**



390

391 **Figure 1 Culture characteristics of the *P. aeruginosa* strain.** The strain exhibit

392 typical green metallic sheen, beta-hemolysis and produce blue-green pigment.

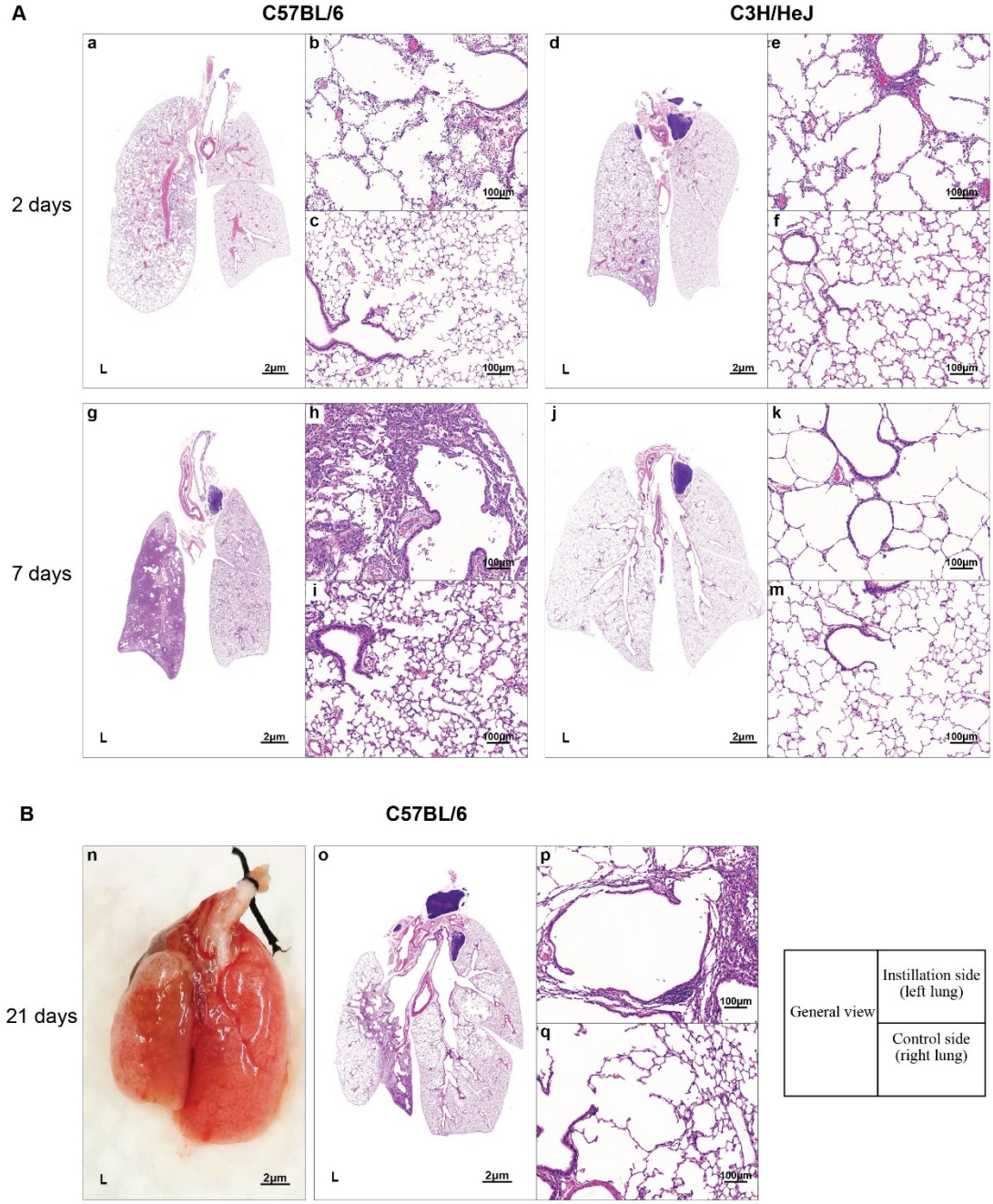
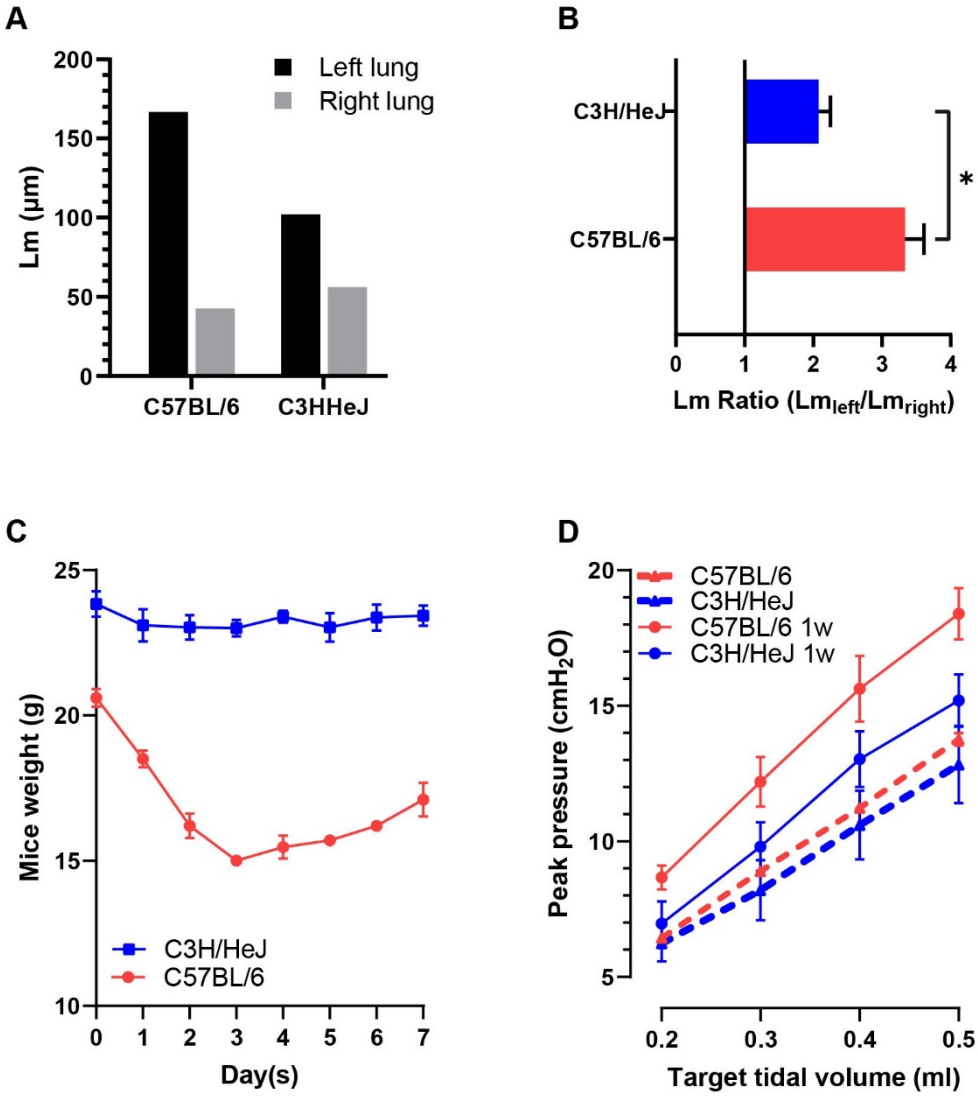


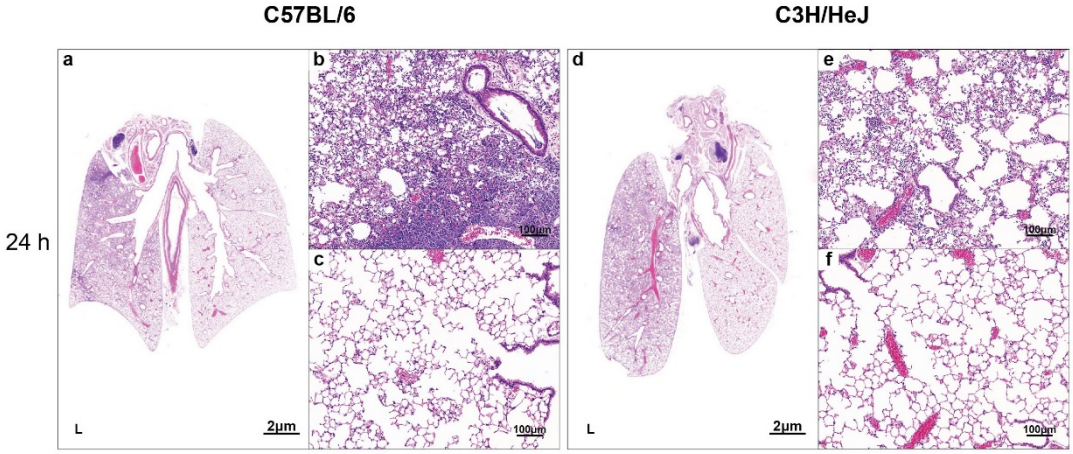
Figure 2 Unilateral pulmonary instillation of *P. aeruginosa* secretion: Two days after instillation, acute panacinar emphysema with inflammation in the left lung of both C57BL/6 mice (b) and C3H/HeJ mice (e). Seven days after instillation, obvious fibrosis and distal emphysema formed in C57BL/6 mice (h) but not in C3H/HeJ (k). Three weeks after instillation, C57BL/6 mice developed large apical and subpleural bullae (n). Subtypes of emphysema coexisting in the left lung (o, p) and compensatory emphysema developed in the right lung (q). All right lungs served as self-control (c, f, i, m, q);

401 Hematoxylin and eosin (H&E) staining;



402

403 **Figure 3: Lm Ratio and dynamic lung compliance:** (A) Lm of the C57BL/6 mice
404 (Figure 2-a) and the C3H/HeJ mice (Figure 2-d) 2 days after instillation. (B) Lm ratio
405 indicates the alveolar of the left lung had enlarged more than three times in C57BL/6
406 group, and two times in C3H/HeJ group. (C) C57BL/6 mice endured obvious weight
407 loss in comparison with C3H/HeJ mice. (D) Dynamic lung compliance of both mice
408 decreased 7 days after modeling. The data represent the mean ± SEM of three animals.
409 *P < .05; **P < .01; ***P< .001;



410

411 **Figure 4 Unilateral live bacteria inoculation:** 24 hours after infection, *P. aeruginosa*
412 didn't induce emphysema in C57BL/6 mice. An abscess full of bacteria at the apex of
413 the left lung formed, but only bronchiectasis-like pathological change (a, b). On the
414 contrary, *P. aeruginosa* inoculation induced prominent centriacinar emphysema in
415 C3H/HeJ mice (d, e). Control lungs (c, f); H&E staining;

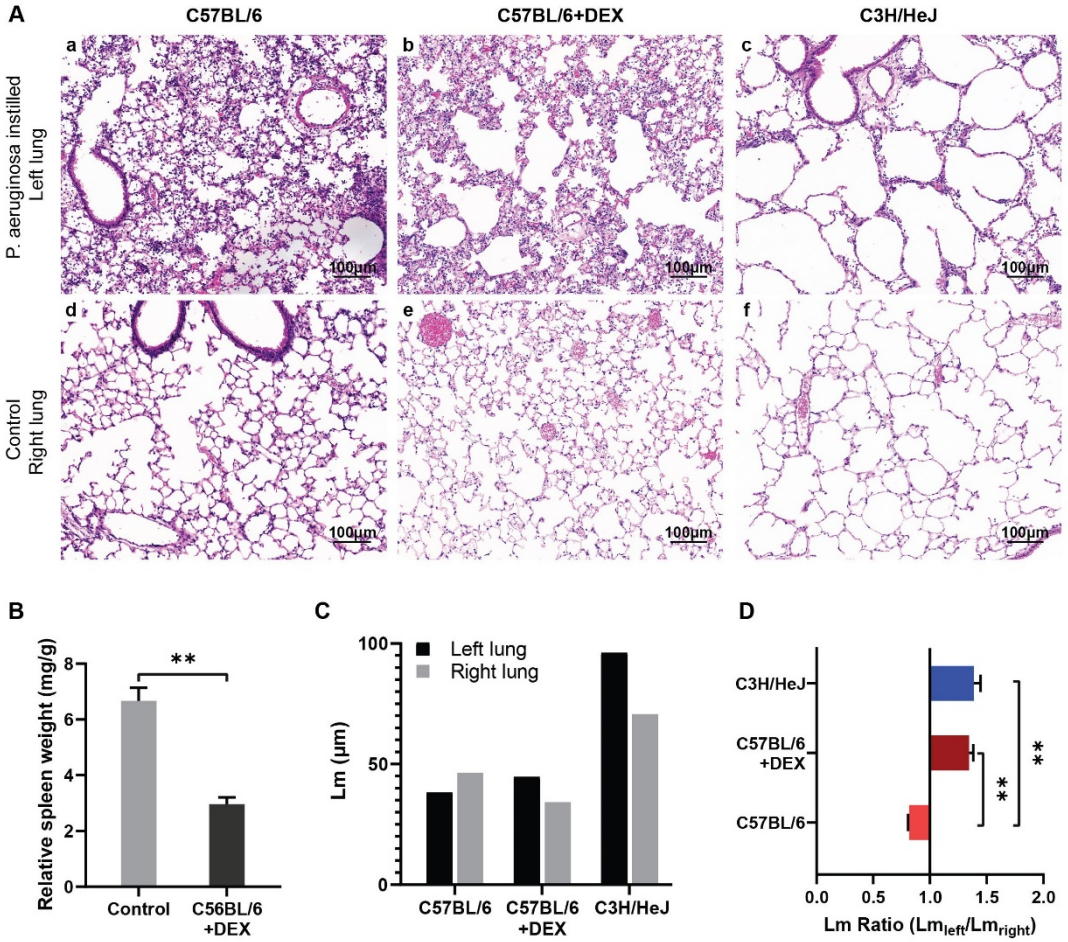


Figure 5 Unilateral live bacteria inoculation in immunosuppressed C57BL/6: (A) Histology of lung of normal C57BL/6 mice, C57BL/6 + DEX mice, and C3H/HeJ mice, Centriacinar pulmonary emphysema developed in both C57BL/6+DEX mice and C3H/HeJ mice, but not in normal C57BL/6 mice. All mice were inoculated with 30μl of 2 MCF *P. aeruginosa* and sacrificed after 9 hours; H&E staining. (B) The decrease of relative spleen weight of C57BL/6 mice treated with DEX shows successful modeling of hypoimmunity. (C) Lm decreased in C57BL/6 mouse. (D) Lm ratio between different groups shows *P. aeruginosa* infection caused significant airspace enlargement in two immunocompromised mice group but airspace compression in the normal C57BL/6 group. The data represent the mean ± SEM of four animals. *P < .05; **P < .01; ***P< .001;