Lung Epithelial Regnase-1 Dampens Local Immune Response but Does Not Worsen Susceptibility to *Klebsiella pneumoniae*

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ABSTRACT

Klebsiella pneumoniae (KP) presents a global health threat, leading to significant morbidity and mortality due to its multidrug-resistant profile and the limited availability of therapeutic options. To eliminate KP lung infection, the host initiates a robust inflammatory response. One of the host's mechanisms for mitigating excessive inflammation involves the RNA-binding protein regnase-1 (Reg1, MCPIP1, or ZC3H12A). Reg1 has an RNA binding domain that recognizes stem-loop structures in the 3′ untranslated region of various proinflammatory transcripts, leading to mRNA decay. However, excessive suppression of inflammation by Reg1 results in suboptimal KP control. Reg1 deficiency within the nonhematopoietic compartment confers resistance to KP in the lung. Given that lung epithelium is crucial for KP resistance, we hypothesized that selective deletion of Reg1 in lung epithelial cells might enhance proinflammatory signals, leading to a better control of KP. Our transcriptomic analysis of epithelial cells in KP-infected wild-type mice revealed the presence of three distinct alveolar type 2 cell (AT2) subpopulations (conventional, inflammatory, and cycling) and enrichment of Reg1 in inflammatory AT2 cells. We conditionally deleted Reg1 in lung AT2 cells (Δ*Reg1*), which amplified the local inflammatory response in the lung and increased macrophage cell numbers compared with controls. However, when Δ*Reg1* mice were subjected to KP infection, there were no significant differences in bacterial burden or survival compared with controls. These findings suggest that the local inflammatory response enhanced by Reg1 deletion in AT2 cells is insufficient to control KP infection. *ImmunoHorizons*, 2024, 8: 89–96.

INTRODUCTION

Klebsiella pneumoniae (KP) accounts for 8–10% of healthcare-associated infections and ranks as the third leading cause of healthcare-associated pneumonia in the United States (1, 2), presenting a significant medical challenge due to its multidrug resistance profile. Although most multidrug-resistant KP strains are hospital acquired and affect immunosuppressed patients, there is a growing awareness of hypervirulent KP strains, which are community-acquired and infect both immunocompetent and immunosuppressed individuals. Historically, hypervirulent KP strains were documented predominantly in Asia. However, more recently, there has been increased recognition of these KP strains in Europe and the United States, with a prevalence of 2.6–3.7% (3). Moreover, some of these hypervirulent KP strains have acquired multidrug-resistant genes, posing a concerning threat (4–7). Consequently, there is a pressing scientific need to expand our understanding of host–pathogen interactions and explore strategies to enhance the immune system's ability to counteract severe KP infections.

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Abbreviations used in this article: AT2, alveolar type 2 cell; KO, knockout; KP, *Klebsiella pneumoniae*; qPCR, quantitative PCR; UTR, untranslated region; WT, wild type.

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During pneumonia, the host initiates a robust inflammatory response for successful pathogen eradication, but this response can come at the cost of tissue injury. During inflammation, the host activates negative feedback mechanisms to mitigate inflammatory collateral damage, such as the endoribonuclease regnase-1 (Reg1, *Zc3h12a*, MCPIP1). Reg1 is an RNA-binding protein that identifies and degrades several proinflammatory mRNAs, such as *Il1b*, *Il12b*, *Il6*, *Nfkbiz*, and its mRNA *Zc3h12a*. Additionally, Reg1 serves as a negative feedback regulator of LPS and IL-17 signal transduction, both essential pathways for KP eradication. Consequently, increased Reg1 can hinder the control of KP infection by excessive suppression of proinflammatory pathways.

Our previous findings in bone marrow chimeras using global heterozygous mice demonstrated that Reg1 deficiency in hematopoietic and nonhematopoietic compartments confers resistance to KP (8). Building on this prior work, we investigated the role of Reg1 in epithelial cells, precisely alveolar type 2 cells (AT2), to control KP infection.

Different AT2 cell clusters start segregating during KP infection, including conventional, inflammatory, and cycling AT2 cells. *Reg1* (*Zc3h12a*) exhibited the highest expression in inflammatory AT2 cells. As a result, we assessed Reg1 in AT2 cells by crossing *Reg1*$^{flox/flox}$ mice with *Sftpc*^{CreERT2} mice (referred to as Δ*Reg1* in this paper) to conditionally delete *Reg1* in AT2 cells. Furthermore, Reg1 deletion in AT2 cells triggered local proinflammatory cytokines and increased alveolar macrophage numbers 48 h after KP infection. However, these enhanced proinflammatory epithelial signals from Δ*Reg1* mice did not impact KP bacterial burden or survival, suggesting that Reg1 deletion in AT2 cells alone is insufficient for controlling KP lung infection.

MATERIALS AND METHODS

Mice

Zc3h12a$^{flox/flox}$ mice are under a material transfer agreement from the University of Central Florida (Orlando, FL). *Sftpc*^{CreERT2} (028054) and *Rosa26*$^{mT/mG}$ (007676) mice were from The Jackson Laboratory. Mice were 8–12 wk old, and both sexes were used. All animal studies were approved by the institutional animal care and use committee of the University of Pittsburgh.

Bacterial infections

KP American Type Culture Collection strain 43816 was grown in Luria-Bertani broth to reach the early log phase. We administered 1–2 × 10^3 CFU/mouse in PBS by deep oropharyngeal aspiration. Where indicated, tamoxifen was dissolved in corn oil and administered i.p. for 4 d and then rested for 7–10 d before induction of infection (dose, 2 mg/mouse).

Bacterial burden, mRNA, and protein analysis

Tissues were homogenized in PBS, and CFU levels were assessed by serial dilution plating. Lung tissues were homogenized in TRIzol (Invitrogen) and subjected to quantitative PCR (qPCR) with SYBR Green probes. Threshold cycle values were normalized to *Gapdh*. ELISA kits were from eBioscience (Thermo Fisher Scientific) and R&D Systems. Abs used for Western blots were ZC3H12A (GeneTex; GTX110807), IκBζ (Cell Signaling Technology; 93726 and 9244), and β-actin (Abcam; ab49900).

Lung tissue processing

To extract lung epithelial cells, murine lungs were perfused with 10 ml of PBS, injected with dispase (2.5 ml/lung), followed by lung extraction and incubation in a water bath for 5 min. Lungs were homogenized in OctoMACS for 5 min. Cell suspensions were passed through 70-μm and a 40-μm cell strainers and treated with RBC lysis buffer to generate a single-cell suspension. Epithelial cells were obtained by negative selection by staining cells for CD45, LyG, CD11b, CD11c, Siglec-F, Ly6C, and MHC class II. Negative selection fractions were stained for CD45, CD31, and Epcam and sorted by flow cytometry (CD45$^-$ CD31$^-$Epcam$^+$) for single-cell RNA sequencing.

For lung hematopoietic cells, after perfusion with PBS, lungs were digested with collagenase and DNase, passed through a 70-μm strainer, and treated with RBC lysis buffer to generate a single-cell suspension. Abs were from eBioscience, BD Biosciences, or BioLegend. Data were acquired on an LSRFortessa and analyzed with FlowJo software.

Deletion of REG1 (ZC3H12A) in a human bronchial epithelial cell line

The deletion of *ZC3H12A* was performed by Synthego using CRISPR-Cas9. Human bronchial epithelial (HBEC3-KT-ATCC) cells were electroporated with Cas9 and a *ZC3H12A*-specific sgRNA (5′-CACCACCCCGCGGGACUAGA-3′). Isogenic negative control cells were electroporated only with Cas9. The cells were expanded to get a knockout (KO) cell pool. Clonal *ZC3H12A*-KO cell lines were generated by limiting dilution. Genomic DNA from monoclonal populations was verified by PCR and Sanger sequencing using *ZC3H12A*-specific primers (forward, 5′-GCCCTGGAACTGCAGATGAA-3′; reverse, 5′-CTTCTTCCAGCCCACCAGAG-3′). The sequencing results were analyzed using the interactive Inference of CRISPR Edits results (www.ice.synthego.com).

Single-cell RNA sequencing

The lungs were removed from four mice 48 h after KP infection. The lungs were processed as above and enumerated by Cellometer2000 right before loading onto a 10× Chromium controller for cell capture (targeting 5000 cells per sample) using the Chromium Single Cell Immune Profiling v2 Dual index kits (catalog no. 1000263). Libraries for gene expression were constructed following protocols from 10× Genomics. Final libraries were quality controlled by Agilent TapeStation, then sequenced on an Illumina NovaSeq 6000, targeting 50,000 reads per cell. Sequencing data were processed with Cell Ranger 7.0 before downstream analysis using Seurat.

Single-cell RNA-sequencing data analysis

Single-cell RNA-sequencing analysis was performed using Seurat 4.0 with R (version 4.1.1). Poor-quality droplets were excluded

from subsequent analysis if a deficient number of the genes and a high percentage of mitochondrial genes were detected. Differential gene expression was performed using a nonparametric Wilcoxon rank-sum test. The results were adjusted for multiple comparisons using the Bonferroni correction. The single-cell sequencing data were deposited in the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE248351).

Statistical analysis

Data were analyzed using Prism (GraphPad Software). Data were analyzed by log-rank test, one-way ANOVA, and Student *t* test as indicated. Each symbol represents one mouse or one well of stimulated cells unless otherwise indicated.

RESULTS

Differential expression of Reg1 in lung epithelial cells during KP infection

We analyzed single-cell transcriptome data from wild-type (WT) mice to gain insight into Reg1 expression within lung epithelial cells during KP infection. Reg1 was highly expressed in AT2 cells compared with ciliated, secretory, and type 1 alveolar epithelial cells. Furthermore, after KP infection, AT2 cells clustered in different groups: conventional AT2 cells (c-AT2) with enriched canonical epithelial genes, inflammatory AT2 cells (i-AT2) with enhanced proinflammatory genes, and proliferative AT2 cells (p-AT2) with associated cell cycle genes (Fig. 1A, 1B). Among these three groups, Reg1 was mainly enriched in inflammatory AT2 cells (i-AT2) (Fig. 1C). Therefore, *Reg1* (*Zc3h12a*) segregates with the inflammatory AT2 cluster during KP infection.

Conditional deletion of Reg1 in alveolar type 2 epithelial cells

Reg1 is ubiquitously expressed. However, its function in the lung structural compartment needs to be better characterized. Because Reg1 was enriched in AT2 cells during KP infection, we conditionally deleted Reg1 in AT2 cells by breeding $Reg1^{flox/flox}$ with $Sftpc^{Cre\ ERT2+}$ or $Sftpc^{Cre\ ERT2-}$ mice (referred to as Δ*Reg1 or WT Reg1* in this paper). Moreover, to evaluate the specificity of $Sftpc^{Cre\ ERT2}$ expression in AT2 cells, Δ*Reg1* mice were crossed with a $Rosa26^{mT/mG}$ reporter mouse, a cell membrane–targeted, two-color fluorescent Cre-reporter allele. When recombined, Cre recombinase-expressing cells revealed GFP (mG) replacing the red fluorescence Td Tomato (mT). An average of 78.7% of AT2

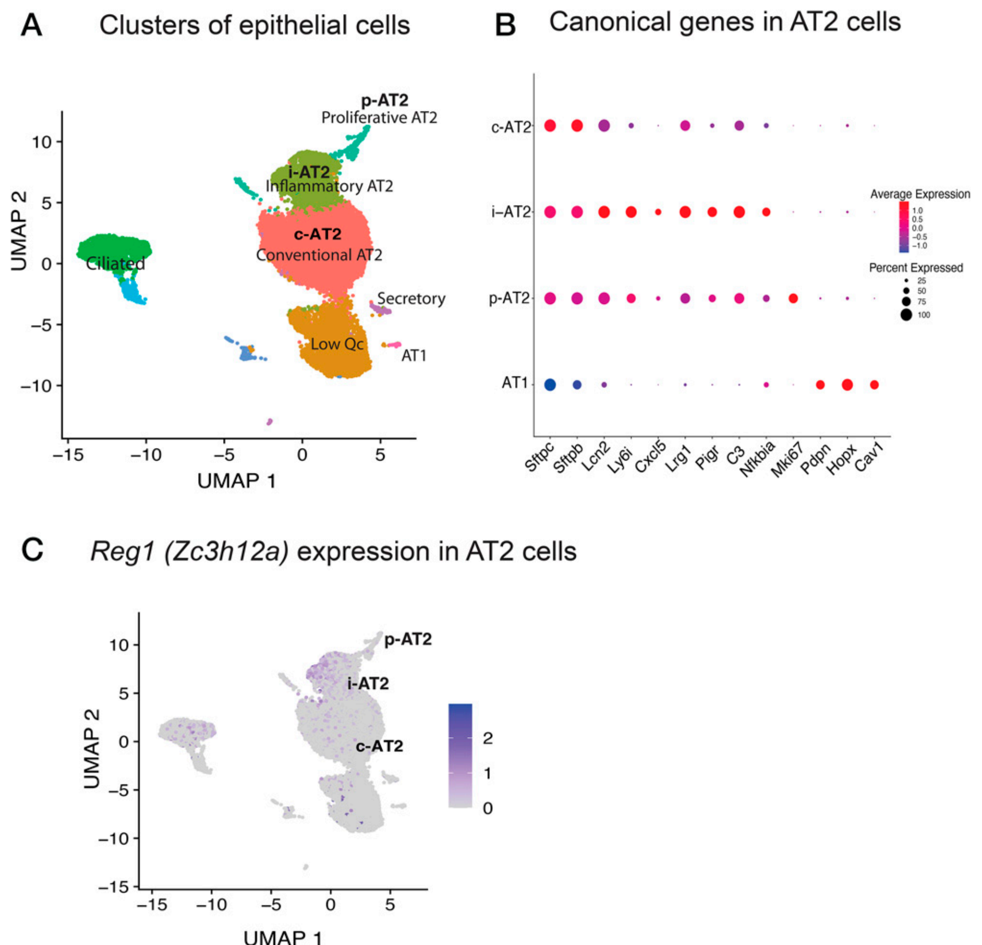

FIGURE 1. Regnase 1 (*Reg1/Zc3h12a*) is expressed in inflammatory AT2 cells (i-AT2). $CD45^{-}CD31^{-}EPCAM^{+}$ cells were identified by single-cell RNA sequencing. (**A**) Clusters of AT2 cells: conventional AT2 (c-AT2), inflammatory AT2 (i-AT2), and proliferative AT2 (p-AT2). Analysis visualized by Uniform Manifold Approximation and Projection (UMAP) and assigned different colors. (**B**) Dot plot showing selected canonical genes in each cluster of alveolar epithelial cells. (**C**) *Reg1* (*Zc3h12a*) expression in each cluster of AT2 cells.

sorted cells ($CD45^{-}CD31^{-}Epcam^{+}MHCII^{hi}$) were GFP^{+} after tamoxifen administration, and the average Cre recombination of the Reg1 floxed region in these GFP^{+} AT2 cells was 87.2% (Fig. 2A). Furthermore, *Reg1* (*Zc3h12a*) expression in sorted epithelial cells ($CD45^{-}CD31^{-}Epcam^{+}$) was significantly downregulated in Δ*Reg1* compared with *WT Reg1* mice (Fig. 2B). Thus, Reg1 conditional deletion in AT2 cells has adequate recombination efficiency.

Reg1 deletion in alveolar type 2 cells and epithelial cells increases local IκBζ expression

Reg1 degrades proinflammatory transcripts through a stem-loop sequence in its 3′ untranslated region (UTR), and Reg1 negatively regulates *Nfkbiz*, which encodes the transcriptional regulator IκBζ (9). As such, we hypothesized that Reg1 deletion in AT2 cells would increase the expression of *Nfkbiz*. IκBζ was increased in sorted epithelial cells ($CD45^{-}CD31^{-}Epcam^{+}$) from Δ*Reg1* mice compared with *WT Reg1* mice at 48 h after KP infection (Fig. 3A).

Moreover, *REG1*-deleted human bronchial epithelial cells (Δ*REG1*) significantly expressed IκBζ compared with *WT REG1* after KP infection. Concomitantly, other targets known to be degraded by REG1 were increased in Δ*REG1* compared with *WT REG1* cells, such as IL-1β, GM-CSF, and *IL6* (Fig. 3B, 3C). Thus, epithelial REG1 controls IκBζ expression during KP infection.

Reg1 deletion in alveolar type 2 cells increases alveolar macrophage numbers

To assess the impact of Reg1 deletion in AT2 cells on lung hematopoietic cells, we assessed Reg1 target proinflammatory genes. *Nfkbiz* and *Il6*, known Reg1 targets, were increased in Δ*Reg1* murine lungs compared with controls at 48 h after KP infection. However, there were no significant differences in *Il1b*, *Tnf*, or chemokines such as *Cxcl1*, *Cxcl5*, and *Ccl2* (Fig. 4A). Neutrophils, inflammatory monocytes, and $CD11b^{+}$ dendritic cells were similarly recruited into the infected lung of *WT Reg1* and Δ*Reg1* mice. Unexpectedly, alveolar macrophage numbers were increased in Δ*Reg1* mice compared with controls at 48 h after KP infection (Fig. 4B). Lung permeability did not account for these findings, because total lung protein and epithelial tight junction expression (*Cldn4*, *Cldn5*) were similar between *WT Reg1* and Δ*Reg1* mice (Supplemental Fig. 1A, 1B). In addition, type I IFN-dependent genes and IFN-β were not affected by the deletion of Reg1 in AT2 cells (Supplemental Fig. 1D, 1E). Thus, selective Reg1 deletion in AT2 cells induces higher alveolar macrophage numbers at 48 h after KP infection.

Reg1 deletion in alveolar type 2 cells does not impact survival upon KP lung infection

Because Reg1 deletion in AT2 cells increased *Nfkbiz* and correlated with increased host alveolar macrophages, we assessed bacterial burden and survival. Bacterial burden in the lung and spleen were similar between *WT Reg1* and Δ*Reg1* mice (Fig. 5A, 5B). Furthermore, there was no difference in survival up to 7 d after KP infection or significant weight loss changes between *WT Reg1* and Δ*Reg1* mice (Fig. 5C, 5D). Thus, Reg1 deletion in AT2 cells is insufficient to ameliorate KP lung infection.

DISCUSSION

Reg1, an RNA-binding protein, is ubiquitously expressed and downregulates inflammation by degrading translationally active transcripts such as *Il1b*, *Il12b*, *Nfkbiz*, and *Il6*, among

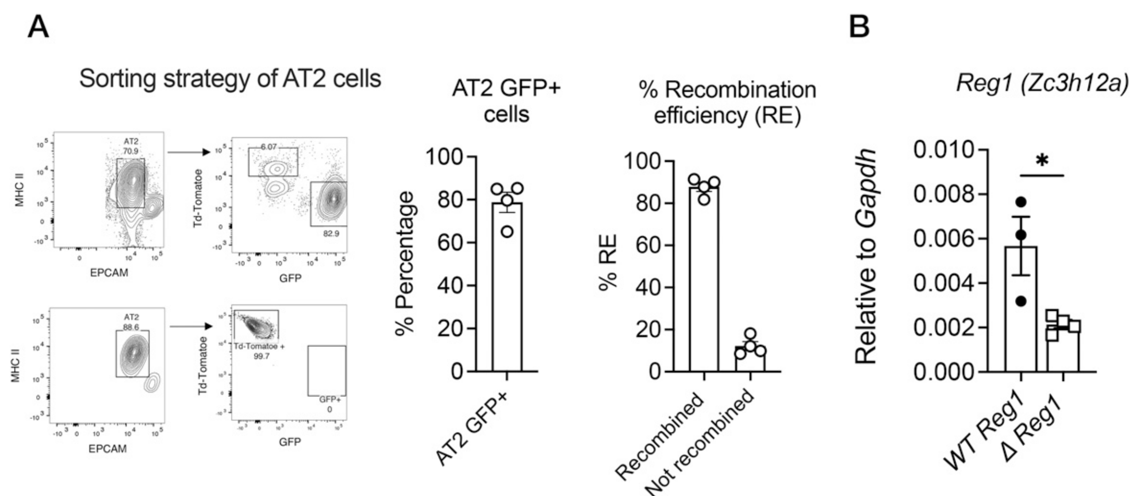

FIGURE 2. Reg1 is efficiently recombined in AT2 cells.
WT Reg1 and Δ*Reg1* mice were crossed with *Rosa26*$^{mT/mG}$ reporter mice. (**A**) Lung epithelial cells ($CD45^{-}$ $CD31^{-}Epcam^{+}$) were assessed by flow cytometry. $Epcam^{+}$ $MHCII^{hi}$ GFP^{+} (AT2 GFP^{+}) and $Epcam^{+}MHCII^{hi}TdTomato^{+}$ (AT2 TdT^{+}) were sorted and evaluated for *Reg1* (*Zc3h12a*) recombination efficiency by qPCR. (**B**) Lung epithelial cells ($CD45^{-}$ $CD31^{-}Epcam^{+}$) from Δ*Reg1* and *WT Reg1* mice were sorted and assessed for *Zc3h12a* expression by qPCR. *$p < 0.05$, unpaired *t* test.

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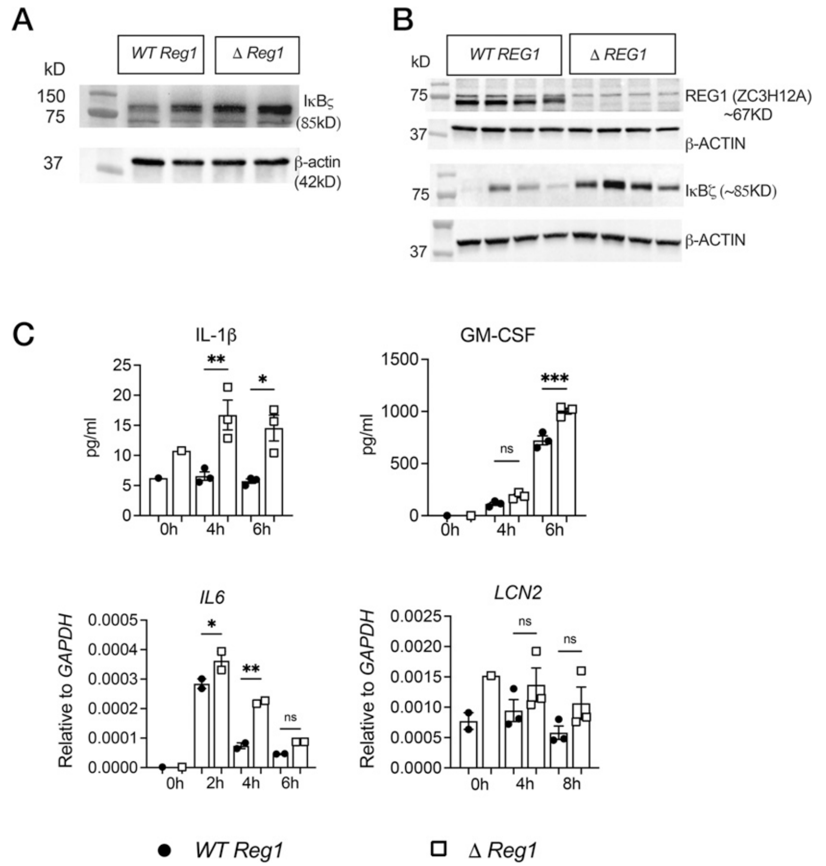

FIGURE 3. Reg1 controls IκBζ in epithelial cells.

WT Reg1 and Δ*Reg1* mice were infected with KP and sacrificed at 48 h. (**A**) Epithelial cells ($CD45^{-}Epcam^{+}MHCII^{+}$) were sorted, and protein was subjected to IκBζ by Western blotting. (**B**) *REG1* (*ZC3H12A*) was deleted in the human bronchial epithelial cell line by CRISPR-Cas9 (Δ*REG1*). The cell line was infected with KP (multiplicity of infection, 10) at the indicated time points and assessed for REG1 (ZC3H12A) and IκBζ by Western blotting. (**C**) IL-1β and GM-CSF by ELISA, and *IL6* and *LCN2* by qPCR. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, one-way ANOVA. Data were repeated twice.

others (9–11). Reg1 has a ubiquitin association domain at the N-terminus, a PIN-like RNase domain along with a conserved CCCH-type Zn finger domain in the middle, and a proline-rich domain at the C-terminus (12). The PIN-like RNase domain is responsible for Reg1 degrading translationally active client mRNAs by recognizing stem-loop structures in 3′ UTRs (13).

In previous work, bone marrow chimera studies revealed that Reg1 in hematopoietic and nonhematopoietic compartments contributes to susceptibility to KP pneumonia (8). This finding prompted the present study of Reg1 in lung epithelial cells. The lung epithelium plays a crucial role in KP resistance through the secretion of neutrophil-chemoattractant proteins and antimicrobial peptides (14, 15). Two pathways crucial for KP resistance converge in the lung epithelium, both recognizing and inducing Reg1, TLR4, and IL-17R, which are crucial for KP resistance (9, 16). On the basis of this premise, we hypothesized that targeting Reg1 deletion in AT2 cells would enhance KP resistance by prolonging the half-life of proinflammatory mediators.

We found that lung AT2 cells are highly dynamic during KP infection, and we detected three distinct populations by single-cell RNA sequencing based on canonical gene expression: conventional, inflammatory, and cycling AT2 cells. The importance of different groups of AT2 cells was highlighted in a model of bleomycin-induced lung injury, where AT2 cells enriched in inflammatory genes, named primed AT2 cells, eventually differentiate into damage-associated transient progenitors crucial for lung regeneration (17). We found a similar subgroup of AT2 cells enriched in inflammatory genes, including *Reg1* (*Zc3h12a*), which we named inflammatory AT2 cells (i-AT2). Ciliated cells also expressed *Reg1* in our dataset, but they were less enriched than AT2 cells. Our data are also supported by publicly available transcriptome information (www.lungmap.net), where *Reg1* is primarily enriched in AT2 cells compared with other epithelial cells. On the basis of these findings, our strategy was to delete *Reg1* (*Zc3h12a*) in AT2 cells.

Although we efficiently deleted Reg1 in AT2 cells, our findings did not support our hypothesis, because there was no difference in bacterial burden or survival between *WT Reg1* and Δ*Reg1 mice*. These results differed from our previous publication in which we noted a modest but significant difference in survival between WT and mice with Reg1 deletion in AT2 cells (8). This difference is due primarily to the increased number of mice per group. In the present study, we included 29 *WT Reg1* mice and 29 Δ*Reg1* mice, representing cumulative findings from four independent experiments. The significant increase in mice eliminated the modest significance seen in our previous publication (8).

Would deletion of Reg1 using a broader epithelial Cre impact KP resistance? The pan-lung epithelial Nkx2.1Cre has been used to delete Reg1 in a model of *Pseudomonas aeruginosa*, with a modest but significant decrease in bacterial burden at 24 h after infection (18). However, survival at day 5 after infection was

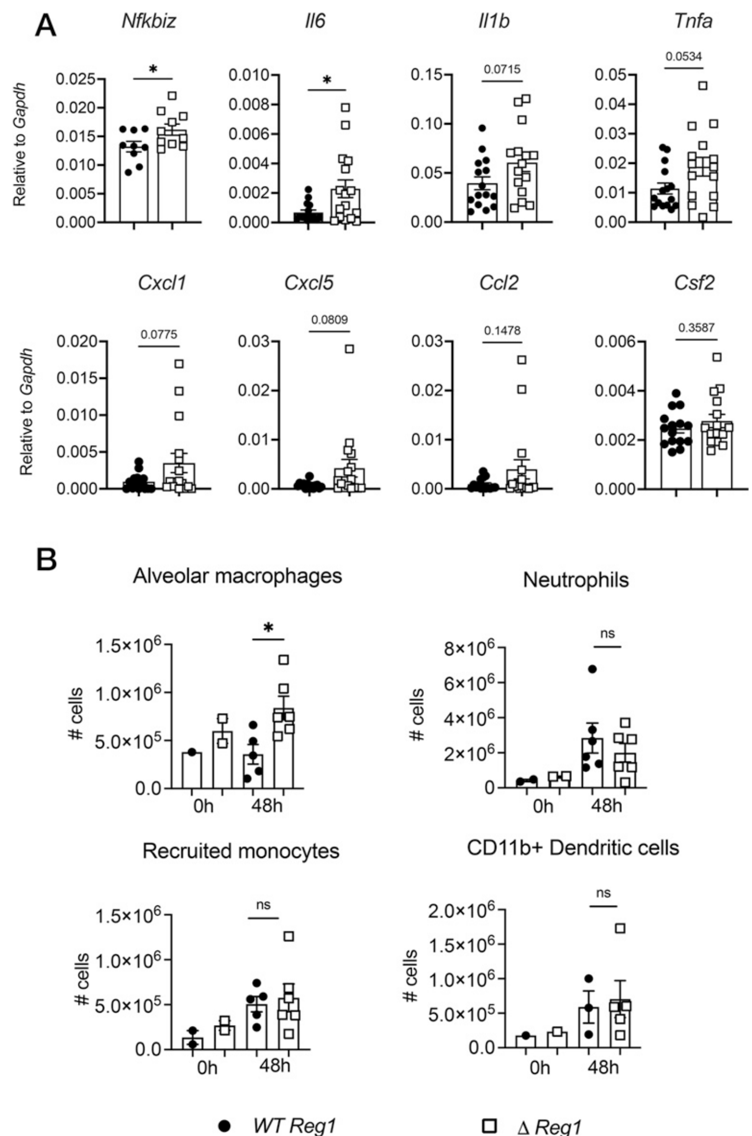

FIGURE 4. Reg1 deletion in AT2 cells associates with increased alveolar macrophages.
(**A**) Total lung tissue was assessed for gene expression of *Nfkbiz*, *Il6*, *Il1b*, *Tnf*, *Cxcl1*, *Cxcl5*, *Ccl2*, and *Csf2* by qPCR at 48 h after KP infection. $*p < 0.05$, unpaired *t* test. (**B**) Absolute numbers of alveolar macrophages ($CD11c^{hi}CD11b^{-}SiglecF^{hi}$), neutrophils ($CD11b^{+}$ $Ly6G^{hi}$), recruited monocytes ($Cd11b^{+}CD11c^{-}Ly6G^{-}MHCII^{-}Ly6C^{hi}$) and $CD11b^{+}$ dendritic cells ($Ly6G^{-}SiglecF^{-}$ $CD11c^{+}CD11b^{+}MHCII^{+}$) recruited to the lung at 48 h after KP infection. $*p < 0.05$, one-way ANOVA. Data are representative of two different experiments.

unchanged between the WT and conditional KO mice. Using a pan-epithelial Cre would not add a significant difference in survival, because Reg1 is mostly expressed in AT2 cells. However, a definitive experiment needs to be performed to rule out that possibility.

Interestingly, other nonhematopoietic cells, such as lung endothelial and mesenchymal cells, express higher levels of Reg1 than lung epithelial cells (www.lungmap.net). Thus, deleting Reg1 in lung endothelial or mesenchymal cells may increase KP resistance more than in epithelial cells. Lung endothelial cells are highly heterogeneous, and recent infection models have started to unravel different subtypes of lung endothelial cells during lung injury (19). Another group of nonhematopoietic cells that express high levels of Reg1 is lung mesenchymal cells. Very few studies have assessed these cells in the context of KP infection. Lung mesenchymal cells have phagocytic capacity and anti-inflammatory activity (20). Thus, exploration of whether deleting Reg1 in these cells would protect or predispose to KP infection has yet to be done.

Our in vivo and in vitro data showed enhanced *Nfkbiz*, which encodes IκBζ. KP-infected Δ*REG1* human bronchial epithelial cells significantly induce IκBζ; this likely results from *Nfkbiz* mRNA stabilization, which is predicted to have two stem-loop structures in the 3′ UTR for Reg1-mediated mRNA decay (13). *Nfkbiz* is induced by TLR4 signaling and IL-17R signaling (9, 21). *Nfkbiz* is induced rapidly, and it is considered a primary response gene (22). It mediates the activation of secondary response genes such as *Il6*, *Il12*, *Ccl2*, and *Csf2*, which are activated at later time points than *Nfkbiz* and are not induced in $Nfkbiz^{-/-}$ cells (21, 23). Furthermore, *Il6* also has a stem-loop in the 3′ UTR that Reg1 recognizes for mRNA degradation. Thus, our findings suggest that *Nfkbiz* and *Il6* expression are

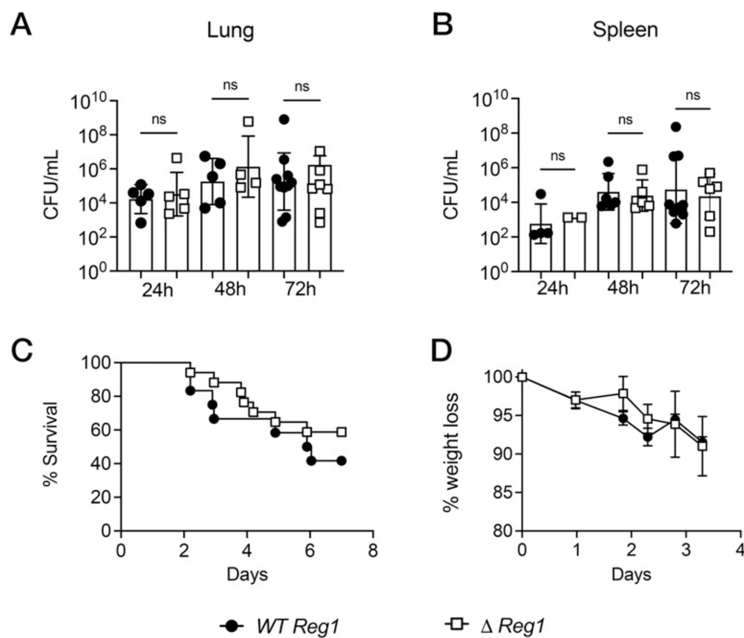

FIGURE 5. Reg1 deletion in AT2 cells does not affect KP resistance.

(**A**) Lung and (**B**) spleen KP burden in DReg1 or WT Reg1 mice up to 72 h postinfection. Each dot and circle represent a mouse. One-way ANOVA, ns, not significant, $p > 0.05$. (**C**) Survival of Δ*Reg1* or WT *Reg1* littermates was assessed up to 7 d after infection ($n = 29$ mice/group). (**D**) Weight loss was assessed at the indicated time points ($n = 29$ mice/group). Data are pooled from four experiments.

locally stabilized due to conditionally deleting Reg1 in AT2 cells.

There was no difference in chemokine expression such as *Cxcl1*, *Cxcl5, or Ccl2*, and, accordingly, the recruitment of neutrophils and inflammatory monocytes was similar between groups. However, alveolar macrophages were higher in Δ*Reg1* mice than in controls. Because the alveolar macrophages have an intact Reg1 protein, we speculate that a prosurvival factor is being secreted in higher amounts from the Δ*Reg1* AT2 cells than the *WT Reg1* and acting on alveolar macrophages. One possible prosurvival factor is GM-CSF, encoded by *Csf2* and enhanced by *Nfkbiz*. Although total lung GM-CSF was similar between *WT Reg1* and Δ*Reg1* mice (Supplemental Fig. 1F), more in-depth functional in vivo experiments and loss-of-function studies are needed to know that for sure.

Notably, the effect of deleting Reg1 in AT2 cells may be offset by other RNA-binding proteins, particularly Roquin 1/2 (RC3H1/2), which share the same 3′ UTR target transcripts for degradation as Reg1 (13). Furthermore, at least in T cells, Reg1 and Roquin cooperate to target the degradation of several proinflammatory transcripts, which is vital to keep autoimmunity in check (24, 25). Would concomitant deletion of Roquin1/2 and Reg1 in AT2 cells have a different outcome? Would the host eradicate KP at the cost of lung injury? These are areas that have not been explored yet.

Overall, the immune system has evolved to balance the vital effects of antimicrobial effector functions with the potential for causing collateral tissue damage. Consequently, activating every immune signaling pathway is accompanied by negative feedback signaling events designed to mitigate inflammation (26, 27). However, allowing localized, heightened inflammation to treat a life-threatening condition may be clinically advantageous. Releasing inflammatory "brakes," ideally in a limited fashion, may be helpful in the context of severe infections such as bacterial pneumonia, and exploring the potential role of Reg1 in other lung structural cells could, in principle, represent a valuable target to achieve this goal.

DISCLOSURES

The authors declare no conflicts of interest.

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