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ImmunoHorizons

RESEARCH ARTICLE

Infectious Disease

Chronic *Pseudomonas aeruginosa* Lung Infection Is IL-1R Independent, but Relies on MyD88 Signaling

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ABSTRACT

Cystic fibrosis is associated with chronic *Pseudomonas aeruginosa* colonization and inflammation. The role of MyD88, the shared adapter protein of the proinflammatory TLR and IL-1R families, in chronic *P. aeruginosa* biofilm lung infection is unknown. We report that chronic lung infection with the clinical *P. aeruginosa* RP73 strain is associated with uncontrolled lung infection in complete MyD88-deficient mice with epithelial damage, inflammation, and rapid death. Then, we investigated whether alveolar or myeloid cells contribute to heightened sensitivity to infection. Using cell-specific, MyD88-deficient mice, we uncover that the MyD88 pathway in myeloid or alveolar epithelial cells is dispensable, suggesting that other cell types may control the high sensitivity of MyD88-deficient mice. By contrast, IL-1R1–deficient mice control chronic *P. aeruginosa* RP73 infection and IL-1β Ab blockade did not reduce host resistance. Therefore, the IL-1R1/MyD88 pathway is not involved, but other IL-1R or TLR family members need to be investigated. Our data strongly suggest that IL-1 targeted neutralizing therapies used to treat inflammatory diseases in patients unlikely reduce host resistance to chronic *P. aeruginosa* infection. *ImmunoHorizons*, 2021, 5: 273 –283.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen known to cause acute pneumonia as well as persistent chronic infections. The prevalence of *P. aeruginosa* infections has increased over the past decade and represent a major clinical concern. *P. aeruginosa* is a frequent infection in intensive care unit and a leading cause of mechanical ventilation, trauma, or viral infections, with a significantly higher mortality compared with other pathogens (1). *P. aeruginosa* is also intrinsically associated with chronic obstructive pulmonary disease and cystic fibrosis (CF) and is a common cause of recurrent infections (1, 2). The

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Abbreviations used in this article: ATI, alveolar type I; ATII, alveolar type II; BALF, bronchoalveolar lavage fluid; CF, cystic fibrosis; IL-1R1, type 1 IL-1R; KC, keratinocyte-derived chemokine; KO, knockout; MPO, myeloperoxidase.

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persistent colonization of the airways by *P. aeruginosa* is associated with chronic inflammation and remodeling accompanied by a progressive decline of respiratory function, leading ultimately to premature mortality (3). Despite considerable advances in antimicrobial chemotherapy, treatment of chronic infections is problematic because of antibiotic resistance (4). Thus, there is a need of novel classes of anti-infectious agents as alternative or complementary strategies to antibiotic. However, the pathophysiological mechanisms that govern host immune responses to chronic *P. aeruginosa* infections remain to be fully deciphered.

Pattern-recognition receptors such as TLR and IL-1R family members are involved in the detection of pathogens and the initiation of inflammatory responses after infections (5). The adapter protein MyD88 (6) is shared by receptors of the TLR and IL-1R families and is critical to control bacterial lung infections, including mycobacterial infection (7–9), but also early acute *P. aeruginosa* infection (10, 11). Type 1 IL-1R (IL-1R1) heterodimerize with IL-1Ra upon IL-1β binding (5). IL-1β is prototypal proinflammatory cytokine that is synthesized as a precursor protein, pro–IL-1b, under the control of the MyD88–NF-κB axis and required a proteolytic cleavage mainly mediated by the class I cysteine protease caspase-1 (ICE1) (12). ICE1 requires activation in response to pattern-recognition receptor signaling, triggered by a multiprotein intracellular platform called inflammasomes (10, 11, 13).

Proinflammatory cytokines, including the ones from the IL-1 family, have been suggested to be critical for the protection against *P. aeruginosa*, although a complete understanding of their effects according to the infectious mode of the bacteria has not been achieved yet. Previous reports suggested that IL-1β expression was mandatory for host protection against acute infection (14). During *P. aeruginosa* chronic colonization, it has been shown that NLRP3 inflammasome is activated leading to the production of IL-1β and the NLRP3 inhibitor MCC950 inhibited inflammation in CF mice (15). In addition, we have reported a critical role of IL-1β in the induction of deleterious inflammatory response in the context of CF-associated mutations (16).

In this study, we revisited the role of IL-1β/IL-1R1 and MyD88 signaling in host resistance against a chronic *P. aeruginosa* lung infection using the clinical strain *P. aeruginosa* RP73. We report in this study that IL-1β is not essential, whereas MyD88 is required to control chronic infection as completed MyD88-deficient mice develop a severe and lethal lung pathology. Interestingly, genetic deletion of MyD88 in alveolar type I (ATI) and alveolar type II (ATII) epithelial cells or in myeloid cells does not impair the control of *P. aeruginosa* chronic infection. Thus, host resistance is independent of MyD88 signaling by IL-1R1 to control the inflammatory response and host resistance to chronic *P. aeruginosa* infection.

MATERIALS AND METHODS

Mice

MyD88 (17) and IL-1R1–deficient mice (18), as well as cell-specific, MyD88-deficient mice generated in-house (19), all on C57BL/6 background, and littermate controls were bred at TAAM (UPS44, CNRS, Orléans, France). All mice were housed under specific pathogen–free conditions and had access to food and water ad libitum. All animals were followed twice daily. All animal experiments complied with the current European legislative, regulatory, and ethical requirements and were approved by Ethics Committee for Animal Experimentation of CNRS Campus Orléans (CLE CCO 2012-042).

Chronic P. aeruginosa *infection*

The *P. aeruginosa* strain RP73 strain (gift from Pr. A. Bragonzi, San Raffalele Hospital, Milano, Italy) was cultured on agarose beads as previously described (20), and mice were infected with freshly prepared inoculum of 1×10^6 CFU by endopharyngeal administration under isoflurane inhalation anesthesia, whereas control mice received uncoated agar beads. As an acute infection control, we used the *P. aeruginosa* 01 strain at 1×10^6 CFU, as previously described (21). Tobramycin (Sigma-Aldrich) was administered by gavage at 100 mg/kg on days 2 and 3 after RP73 *P. aeruginosa* infection, and mice were killed 6 h after the last administration.

IL-1β Ab administration

Mice were injected i.p. with anti–IL-1β Ab (gift from Dr. H. Gram, Novartis Pharma, Basel, Switzerland) at the dose of 10 μg/g body weight in 200 μl. Control mice received the same dose and volume of the isotype control Ab.

Bacterial load in lung

Lung total weights were recorded at sacrifice and expressed as a percentage of body weight. Lung homogenates were prepared in 1.5 ml of isotonic saline solution using a Dispomix tissue homogenizer (Medic Tools, Bern, Switzerland). The 10-fold serial dilutions of lung homogenates were plated on brain heart infusion agar plates (BioValley France). Plates were incubated at 37°C and 5% CO_2, and the numbers of CFU were enumerated after 24 h. In vitro antibiotic resistance was assessed in bacterial suspension cultures with increasing tobramycin concentrations, known as minimal inhibitory concentration assay.

Bronchoalveolar lavage

After sacrifice, bronchoalveolar lavage fluid (BALF) was collected by cannulating the trachea and washing the lung with 1 ml saline at room temperature. The lavage fluid was centrifuged at $400 \times g$ for 10 min at 4°C, and the supernatant was stored at −80°C for analysis. The cell pellet was resuspended in 1× PBS and counted in a hemocytometer chamber, and cytospins were prepared to count the neutrophils, macrophages, lymphocytes, and eosinophils as described before (16).

Cytokine and chemokine measurement

TNF-α, IL-1β, IL-6, and keratinocyte-derived chemokine (KC; CXCL-1) as well as myeloperoxidase (MPO) levels in lung homogenates were measured by ELISA (Duoset Kit; R&D

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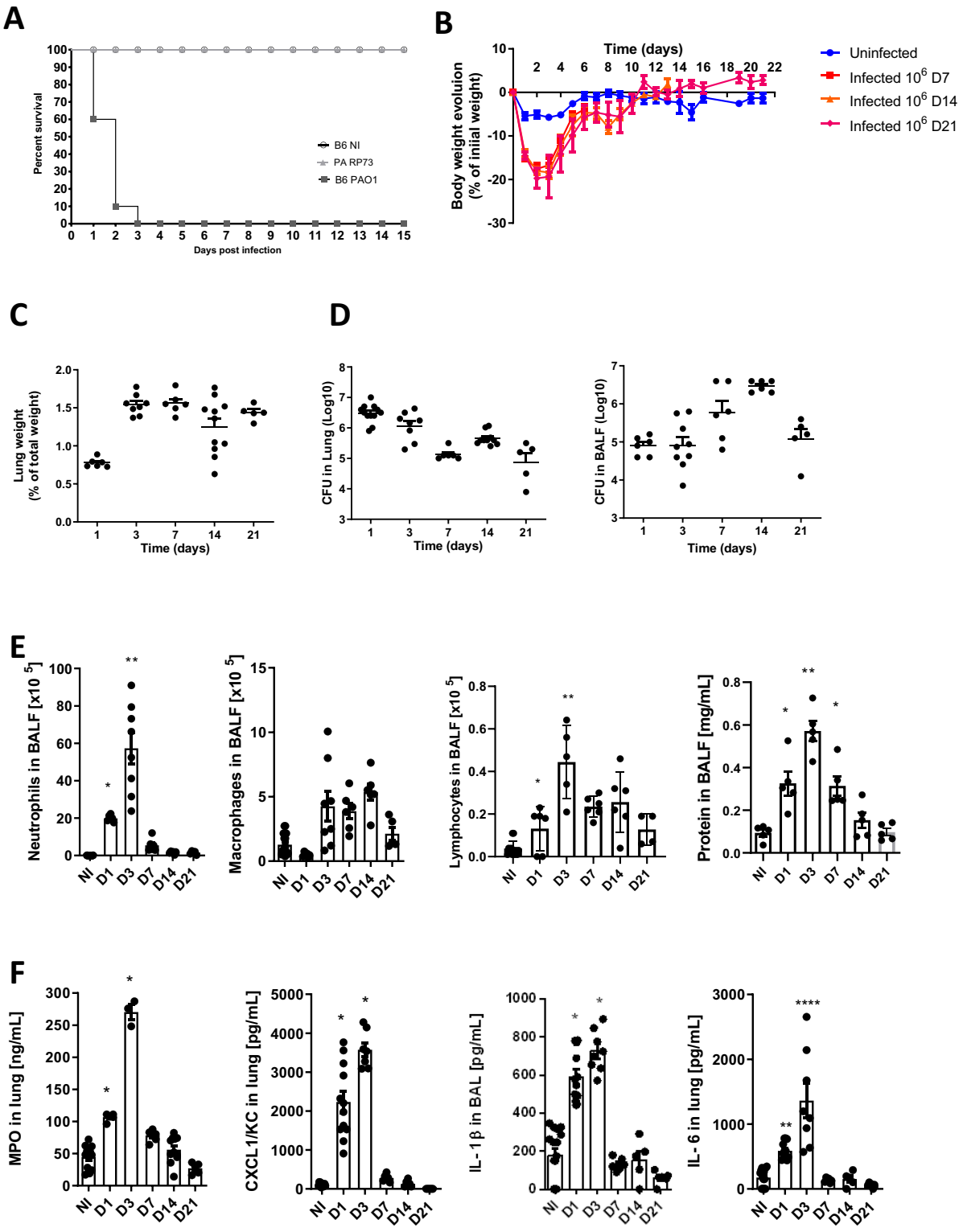


FIGURE 1. Establishment of chronic *P. aeruginosa* infection with the RP73 strain in BL6 mice.

BL6 mice were infected with PAO1 (only survival) or *P. aeruginosa* RP73 strain (1×10^6 CFU on agar beads by endotracheal instillation) and analyzed at 1, 3, 7, 14, and 21 d. (**A**) Comparison of PAO1 or *P. aeruginosa* RP73 strain in survival. (**B**) Body weight. (**C**) Lung weight (percentage of initial weight). (**D**) Bacterial load in BALF and lung (CFU). (**E**) Neutrophils, macrophage, and lymphocyte counts and total protein levels in BALF. (**F**) MPO, CXCL1/KC, IL-1β, and IL-6 in lung. Data from three representative experiments are shown and expressed as mean ± SEM, with at least six mice per group. $*p < 0.05$, $**p < 0.01$.

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Systems, U.K.) according to the manufacturer s instructions. Total protein concentration to assess respiratory barrier integrity was analyzed in BALF using Bradford assay with BIORAD DC Protein assay kit (Bio-Rad Laboratories, France), according to the manufacturer s instructions.

Microscopy

Lungs were fixed in 4% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Sections (3 μm) were stained with H&E, and all lung sections were evaluated by light microscopy by three independent observers (J.P., T.M., and B.R.) for recruitment of inflammatory cells in bronchi, bronchiolitis, and respiratory epithelial injury using semiquantitative severity score (0–5) as described before (16).

Statistical analysis

Statistical evaluation of differences between the experimental groups was done by Mann–Whitney nonparametric test or log-rank test for survival. All tests were performed with GraphPad Prism (GraphPad Software, San Diego, CA; www.graphpad.com). A p value <0.05 was considered significant and was symbolized with an asterisk (*), and $^{**}p < 0.01$ and $^{***}p < 0.001$. A p value >0.05 was considered NS.

RESULTS

Establishment of chronic P. aeruginosa *RP73 respiratory infection in mice*

To establish a chronic model, we prepared agar beads coated with the clinical mucoid RP73 strain (provided by Prof. A. Bragonzi). First, the course of infection with the nonmucoid PAO1 (not on beads) and the mucoid RP73 strain was compared. Agar beads coated with the *P. aeruginosa* RP73 strain were administered at 1×10^6 CFU by endopharyngeal instillation to BL6 mice. Infection with both strains caused clinical signs disease, but all mice inoculated with the *P. aeruginosa* RP73 strain survived, whereas mice infected with the 1×10^6 CFU PAO1 strain died within 2 d upon infection (Fig. 1A), as reported earlier (21). Infected BL6 mice lost ∼15% of body weight at 48 h but recovered over the next 10 d and survived the infection (Fig. 1A, 1B). Lung weights as surrogate marker of inflammation were increased in *P. aeruginosa* RP73 strain–infected mice on days 1 and 3 and were elevated up to day 21, suggesting chronic lung infection (Fig. 1C). To ascertain the chronicity of the infection, the viable bacteria recovered from BALF and lung homogenates were assessed. Viable *P. aeruginosa* RP73 were detected in the lung at day 1 at 3×10^6 CFU counts and decreased to 1×10^6 CFU at day 7 and were stable till day 21. The BALF CFU counts at day 1 were 1×10^5 CFU and increased significantly to 3×10^6 CFU at day 14 and declined to 1×10^5 CFU at day 21 (Fig. 1D). Inflammatory cell recruitment in the BALF was initially characterized by abundant neutrophils peaking at 5×10^6 on day 3 and reduced on day 7. By contrast, macrophages were increased from day 3 to 14, whereas lymphocyte counts were elevated from day 1 to 14. Total protein in BALF as a measure of respiratory barrier damage was detected from day 1 to 7 (Fig. 1E). Further, *P. aeruginosa* RP73 strain infection was associated with significantly increased MPO as a marker of neutrophil infiltration, CXCL1/KC, IL-1β, and IL-6 in lung (Fig. 1F).

Therefore, the data demonstrate that the mucoid *P. aeruginosa* RP73 strain causes chronic lung injury, chronic infection, bronchitis, and pneumonia in BL6 mice.

Chronic P. aeruginosa *lung injury and infection is resistant to chemotherapy*

To characterize the inflammation in chronic *P. aeruginosa* infection, we investigated the infected lung tissue in BL6 mice. Unlike the empty agar beads, which do not cause any significant tissue reaction, the *P. aeruginosa* RP73–loaded beads elicit respiratory epithelial cell damage with cell desquamation visible at day 1 and 3, followed by repair on day 7 (black arrows).

Epithelial injury is associated with acute bronchitis and bronchiolitis, with infiltration by neutrophils, macrophages/monocytes, and lymphocytes in the first few days, which then develops into a chronic biofilm infection with pneumonia at ∼7 d (red arrows), which declines at day 21 (Fig. 2A, 2B).

Biofilm infections are usually associated with antibiotic resistance. We tested the resistance to tobramycin in suspension culture of the *P. aeruginosa* RP73 strain and confirm that this strain is sensitive to tobramycin (Fig. 2C). Furthermore, administering a high-tobramycin dose (100 mg/kg on days 2 and 3 by gavage) to mice with established chronic RP73 *P. aeruginosa* lung infection had no effect on the bacterial burden, relative lung weight, and lung inflammation at day 7 postinfection (Fig. 2D). These data reproduce the important feature of an antibiotic resistance of clinical biofilm infection and are in line with previous reports that *P. aeruginosa* RP73 biofilm infection is resistant to antibiotic treatment.

Uncontrolled lung P. aeruginosa *infection in the absence of MyD88*

Having established the chronic biofilm infection model, we asked which factors might reduce host resistance. The common TLR adapter protein MyD88 is critically involved in acute *P. aeruginosa* infection (22). We report in this study that *P. aeruginosa* RP73 strain (1×10^6 CFU)–infected, MyD88-deficient mice are highly susceptible to infection and succumb to infection within 4 d, whereas littermate control mice survive (Fig. 3A). MyD88-deficient mice present increased lung weight as surrogate marker of lung inflammation. Total protein levels in BALF were increased indicating respiratory barrier injury/disruption and increased numbers of neutrophils, macrophages, and lymphocytes (Fig. 3B) associated with apoptosis and cell death of neutrophils and epithelial cells (Supplemental Figs. 1, 2). MPO, CXCL1/KC, and IL-6 levels in lung were increased in RP73 *P. aeruginosa*–infected, MyD88-deficient mice, whereas IL-1β and IL-17A levels were not different (Fig. 3C). The infection was uncontrolled with a massive

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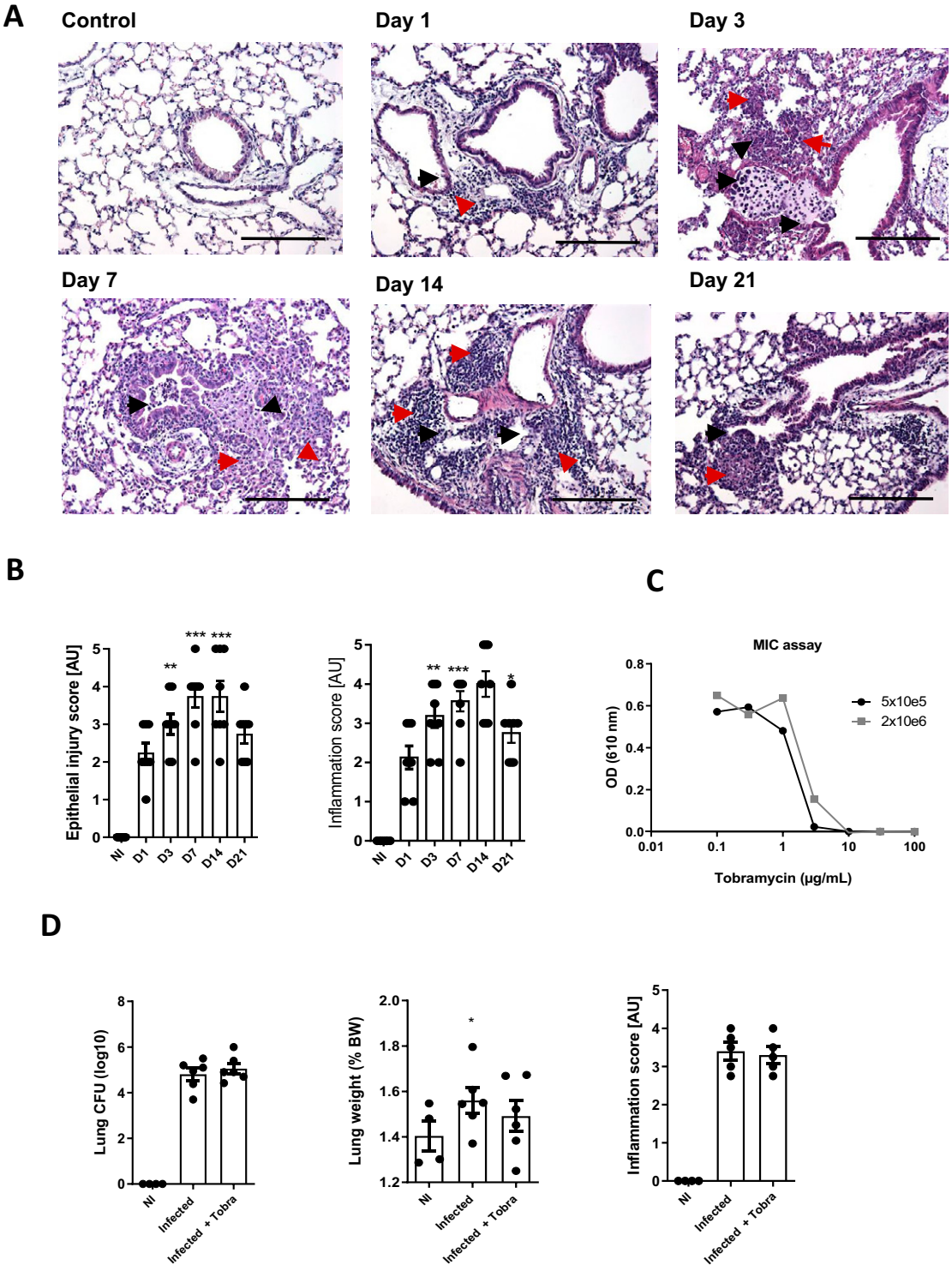


FIGURE 2. Microscopic analyses of chronic lung infection and tobramycin resistance upon *P. aeruginosa* RP73 strain infection (1×10^6 CFU) in BL6 mice.

(**A**) Microscopic changes of the *P. aeruginosa* RP73 infected lung at 1, 3, 7, 14, and 21 d. (**B**) Semiquantitative score of epithelial lesions and bronchial and peribronchial inflammation. Epithelial injury (black arrows) and inflammation (red arrows). Scale bar, 100 µm. (**C**) Tobramycin inhibition assay of *P. aeruginosa* in suspension culture, expressed as OD. (**D**) Tobramycin resistance in chronic *P. aeruginosa* infection in vivo with bacterial burden, lung weight, and inflammation score. The data are expressed as mean ± SEM, with $n = 6$ mice per group, and are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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increase of the bacterial load in lung and BALF (3 log CFU), as compared with littermate control mice (Fig. 3D). Microscopic analysis of the lung of MyD88-deficient mice at day 3 showed a trend for increased acute inflammatory cell infiltration, epithelial cell injury, acute alveolitis, and bronchiolitis as compared with infected littermate controls (Fig. 3E). Therefore, the data demonstrate that MyD88 signaling is critical to control chronic RP73 *P. aeruginosa*–induced lung epithelial damage, inflammation, and lethal infection.

Lung epithelial and myeloid cell MyD88 deficiency is not responsible for uncontrolled chronic P. aeruginosa *infection*

Having demonstrated that MyD88 is essential to control infection, we asked whether lung epithelial cells are involved in the inflammatory response and control of chronic *P. aeruginosa* RP73 infection. To address this question, we used cell-specific, MyD88-deficient mice developed in the laboratory and described before (19).

Importantly, we find that *P. aeruginosa* RP73 strain infection is controlled in mice deficient for MyD88 in $AQP5^+$ ATI lung cells (MyD88 floxxAQP5-cre mice, in short MyD88-AQP5 knockout [KO]). Clinical signs and body weight loss, lung weight, and inflammation with macrophage and neutrophil recruitment in the bronchoalveolar lavage and protein extravasation in the airways after *P. aeruginosa* RP73 infection were reduced in MyD88-AQP5 KO mice as compared with littermate controls (Fig. 4A, 4B). Although MPO in the lung tissue was increased in infected mice, the increase of CXCL1/KC and IL-6 level in the lung seen in complete MyD88-deficient mice was attenuated in MyD88-AQP5 KO mice upon infection, comparable to littermate control mice, whereas IL-1β and IL-17A levels were not different (Fig. 5C). In addition, MyD88-AQP5 KO mice controlled bacterial load in the lung and BALF similar to littermate infected control mice (Fig. 4D). Histologically, the extent of epithelial injury, inflammatory cell infiltration, and bronchiolitis and pneumonia was attenuated in MyD88-AQP5 KO mice comparable to littermate controls, in contrast to complete MyD88 KO mice (Fig. 4E). Therefore, the data obtained from MyD88-AQP5 KO mice show that MyD88 signaling by lung epithelial ATI cells do not contribute to the control of lung inflammation and infection by the *P. aeruginosa* RP73 strain in mice.

Furthermore, myeloid cell–specific, MyD88-deficient mice (MyD88 floxxlysM-Cre, in short MyD88-lysM KO) controlled infection and inflammation similar to MyD88-AQP5 KO and wild-type littermate controls, unlike complete MyD88-deficient mice (Fig. 4A–E). Therefore, although complete absence of MyD88 pathway impairs the control of chronic *P. aeruginosa* infection, MyD88 in myeloid cells are dispensable to control chronic *P. aeruginosa* RP73 infection. However, the LysM promotor is not restricted to myeloid cells but is also expressed in ATII epithelial cells (Supplemental Fig. 3) (23, 24), suggesting that ATII epithelial cells are not required.

Therefore, our data suggest that MyD88 signaling in ATI or ATII epithelial cells, as well as in myeloid cells, is not required because in their absence, *P. aeruginosa* RP73 infection allows control of inflammation and infection, and the mice survive at least for 3 wk (data not shown), unlike complete MyD88-deficient mice.

IL-1 R is not required for host resistance to chronic P. aeruginosa *lung infection*

We published before that IL-1β is required to control in acute *P. aeruginosa* infection using PAO1 strain using IL-1R1 KO mice (16) and report in this study increased IL-1β in bronchoalveolar lavage upon *P. aeruginosa* RP73 strain infection (Fig. 1F). Therefore, we asked whether IL-1 plays a role in chronic *P. aeruginosa* RP73 biofilm infection and whether the high susceptibility to infection of MyD88-deficient mice is related to IL-1R signaling. Therefore, we infected IL-1R1–deficient mice and found that they survived chronic *P. aeruginosa* RP73 infection as BL6 mice (data not shown). Lung weight, neutrophil, and lymphocyte recruitment in BALF and lung bacterial load were similar in *P. aeruginosa* RP73–infected, IL-1R1–deficient and littermate wild-type control mice (Fig. 5A). The increase of MPO, CXCL1/KC, and protein leak in BALF as well as the lung injury and inflammation were similar in infected IL-1R1–deficient and in littermate control mice (Fig. 5B). Thus, IL-1R1–deficient mice control chronic *P. aeruginosa* infection, as infected BL6 control mice, unlike complete MyD88-deficient mice, which succumb to infection within 4 d.

Finally, to corroborate these data, we asked whether neutralizing IL-1β Ab alters host inflammation and resistance to *P. aeruginosa* RP73 lung infection in BL6 mice. Blockade of IL-1β by neutralizing Abs (200 μg, injected daily i.p.) did not increase the sensitivity of BL6 mice to *P. aeruginosa* RP73 infection, as all the inflammatory and infection parameters measured were comparable to untreated infected BL6 mice (Fig. 5C, 5D).

Therefore, our data demonstrate that IL-1R1 signaling is not required to control chronic *P. aeruginosa* RP73 infection, and IL-1β Ab blockade does not reduce host resistance to chronic *P. aeruginosa* infection in control B6 WT mice.

DISCUSSION

Chronic biofilm lung infection with *P. aeruginosa* is a common complication in patients with CF or other predisposing factors and represents a therapeutic challenge. We established a chronic lung inflammation in BL6 mice using the clinical *P. aeruginosa* RP73 strain to investigate the role of innate immune pathway MyD88/IL-1R1 in epithelial barrier injury, inflammation, and host resistance. A critical role of MyD88 is established in response to endotoxin (17) and several bacterial infections in mice, like with Burkholderia (9), *Listeria* (25, 26), *Legionella* (27), or *Mycobacterium tuberculosis* (8), to mention a few major infections. A role of MyD88 was shown in the early response to acute *P. aeruginosa* aerosol infection with defective NF-κB, IL-1β, or TNF response in the absence of the MyD88 pathway (11) while dispensable at 48 h postinfection (10).

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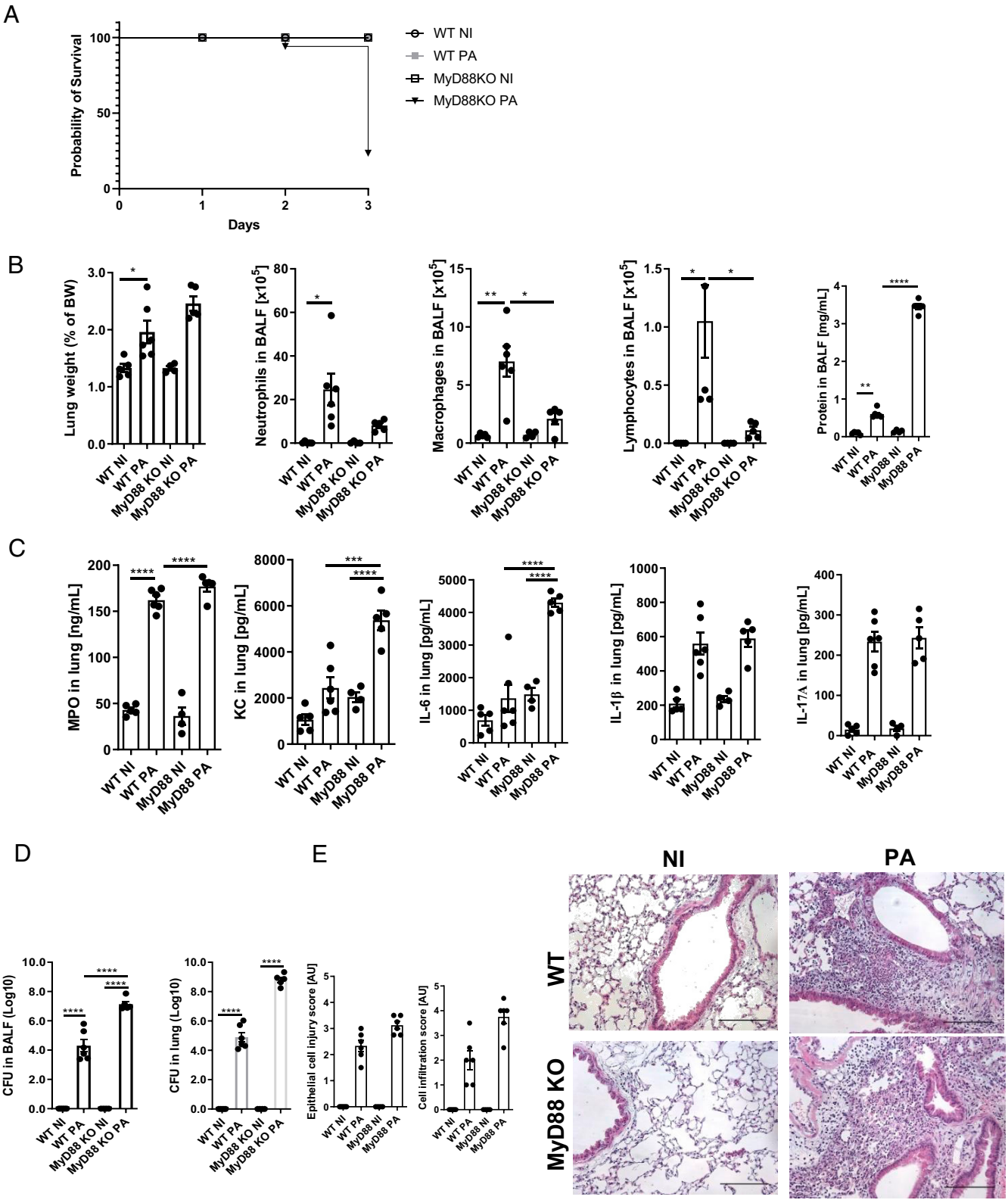


FIGURE 3. *P. aeruginosa* RP73 strain induced uncontrolled infections in absence of MyD88.

BL6 and MyD88 KO were infected with *P. aeruginosa* RP73 strain on agarose beads (1×10^6 CFU) and analyzed at 3 d postinfection. (**A**) Survival. (**B**) Relative lung weights, neutrophil, macrophage, and lymphocyte counts and total protein levels in BALF. (**C**) MPO, CXCL1/KC, IL-6, IL-1β, and IL-17A in lung. (**D**) Bacterial load in lung and BALF. (**E**) Lung epithelial damage and inflammatory cell scores with representative lung micrographs. Scale bar, 100 μm. Results are expressed as mean ± SEM, with n = 5–7 mice per group, and are representative for three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$.

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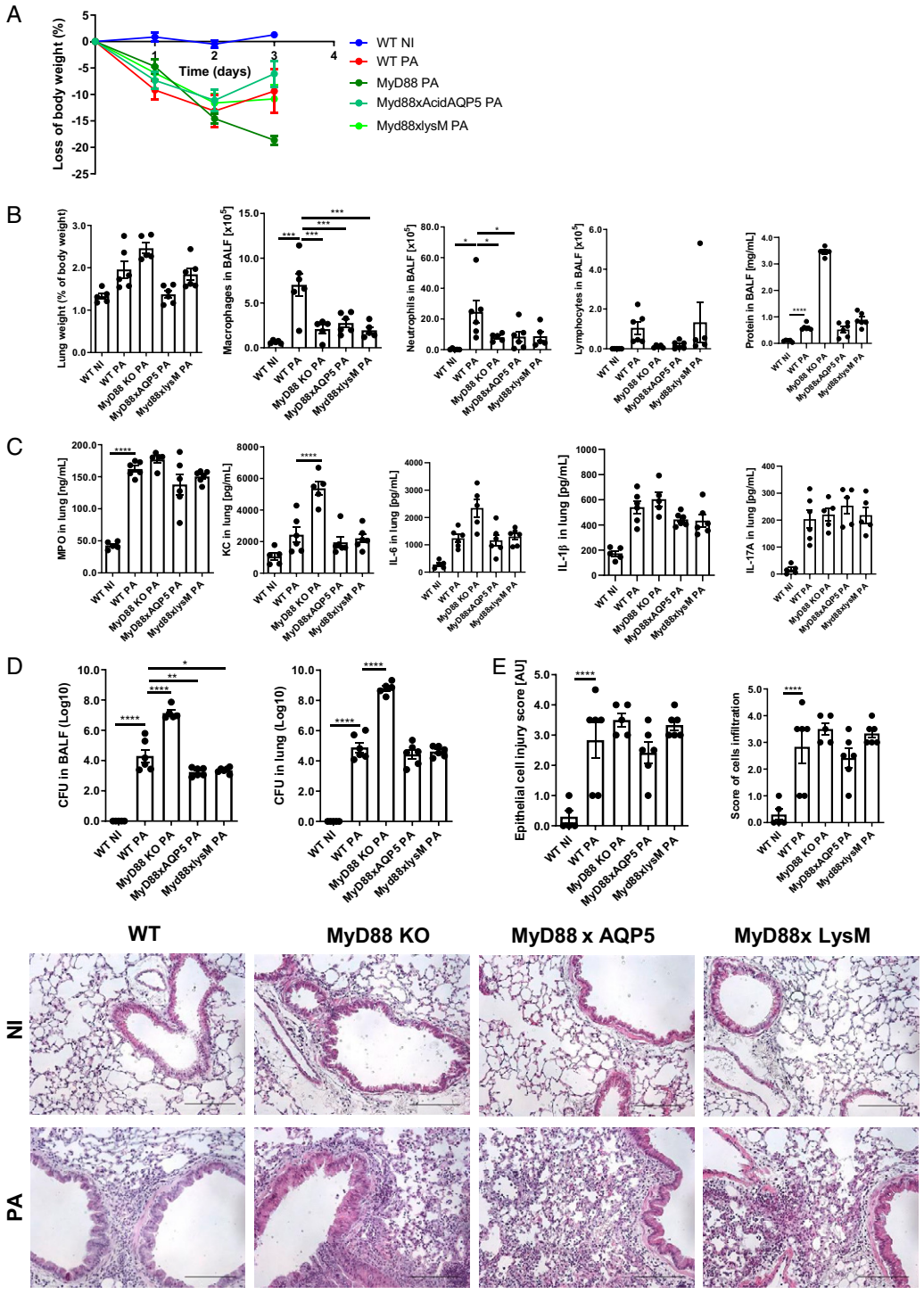


FIGURE 4. Absence of MyD88 expressed in ATI and -II epithelial and myeloid cells controls *P. aeruginosa* RP73 infection.

MyD88-deficient mice, ATI lung epithelial cell–specific AQP5fcrexMyD88flox and myeloid, and ATII-specific MLys-crexMyD88 flox–deficient mice (19) were analyzed 3 d postinfection with the RP73 strain *P. aeruginosa* (1×10^6 CFU). (**A**) Body weight loss. (**B**) Relative lung weights; macrophages, neutrophils, and lymphocyte counts and protein in BALF. (**C**) MPO, CXCL1/KC, IL-6, IL-1β, and IL-17A in lung. (**D**) Bacterial load in lung and BALF. (**E**) Lung epithelial injury and inflammation scores and representative lung micrographs. Scale bar, 100 μm. Results are expressed as mean ± SEM, with n = 5–7 mice per group, and are representative for three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

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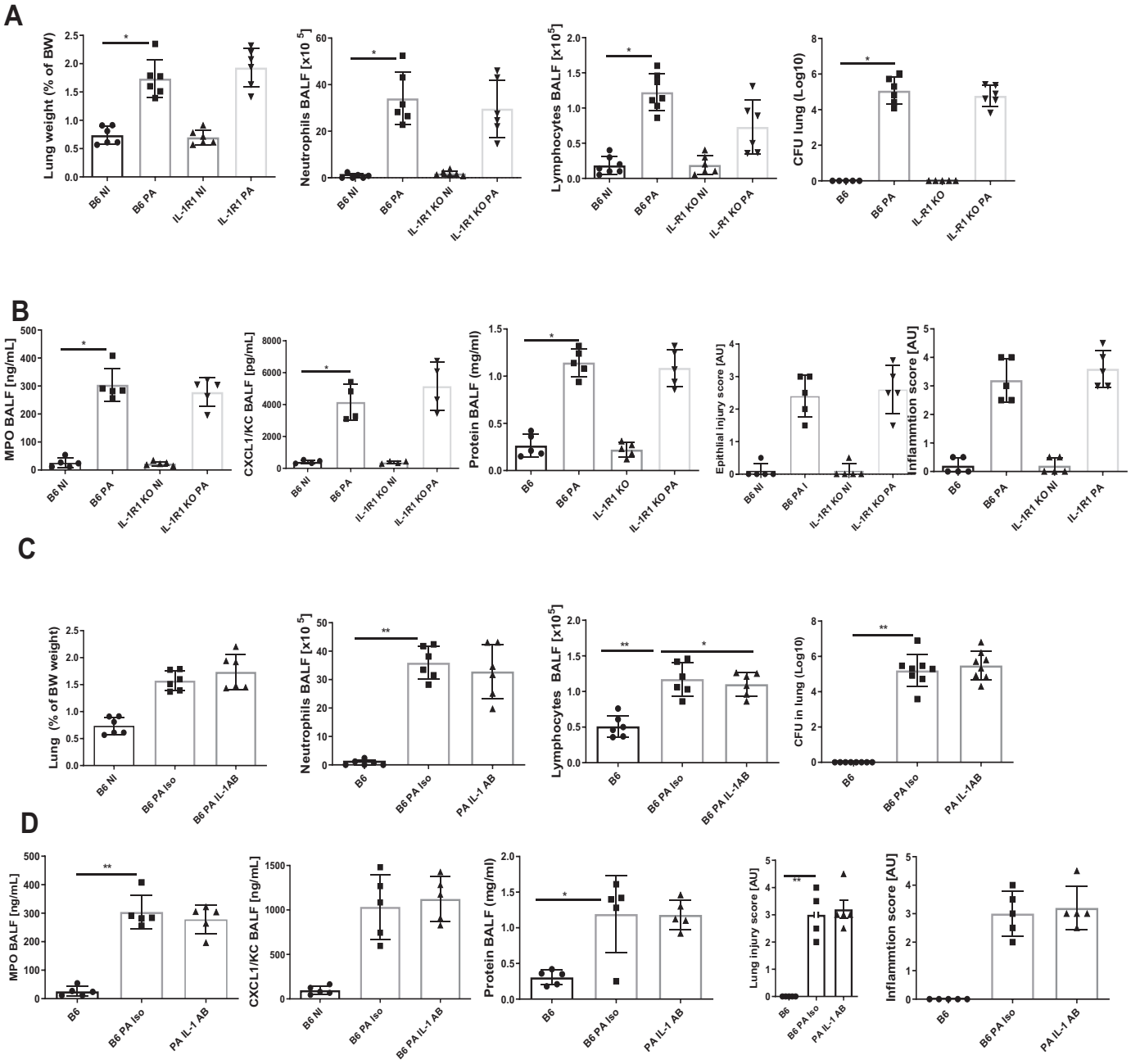


FIGURE 5. Role of IL-1 in host resistance to chronic RP73 *P. aeruginosa* infection.

IL-1R1 KO mice (**A** and **B**) and BL6 mice injected with neutralizing IL-1β Ab or isotype IgG1 Ab 200 μg i.p. (**C** and **D**) were infected with *P. aeruginosa* RP73 strain (1×10^6 CFU) and analyzed 3 d postinfection. (A) Lung weight, neutrophil, and lymphocyte counts in BALF and bacterial load (CFU) in lung. (B) MPO, CXCL1/KC, and protein in BALF, epithelial damage, and inflammation score of the lung in BL6 and IL-1R1–deficient mice. (C) Lung weight, neutrophil, and lymphocyte counts in BALF and bacterial load in lung. (D) MPO, CXCL1/KC, and protein in BALF, epithelial damage, and inflammation score in BL6 mice injected i.p. 200 μg IL-1β Ab (IL-1 AB). Results are expressed as mean ± SEM, with n = 4-6 mice per group; data are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$.

Because chemotherapy resistance is a major therapeutic issue of chronic *P. aeruginosa* biofilm infection in patients, we first investigated whether the clinical *P. aeruginosa* RP73 strain infection is sensitive to chemotherapy. We found that although the *P. aeruginosa* strain in suspension culture is sensitive to tobramycin, in vivo chronic *P. aeruginosa* RP73 lung infection is resistant to tobramycin treatment. We demonstrated before that the acute PAO1 infection is sensitive to meropenem and

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tobramycin (28). Increased antibiotic resistance of bacteria with biofilm growth is a major therapeutic challenge (29).

In this study, using cell-specific deletion of MyD88 signaling in ATI or ATII epithelial cells, as well as of myeloid cells (19), we show that these cells are not involved in host resistance because in their absence, *P. aeruginosa* RP73 infection and inflammation was controlled, and the mice survived at least for 3 wk, unlike complete MyD88-deficient mice.

Previous studies focused on the role of innate pattern-recognition receptors such as TLRs in *P. aeruginosa* infection. The consensus of these studies was that the sensing of *P. aeruginosa* is more complex and not restricted to a single TLR, but it appears the MyD88 adapter protein is essential to initiate the cytokine inflammatory response and control acute *P. aeruginosa* infection (10, 11, 22). In this study, we extend this finding and report for the first time, to our knowledge, that MyD88 is essential to control chronic *P. aeruginosa* RP73 biofilm lung infection.

Importantly, we demonstrate that MyD88 signaling in lung epithelial cells is not required to control lung inflammation and infection because ATI-specific, MyD88-deficient mice control inflammation and infection as littermate control and BL6 mice. We confirm that myeloid cells play a redundant role because MlyscrexMyD88-deficient mice control inflammation and infection. Because the LysM promotor is also expressed in ATII cells, we also include that both alveolar cell types are not required (23, 24).

Therefore, we conclude that MyD88 signaling in alveolar epithelial cells, the first contact site for the bacteria and in myeloid cells, is not required to control *P. aeruginosa* RP73 chronic infection. Previous studies report that radioresistant stromal cells in MyD88 bone marrow chimeric mice control acute *P. aeruginosa* infection (30). By contrast, Mijares et al. (22), using a transgenic expression of MyD88 by a CC10/Scgb1a1 promoter in the secretory club/basal cells and goblet cells of the bronchiolar region, but not in alveolar AT1 and -2 cells, restored MyD88-deficient phenotype in the acute *P. aeruginosa* infection model. In this study, using biofilm *P. aeruginosa* RP73 infection, we show directly that MyD88 signaling in the alveolar epithelium, in either ATI or ATII cells and myeloid cells, plays no role for the high susceptibility to chronic RP73 *P. aeruginosa* infection, as in their absence, *P. aeruginosa* infection is controlled.

Single-cell transcriptomic analysis of mouse lungs revealed that LysM is not only expressed in myeloid cells but also in ATII epithelial cells (31, 32). Therefore, we postulate that both myeloid cells or ATI and ATII cells are not required to control inflammation and infection because in their absence, the uncontrolled infection found in complete MyD88-deficient mice is drastically attenuated and comparable to littermate controls.

We did not investigate the role of other cytokines in chronic *P. aeruginosa* infection but demonstrated a protective role of IL-1, IL-17, and TNF in unrelated bacterial infections. We reported that IL-1 is required to control mycobacterial infection because IL-1R1–deficient mice are highly sensitive to *M. tuberculosis* (7, 33), whereas other IL-1 family members, including IL-36R, exert no significant effect on mycobacterial infection (34). Further, we demonstrated that both T cell and myeloid cells expressing TNF and TNR1 are essential to control mycobacterial infection (35, 36). We reported before that IL-1 is required for IL-17A differentiation (37). Although Th17 cells play no significant role in *M. tuberculosis* infection (36), IL-17A is required for host resistance to chronic *P. aeruginosa* lung infection (38).

Because the MyD88 adapter protein is involved in signaling of IL-1R family members, we investigated the role of IL-1 and report in this study that IL-1R1 signaling is not required for the control of chronic *P. aeruginosa*–induced lung inflammation and infection. Furthermore, IL-1β Ab blockade did not diminish host resistance in BL6 mice. Therefore, the role of other cell types or other members of the IL-1R family or TLR family depending on MyD88 signaling need to be explored in future studies.

In conclusion, the high susceptibility to chronic *P. aeruginosa* infection observed in complete MyD88 KO mice is not found in cell-specific deletion of MyD88 in ATI or ATII epithelial and myeloid cells, suggesting that these cells are not required for infection control. Furthermore, host resistance is IL-1R1 independent. Thus, IL-1–targeted therapy for inflammatory diseases in patients with autoimmune inflammatory disease may not increase the susceptibility to chronic *P. aeruginosa* biofilm infection.

DISCLOSURES

The authors have no financial conflicts of interest.

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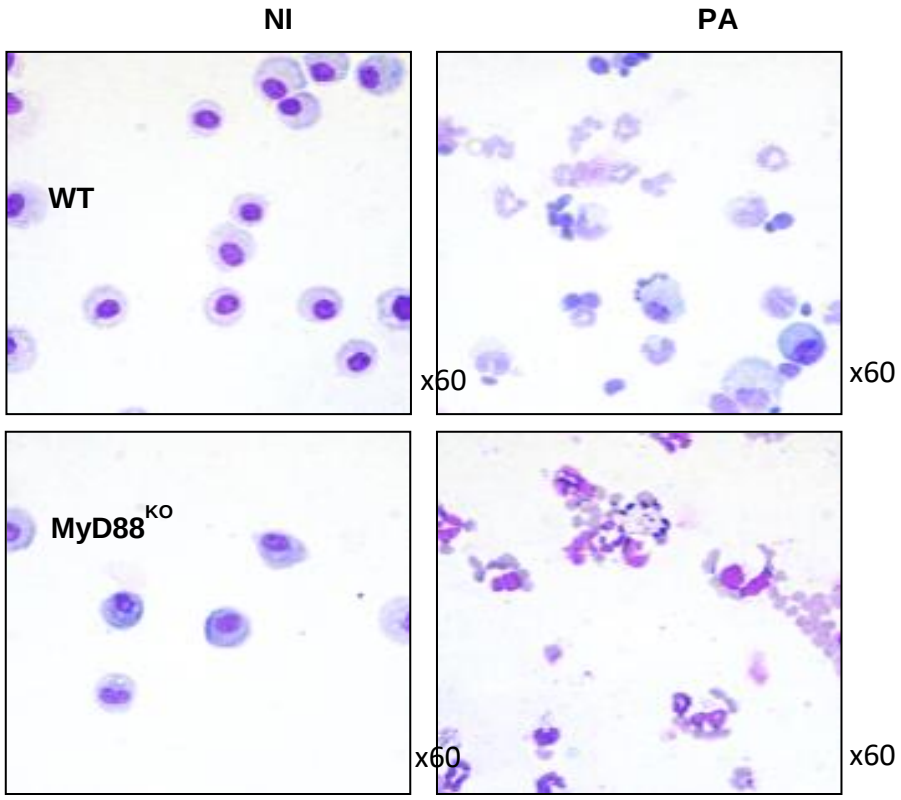
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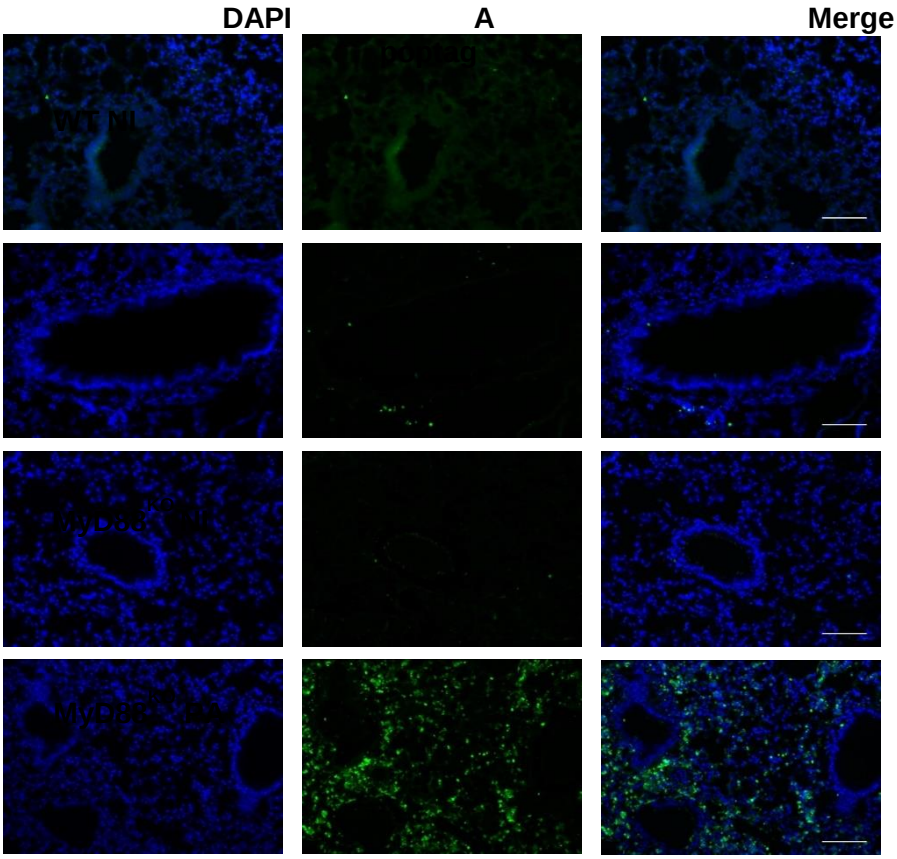
Supplemental Figure

Chronic *Pseudomonas aeruginosa* lung infection is IL-1R independent, but relies on MyD88 signaling

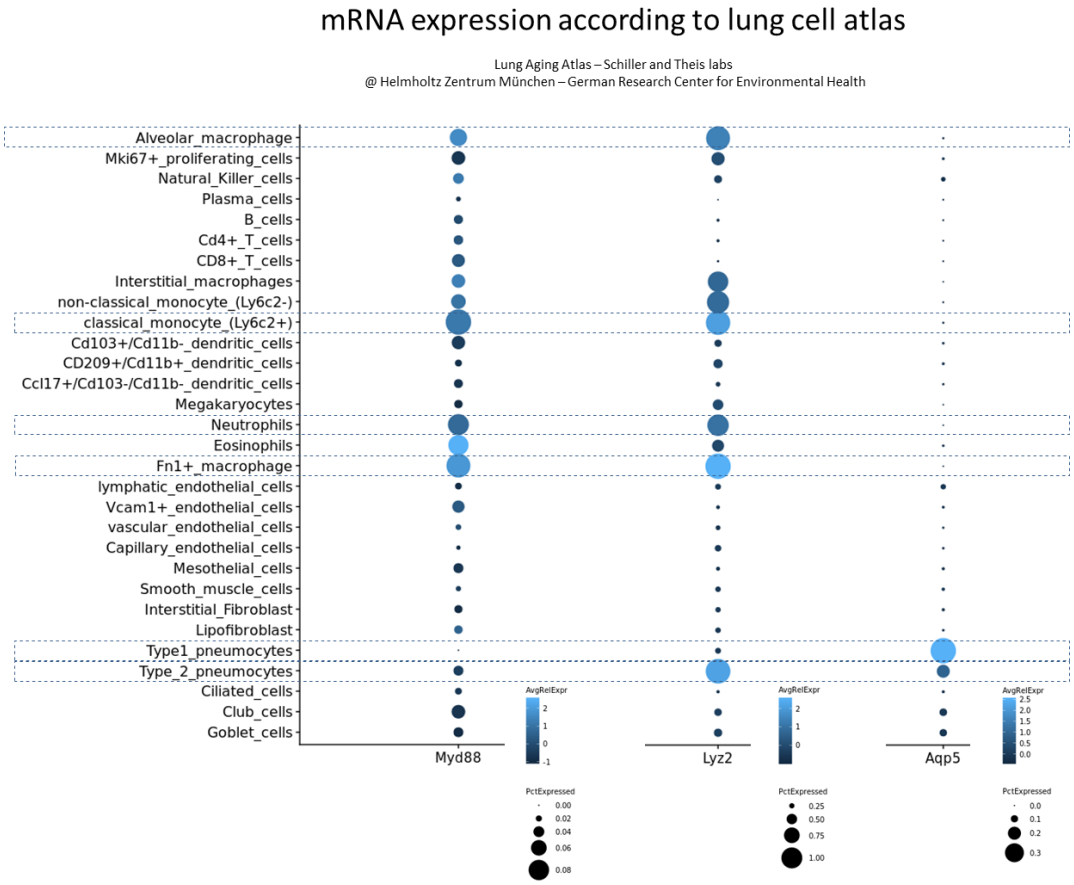
Supplemental Figures



Supplemental Figure 1 Cytospin preparation of BAL cells from MyD88 deficient mice at day 3 post PA infection with abundant neutrophils undergoing cell death.



Supplemental Figure 2 Enhanced apoptosis and repair in the lung of PA73 PA infected MyD88 deficient mice as compared with BL6 control mice at day 3 post infection.



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Supplemental Figure 3 Relative gene expression levels of Lyz2 (LysM) and Aqp5 mapped to 30 distinct cellular identities of the lung, modified from (Angelidis et al., 2019).