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RESEARCH ARTICLE

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Increased lethality of respiratory infection by *Streptococcus pneumoniae* in the Dp16 mouse model of Down syndrome

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Abstract

Objectives: We sought to investigate whether the Dp16 mouse model of Down syndrome (DS) is more susceptible to severe and lethal respiratory tract infection by *Streptococcus pneumoniae*.

Study Design: We infected controls and Dp16 mice with *Streptococcus pneumoniae* and measured survival rates. We compared cytokine production by primary lung cell cultures exposed to *Streptococcus pneumoniae*. We examined lung protein expression for interferon signaling related pathways. We characterized the histopathology and quantified the extent of bronchus-associated lymphoid tissue. Finally, we examined mouse tissues for the presence of oligomeric tau protein.

Results: We found that the Dp16 mouse model of DS displayed significantly higher susceptibility to lethal respiratory infection with *Streptococcus pneumoniae* compared to control mice. Lung cells cultured from Dp16 mice displayed unique secreted cytokine profiles compared to control mice. The Dp16 mouse lungs were characterized by profound lobar pneumonia with massive diffuse consolidation involving nearly the entire lobe. Marked red hepatization was noted, and Dp16 mice lungs contained numerous bronchus-associated lymphoid tissues that were highly follicularized. Compared to uninfected mice, both control mice and Dp16 mice infected with *Streptococcus pneumoniae* showed evidence of oligomeric tau aggregates.

Conclusions: Increased susceptibility to severe respiratory tract infection with *Streptococcus pneumoniae* in Dp16 mice closely phenocopies infection in individuals with DS. The increase does not appear to be linked to overexpression of mouse interferon genes syntenic to human chromosome 21.

1 | INTRODUCTION

Trisomy 21, or Down syndrome (DS), is a condition caused by the presence of an extra copy of human chromosome 21 (Hsa21). People with DS are born with congenital abnormalities that involve many organ systems including the heart and lungs.[1] In the middle of the last century, the life expectancy for people with DS was ~12 years. Due to tremendous progress in medicine and changes in social attitudes, life expectancy for persons with DS is now greater

than 60 years of age. Between 1979 and 2003, the number of babies born with DS increased by about 30% (CDC). Given these data, there is a profound unmet need to better address medical issues confronted by people with DS across their lifespan.

In addition to intellectual disability, people with DS experience increased hearing loss, ear infections, eye diseases, heart defects at birth, thyroid disease, anemia, leukemia, and the development of Alzheimer's disease.[2] However, the major health threat for all people with DS at any age is respiratory tract infection (RTI).[3,4] For children with DS, RTI accounts for over half of hospital admissions, with pneumonia most commonly implicated for intensive care unit admissions and longer hospital stays.[5] Similar data are observed in adults with DS.[6–8] In a recent study, the incidence of all-cause pneumonia was increased five-fold in persons with DS compared to those without DS.[9] Unfortunately, the specific mechanisms underpinning the severity and lethality of infectious lung disease for persons with DS are largely unexplored.

Mouse models of DS have been invaluable in the pursuit of understanding the biology of DS. The genes on Hsa21 that are syntenic in mice are spread over chromosomes 10, 16, and 17. Mice have been created with segmental duplications of these chromosomes, with the goal of examining overdosage of genes orthologous to those on Hsa21. The challenges in experimental design and interpretation of models of DS are numerous and significant.[10] Recently, the Dp16 mouse model of DS has been invaluable in exploring the extent to which DS can be considered an "interferonopathy", given that several interferon signaling genes are located on Hsa21.[11] Indeed, people with DS appear to have hypersensitivity to type I interferons.[12] Interferons are cytokines that bind interferon receptor types I (IFNARI), II (IFNGR2), and III (IL-10Rb), and among many other functions, activate antiviral activity. During viral infection, interferon-stimulated genes directly inhibit viral biology but render individuals susceptible to secondary infection by bacteria such as *Streptococcus pneumoniae*.[13] For people with DS, the recent SARS-CoV-2 pandemic resulted in a ~ten-fold increased risk for COVID-19-related death, and in major part due to pneumonia.[3]

We recently characterized the Dp16 mouse model of DS with regard to lung and heart biology with an emphasis on interferon signaling.[14] We found that the Dp16 mouse displays lung dysmorphology similar to DS, including alveolar simplification. In addition, we found significantly expanded bronchus-associated lymphoid tissue (BALT). Interestingly, at the lung tissue-level, we found no evidence of constitutively-activated interferon signaling in Dp16 mice. Several recent studies have arrived at similar conclusions using cultured cells from people with DS and by reanalyzing published datasets.[15–17] Here, we test the hypothesis that Dp16 mice phenocopy the increased susceptibility to severe respiratory tract infection with *Streptococcus pneumoniae* in Dp16 mice observed in individuals with DS. We conclude that the increased susceptibility to mortal RTI in people with DS is directly linked to changes in pulmonary physiology, histology, and cell molecular biology that result from deranged expression of Hsa21 and non-Hsa21 encoded genes. Furthermore, we uncover a potential link between recurrent RTI and cognitive dysfunction in DS.

2 | METHODS

2.1 | Mice

Dp(16Lipi-Zfp295)1Yey (abbreviated Dp16) mice (The Jackson Laboratory) were housed in pathogen-free rooms under a 12-h light/dark cycle photograph period. The mice were provided with Teklad Global Soy Protein-Free Extruded Rodent Diet Irradiated (2920X) and sterilized water ad libitum. All mice were treated in accordance with the Guide for the Care and Use of Laboratory Animals, Animal Welfare Act and PHS Policy. Littermates (C57/Bl6 strain background) lacking segmental duplication of chromosome 16 were used as controls. For euthanizing mice, Sleep Away euthanasia solution was injected (intraperitoneal) at 200 mg/kg. Mice were placed in a weigh boat for mass measurement. After ataxia passed, the mice were then placed in a prone position with their limbs maintained in a natural posture. The mice were measured from tip of the nose to the posterior section where the tail begins in centimeters using a standard ruler. Time until nonresponsiveness was measured by starting a timer at injection and concluding when the mice did not respond to any stimulus such as ocular touch or a toe pinch.

2.2 | Infection with Streptococcus pneumoniae

In three independent experiments, 12 male and 12 female each of C57bl/6 littermate control mice and Dp16 mice at 8–10 weeks of age were challenged with 1.7×10^7 colony-forming units (CFU) intranasal *S. pneumoniae* (exponential growth phase) in 50–100 µL sterile saline. Mice were closely monitored for weight loss and other moribund criteria. Seventy-two hours postinfection, mice were euthanized as described above and tissues were prepared for the study as described below.

2.3 | CFU

Mouse lung tissues were weighed and homogenized with four times the titer of sterile PBS and subjected to serial dilution. The diluted homogenates were spread on 5% sheep blood agar plate and incubated for 24 h at 37°C with 5% CO_2. The *S. pneumoniae* colonies were then counted.

2.4 | Mesoscale discovery ELISA

To obtain cell cultures from whole lung, mouse left lungs were flushed with sterile 1X PBS, put through lung tissue digestion procedure, spun down at 300 × g for 10 min at RT, passed through 100 µm and 70 µm nylon screens and then split into plates. Multiple cell types grew until 80%–90% confluency. Cells were washed twice before addition of culture media lacking antibiotics. Coincubation of cells with *S. pneumoniae* was performed as previously described.[18] To collect whole lung cell culture supernatants, 0.5% serum media was place on mouse whole lung adherent cells for 18 h before supernatants were collected and spun at 400 × g to remove any debris. Supernatants were used neat in the MSD MULTI-SPOT Assay System. Cytokines were assayed using the V-Plex Proinflammatory Panel 1 Kit according to manufacturer's protocol (Mesoscale Discovery) using samples diluted 50:50 into diluent. All standards and samples were run in triplicate. In cases where an analyte was above or below the range of the kit standards, samples were run again at dilutions suitable for the dynamic range of the kit for that analyte.

2.5 | Sarkosyl precipitation of tau

Sarkosyl-insoluble tau was precipitated from 1.5 mL of pooled plasma and from tissue homogenates as previously described.[19] Lungs and hearts were weighed and suspended in 4× volume of homogenization buffer containing 1 mM phenylmethylsulfonyl fluoride and 1 µg/mL each antipain, chymostatin, leupeptin, and pepstatin. Tissues were homogenized by 10 s pulsing with a PowerGen 125 homogenizer (Fisher) on ice. The soluble tau fraction was collected in the supernatant following centrifugation at 27,000 × *g* for 20 min at 4°C. The pellet was homogenized in homogenization buffer (10 mM Tris pH 7.4, 800 mM NaCl, 10% sucrose, 1 mM ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid, 1 mM phenylmethylsulfonyl fluoride), and centrifuged as above. The supernatants were collected, sarkosyl was added to a final concentration of 1%, incubated at 37°C for 1 h, and then centrifuged at 21,000 rpm for 6 h at 4°C. The pellet was resuspended in 200 µL of resuspension buffer representing the sarkosyl insoluble tau fraction. Samples were assayed for total protein concentration using the lowry protein assay and stored at −80°C until used in immunoblot analyses.

2.6 | Immunoblot

Mice lung protein lysates were prepared by addition of RIPA buffer supplemented with protease, kinase, and phosphatase inhibitors (HALT, Pierce-Thermo Scientific). 25 µg of sample protein per lane was electrophoresed on 4%–12% gradient Bio-Tris gels (NOVEX, Invitrogen) followed by transfer to PVDF membrane (NEN Life Science Products) in buffer (Tris-glycine, NOVEX, Invitrogen) with 10% methanol. Prestained molecular mass marker proteins (Bio-Rad) were used to estimate band sizes. Luciferase chemiluminescence utilized Western Blot Chemiluminescence Reagent (GE Amersham). Antibodies against beta actin were used to probe blots and estimate loading equivalencies (see Table S1 for antibody details). Scanned images of gels were acquired (iBright CL1000, Thermo) from each blot of three independent experiments. Densitometry was performed according to manufacturer's protocol (Thermo) using normalization by beta actin protein expression (all antibodies used in the study are listed in Table S1).

2.7 | Histomorphometry

Lung histopathology scoring: Seven Dp16 (three males and four females) and seven control (three males and four females) mouse lungs were harvested from mice that were 9–13 weeks old and cut at the pulmonary artery, fixed in buffered formalin, and paraffin-embedded. These samples were cut with a microtome and stained with hematoxylin and eosin. Stained sections were examined and images were acquired at room temperature using a Revolution microscope (Echo, San Diego, CA, USA) fitted with a 4 × 0.13 numerical aperture objective and a five megapixel CMOS color bright-field camera. Histopathological findings were scored on a scale of 0–9 as previously described.[20] Every slide was scored 0–3 in the following three categories by three blinded investigators: the degree of tissue consolidation, severity of bronchovascular bundle inflammation, and the percentage of bronchovascular bundles inflamed.

Bronchus-associated lymphoid tissue (BALT): Seven Dp16 (three males and four females) and seven control (three males and four females) mouse lungs were harvested from mice that were 9–13 weeks old and cut at the pulmonary artery and frozen in OCT. These samples were

sectioned at 10 microns with a cryostat and stained with hematoxylin and eosin. The section inferior of the pulmonary artery was examined at 10× magnification for BALT. BALT was found in four samples, three female and one male. The BALT was measured semiautomatically with a custom MATLAB program that measures BALT in lung sections by looking for densely grouped nuclei in hematoxylin and eosin stained samples and further annotated to encompass the area of the BALT by an observe blinded to the identity if the tissue specimen.

2.8 | Statistics

For comparison between two groups, student's *t*-test was used, with significance selected as $p < 0.05$ (Prism 9). For multiple comparisons, an ordinary one-way ANOVA was used, with significance selected as $p < 0.05$.

3 | RESULTS

The general phenotypic features of our Dp16 mouse cohort, including littermate controls, are presented in Table 1. No differences were noted in sexes of the offspring among control mice and Dp16 mice cohorts.

Our study design is presented in Figure 1. C57bl/6 control mice and Dp16 mice at 8–10 weeks of age were challenged with intranasal *S. pneumoniae* and carefully followed for 72 h. Surviving mice were sacrificed humanely and tissues processed for downstream endpoints. Two subsequent independent groups were similarly challenged and the data pooled; however, due to the surprising lethality observed in the first group of Dp16 mice, we carefully monitored mice and sacrificed them prior to death to maximally preserve tissue integrity.

People with DS are highly susceptible to lethal infectious lung disease throughout their lives. We challenged control and Dp16 mice with intranasal instillation of *S. pneumoniae* and measured survival rates. We found that Dp16 mice were significantly more susceptible to mortal

TABLE 1 General characteristics of control and Dp16 mice.

	Control		Dp16	
	M	F	M	F
Mean age, days	119	108	104	107
Mean body weight, g	32.2	24.2	31.0	23.3

Note: All mice in the study were age- and sex-matched as closely as possible. There were no significant differences in age. There were no significant differences in mean body weight between controls and Dp16 mice of the same sex. The mean body weights of female mice were significantly lower than male mice for both control and Dp16 groups.

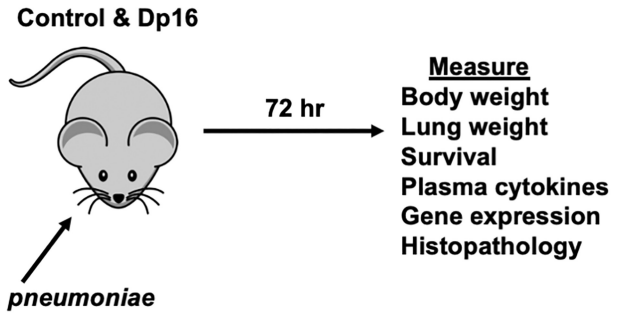

FIGURE 1 Study design. Littermate control and Dp16 mice were challenged with intranasal *S. pneumoniae* in saline. Mice were carefully monitored and, after 72 h, were evaluated for respiratory tract infection utilizing the endpoints listed.

RTI compared to controls (Figure 2). In the second experiment, moribund criteria were reached prior to mice succumbing to *S. pneumoniae*. These data indicate that segmental duplication of genes on mouse chromosome 16 is sufficient to render mice significantly more susceptible to lethal lung infection with *S. pneumoniae*. We observed no significant differences in these endpoints between males and females.

To begin to examine differences in molecular response to infection with *S. pneumoniae*, we measured a panel of plasma cytokines as we had previously reported in DS and in Dp16 mice at baseline (i.e., in the absence of infection). Upon infection with *S. pneumoniae*, we found differences in primary lung cell secretion of IL-5, IL-10, IL-1beta, KC-GRO and IL-12p70 (Table 2). These data indicate that the secreted cytokine response to infection with *S. pneumoniae* by Dp16 mice is unique compared to controls.

Dp16 mice carry a segmental duplication of chromosome 16 that includes several members of types I, II, and III interferon signaling pathways. We previously reported that although interferon receptors IFNAR1, IFNGR2, and IL-10Rb were overexpressed at the mRNA level in Dp16 mice, their corresponding protein levels were not. Following infection with *S. pneumoniae*, we found no differences between controls and Dp16 mice in lung protein expression levels of IFNAR1, STAT1, and phospho-STAT1 by immunoblot (Figure 3A). We found decreased levels of STAT3 and phospho-STAT3 protein expression in female Dp16 mice compared to controls. We found that IL-10Rb protein was significantly higher in male Dp16 mice compared to controls (Figure 3B). Collectively, these data suggest that lethal bacterial lung infection in Dp16 mice is not caused by increased amounts of interferon signaling constituents or by increased activation of STAT1 or STAT3.

Platelet-activating factor receptor (PAFR) is a rhodopsin-like g protein-coupled receptor that binds the phospholipid platelet-activating factor.[21] Airway epithelial expression of PAFR has been linked to increased adhesion

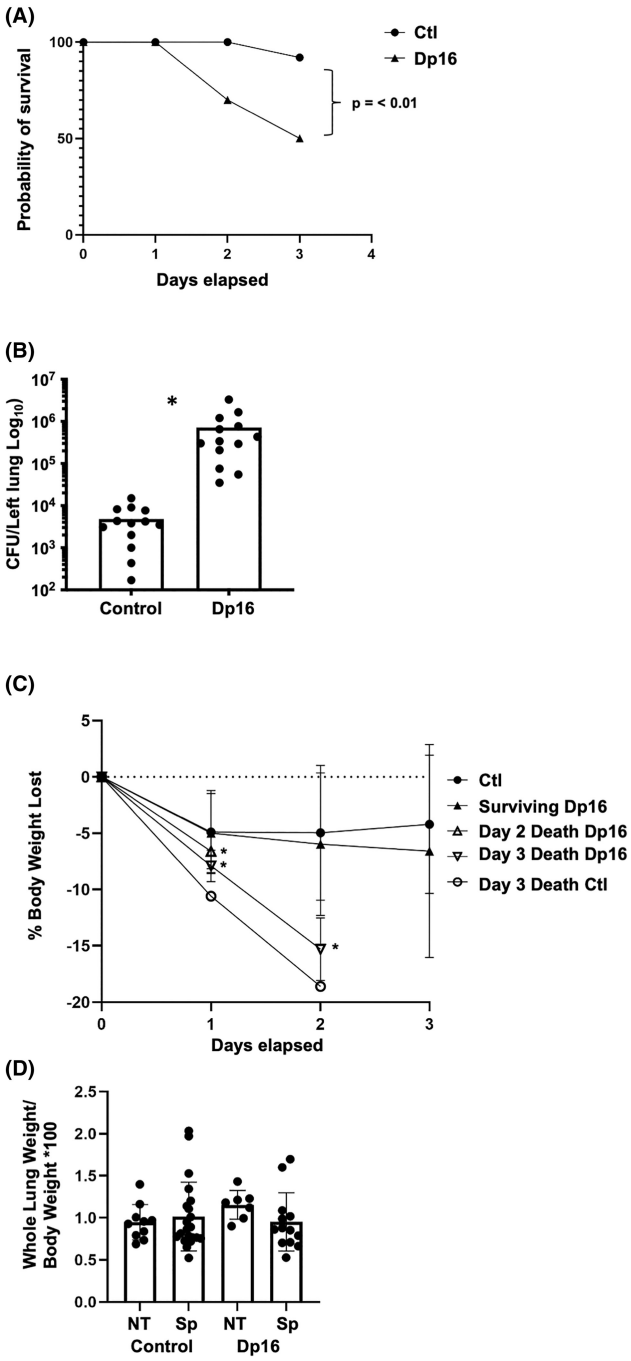

FIGURE 2 Increased lethality of *S. pneumoniae* infection for Dp16 mice compared to controls. (A) The probability of survival after challenge with *S. pneumoniae* was significantly lower for Dp16 mice ($n = 36$) compared to controls ($n = 36$). (B) Postinfection, Dp16 mice lungs contained significantly higher colony-forming units (CFUs) compared to controls. (C) Postinfection, Dp16 mice that died demonstrated a significant percent weight loss compared to Dp16 mice that survived. (D) There were no significant differences in the ratio of lung weight to body weight. SP = *S. pneumoniae* treated; NT = saline control. There were no significant differences in these endpoints between males and females.*$p < 0.05$.

of bacteria,[22] although this finding has been disputed.[23] We previously found that PAFR is decreased in the lungs of male Dp16 mice compared to controls, and that female Dp16 mice lungs showed no increase in PAFR expression. Following *S. pneumoniae* infection, we found that PAFR was not significantly increased in lungs of Dp16 mice versus controls (Figure 3C). These data suggest that pneumococcal infection in DS likely does not involve increased expression of PAFR in the lungs.

Next, we examined lung tissue sections for histopathological changes stemming from infection with *S. pneumoniae*. In both untreated controls and Dp16 mice, we found normal lung architecture throughout, with the notable exceptions of alveolar simplification and the presence of large, prominent bronchus-associated lymphoid tissue (BALT) in Dp16 mice. In both controls and Dp16 mice challenged with *S. pneumoniae*, we found significant lung pathology characterized by destruction of alveolar architecture, and large collections of inflammatory cell infiltrates. (Figure 4A). The lungs of Dp16 mice showed significantly worse pathology characterized by massive lymphoid aggregates, severe edema and hemorrhage, and the appearance of hyaline membranes (Figure 4B). The hearts of both control and Dp16 mice showed no increase in inflammatory cell infiltrates in both ventricles (Figure 4C), suggesting that *S. pneumoniae* infection was restricted to the lung.

We and others have previously reported that bronchus-associated lymphoid tissue (BALT) is prominent in the lung in a variety of infectious and noninfectious experimental settings.[24] We found that Dp16 mice with and without pneumonia displayed prominent and follicularized BALT in and around large, medium, and small caliber airways, as well as in numerous ectopic regions throughout the lung parenchyma (Figure 5A). Dp16 BALT were more numerous and significantly larger compared to controls (Figure 5B). Collectively, these data suggest unique roles for BALT in orchestrating lung immunity in Dp16 mouse lungs before and after infection with *S. pneumoniae*.

RTI is associated with development of cognitive dysfunction in typical individuals[25] as well as those with DS.[26] Recently, it has been determined that pneumonia can induce a tauopathy due to release of cytotoxic lung endothelial tau,[27,28] and, in separate studies, it has been shown that persons with DS circulate elevated Tau in peripheral blood.[29,30] We precipitated tau from plasma, lung, and brain of both control and Dp16 mice groups that were infected with *S. pneumoniae* but not in uninfected mice (Figure 6). These studies confirm that RTI elicits significant increases in lung tau in mice and that tau oligomers are distributed in peripheral tissues, potentially including brain.

TABLE 2 Whole lung cell cultures from control mice and Dp16 mice respond exhibit unique cytokine responses when treated in vitro with *S. pneumoniae*.

	0 h pg/mL		4 h pg/mL		18 h pg/mL	
Secreted Cytokine	**Ctl**	**Dp16**	**Ctl**	**Dp16**	**Ctl**	**Dp16**
IL-2	1.87 ± 0.9	1.7 ± 1.4	1.77 ± 1.4	1.67 ± 0.9	2.27 ± 0.7	2.77 ± 0.8
IL-4	ND	ND	ND	ND	ND	ND
IL-5	0.37 ± 0.2	0.087 ± 0.4	0.37 ± 0.4	0.057 ± 0.1	0.87 ± 0.1	0.47 ± 0.4
IL-6	4207 ± 146	3427 ± 189	4357 ± 133	2867 ± 177	5857 ± 249	6907 ± 124
IL-10	0.77 ± 0.2	2.47 ± 0.4	1.37 ± 0.2	**2.07 ± 0.3**	5.47 ± 0.3	8.87 ± 4.4
IL-1 beta	0.17 ± 0.3	0.17 ± 0.2	0.137 ± 0.3	**0.057 ± 0.2**	0.167 ± 0.4	0.247 ± 0.3
IL-12p70	157 ± 0.6	10.87 ± 0.4	13.57 ± 0.5	10.77 ± 0.4	29.27 ± 0.6	34.87 ± 0.7
IFN-gamma	0.057 ± 0.2	ND	0.37 ± 0.7	0.27 ± 0.5	0.17 ± 0.8	0.17 ± 0.8
KC-GRO	997 ± 29	**35.97 ± 28**	997 ± 30	28.47 ± 19	4297 ± 98	2467 ± 126
TNF-alpha	0.87 ± 0.6	0.97 ± 0.4	0.87 ± 0.4	0.97 ± 0.3	2.77 ± 1.4	2.57 ± 1.5

Note: Supernatants from Dp16 mice lung cells showed decreased IL-5 before and after exposure to *S. pneumoniae*. Supernatants from Dp16 mice lung cells showed increased IL-10 before and after exposure to *S. pneumoniae*. Cytokine production of IL-1beta, KC-GRO, and IL-12p70 was variable. No differences were found between control and Dp16 lung cell culture supernatants for IL-2, IL-6, IFN-gamma, or TNF-alpha. – indicates analyte was below the level of detection. ND = not detected. Bold = $p < 0.05$.

4 | DISCUSSION

Respiratory tract infection is the most lethal medical condition for all persons with DS regardless of age. We report here that the Dp16 mouse model of DS is significantly more susceptible to lethal RTI with *S. pneumoniae* compared to controls. The severe course of RTI in Dp16 mice is associated with massive expansion of bronchus-associated lymphoid tissue. Furthermore, we show that RTI in Dp16 mice is not associated with increased levels of interferon receptors or STAT 1 or STAT 3 proteins. Dp16 mice do not overexpress PAFR in their lungs during infection. RTI in both controls and Dp16 mice groups resulted in increases in tau in lung, heart, and plasma. To our knowledge, our report is the first to describe such unique pathological changes following bacterial RTI in any preclinical model of DS.

Recently, the INCLUDE (INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE) program at NIH has ushered in a renaissance of new investigators, new ideas, and increased funding with the goal of helping persons with DS live longer and healthier lives. For example, trisomy 21 and the Dp16 mouse of DS have been associated with consistent activation of interferon responses.[11,31] Despite insights gained from studies on congenital abnormalities of airways and on immune cell defects, there are unfortunately few studies yet available that illuminate why people with DS are so susceptible to severe, lethal, and recurrent respiratory tract infection. To begin to address this major unmet clinical need, we hypothesized that overexpression of interferon receptors in people with DS might render them more susceptible to bacterial pneumonia. Our finding that Dp16 mice are highly susceptible to lethal pulmonary infection with *S. pneumoniae* is consistent with this paradigm, despite lack of interferon activation.

Type I interferons are principal orchestrators of immune responses in infectious diseases. Specific outcomes of type I IFN responses are highly context-dependent.[32] Type III interferons are produced in response to microbial ligands, signal through receptors principally expressed by lung epithelium, and can be beneficial or detrimental depending on timing and biological contexts.[33] Several IFN genes are encoded on Hsa21, including IFNAR1, IFNAR2, IFNGR2, and IL-10Rb. Type I IFN signaling is detrimental in highly susceptible SARS-CoV-infected BALB/c mice, in large part by promoting the influx of pathogenic inflammatory monocyte-macrophages (IMMs).[34] On the contrary, mice lacking the receptor for IFNAR1, or which received antibodies that interfere with receptor activation, showed increased development of bacteremia upon lung infection with *S. pneumoniae*.[35] Treating mice, or cell lines, with type I IFN protected against bacterial migration across epithelial and endothelial cell barriers and correlated with increased lung barrier function.

In our study, we were unable to find strong linkage between overexpression of interferon receptor protein levels and increased mortality of Dp16 mice in response to infection with *S. pneumoniae*. There are a few potential explanations for this. First, we measured protein levels after ~72 h postinfection, long after lung and lung immune cell pattern recognition receptors and toll-like receptors are activated. In a previous paper, we reported highly variably protein expression

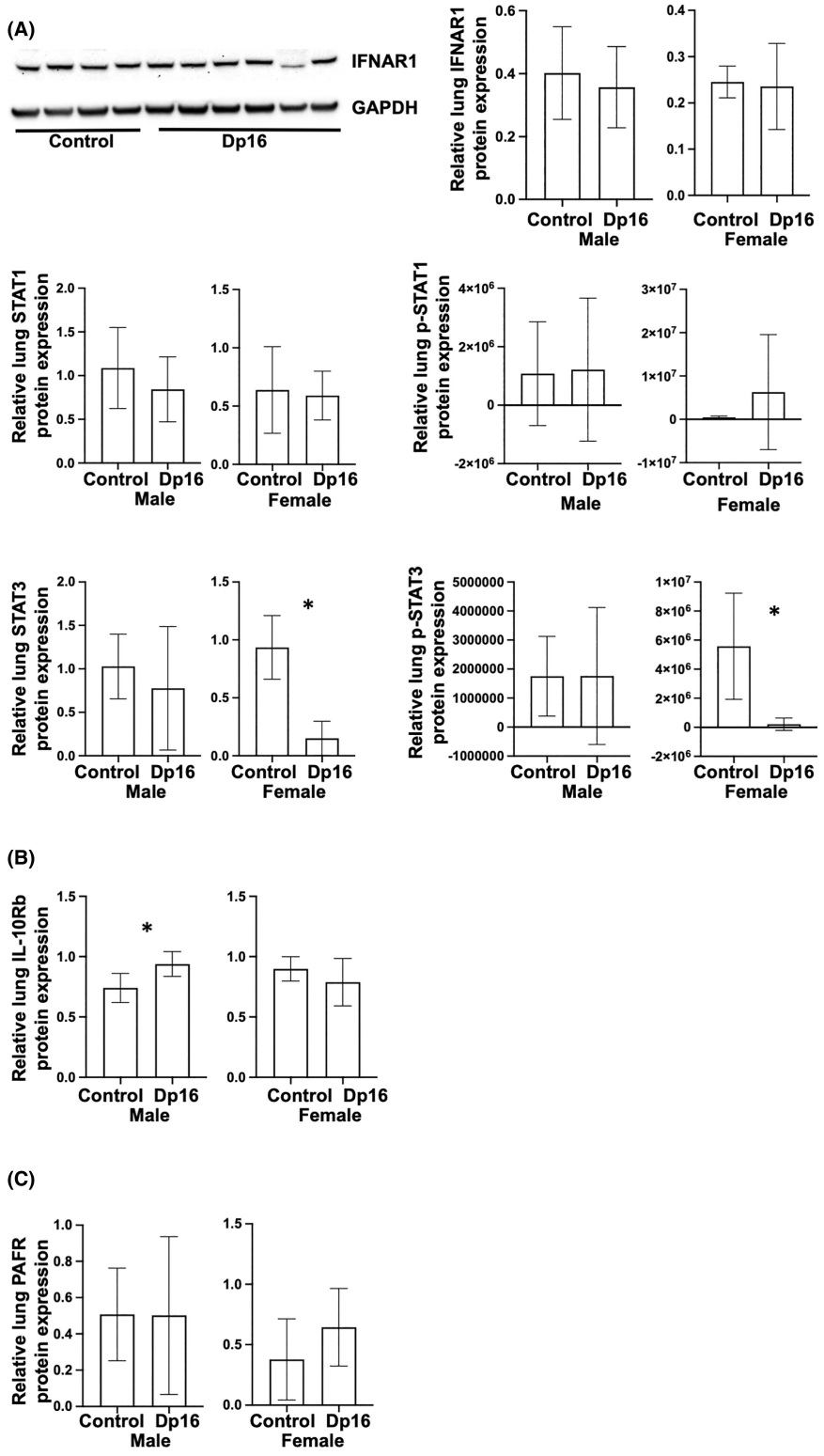

FIGURE 3 Infection with *S. pneumoniae* is variably associated with changes in lung protein levels of interferon signaling genes in Dp16 mice. (A) (Left) Representative immunoblots for IFNAR1 and GAPDH. (Bar graphs) We found no differences between controls and Dp16 mice in lung protein levels of IFNAR1, STAT1, and phospho-STAT1 by immunoblot. STAT3 and phospho-STAT3 proteins were decreased in female Dp16 mice compared to controls. (B) IL-10Rb protein was significantly higher in male Dp16 mice compared to controls. (C) PAFR protein was not significantly increased in lungs of Dp16 mice versus controls. $n = 6$ mice per group each for males and females.

of mouse chromosome 16 genes syntenic to Hsa21, including interferon receptors. Thus, one potential conclusion is that, at least in Dp16 lungs, there is no protein level interferonopathy. A second potential explanation comes from studies on simian immunodeficiency virus. Administration of IFN alpha2a initially upregulated antiviral genes, but with continued treatment animals became desensitized to IFN alpha and antiviral gene expression decreased.[36] Thus, the timing of IFN-induced immune responses is critically important in determining disease outcome. In people with DS, it is unclear how chronic (over-) expression of interferon receptors contributes to disease spectrum. Our data in the Dp16 mouse support a model in which some level

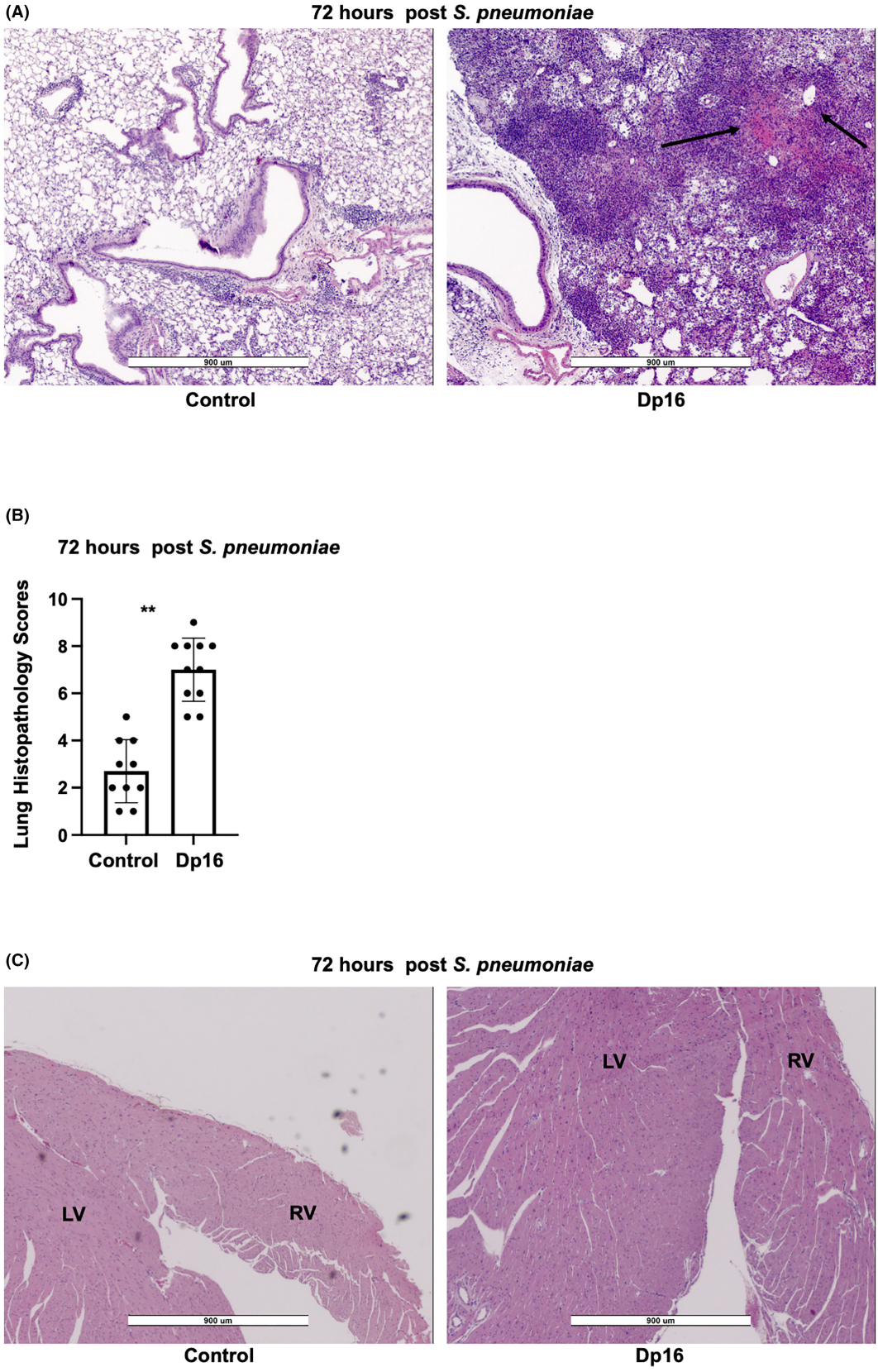

FIGURE 4 Compared to controls, Dp16 mice display significantly worse lung pathology when challenged with *S. pneumoniae*. (A) Representative lung histopathology images from controls (left image) and Dp16 mice (right image) showing Dp16 mice lungs with necrotizing vasculitis, hemorrhage, and hepatization (arrows). (B) Quantification of lung histopathology postinfection indicates Dp16 mice exhibit significantly greater lung pathology compared to controls. (C) We found no evidence of cardiac histopathology postinfection in controls (left image) and Dp16 mice (right image). $^{**}p < 0.01$. scale bar = 900 μm. LV, left ventricle; RV, right ventricle.

of desensitization or accommodation of lung interferon receptors limits their overexpression at the protein level, yet is still associated with a state of susceptibility to bacterial infection. This has been recently demonstrated as "oscillations" of hyper- and hypo-response to type I IFN stimulation due, at least in part, to triggering of negative feedback.[17]

Type III interferon signaling has recently been linked to increased inflammation and decreased epithelial barrier function.[20,37] Similar to type I interferons, our data does not directly link overexpression or overactivation of type III interferons to severe RTI with *S. pneumoniae*. Nevertheless, influenza viral infection and subsequent upregulation of type III interferons compromised barrier function and increased inflammation resulting in superinfection with *S. pneumoniae*. We did not assess epithelial barrier damage in our studies. We also did not assess potential impairment of neutrophil recruitment and/or phagocytic function. Type III interferon signaling has been shown to impair both.[38,39]

We found increased secretion of IL-10 by primary lung cells upon infection with *S. pneumoniae*. It is therefore possible that overexpression of IL-10Rb by Dp16 mouse cells with excess IL-10 ligand stimulated a type III IFN response. Since we only measured IFN responses by immunoblot 72 h after infection, we likely missed such a potential phenomenon. The differences in secretion of IL-5, IL-12p70, KC-GRO, and IL-1b beta between control and Dp16 mouse lung cultures could be due to several factors. First, the numbers and

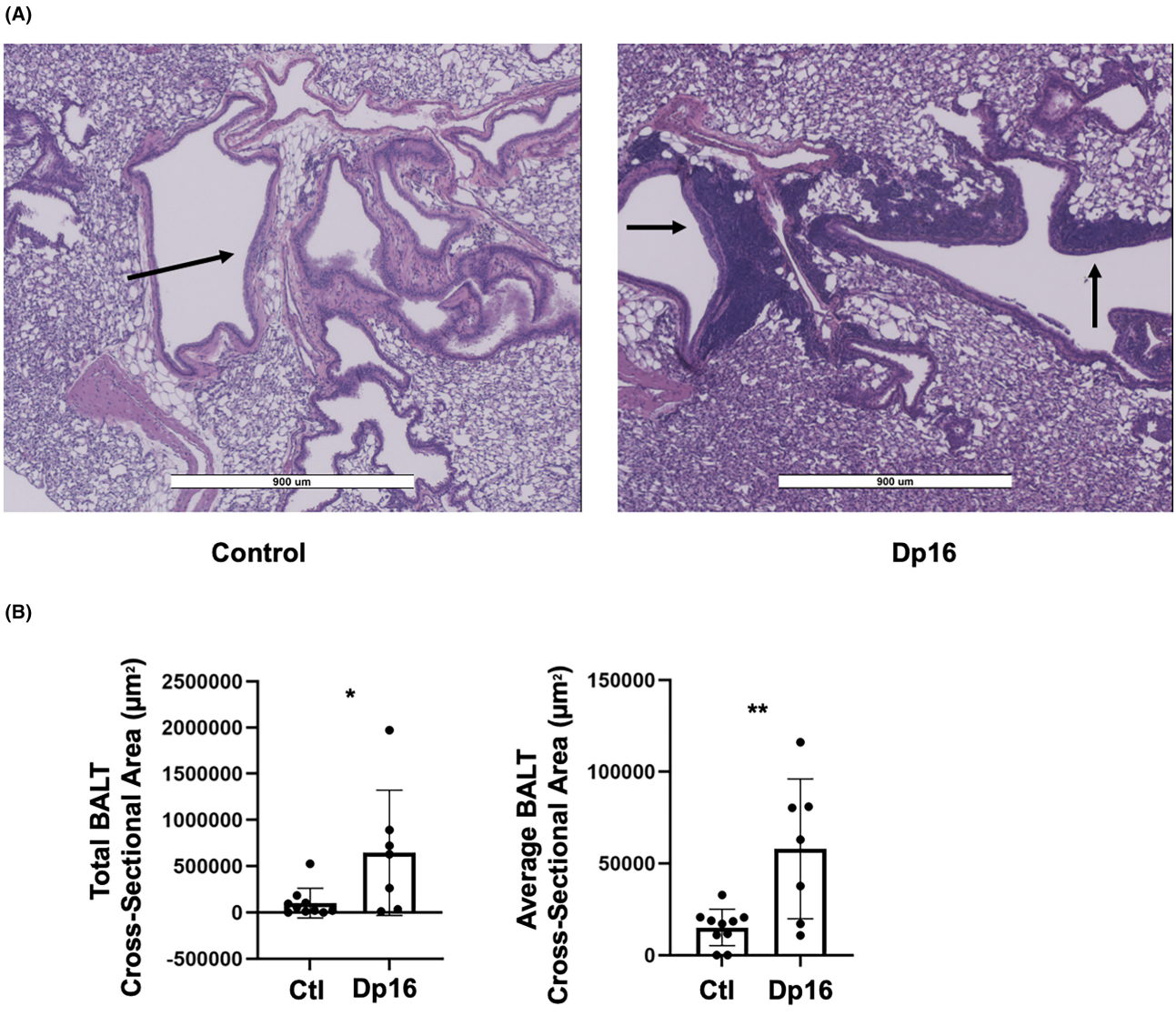

FIGURE 5 Prior to infection, the lungs of Dp16 mice are replete with bronchus-associated lymphoid tissue (BALT). (A) Representative photomicrographs of bronchovascular histology. Note the expansive BALT in Dp16 (right) versus controls (left). (B) Graphs depicting significant increases in total BALT per cross sectional area (left) and average BALT per cross sectional area in Dp16 mice compared to controls. *$p < 0.05$, **$p < 0.01$. scale bar = 900 µm.

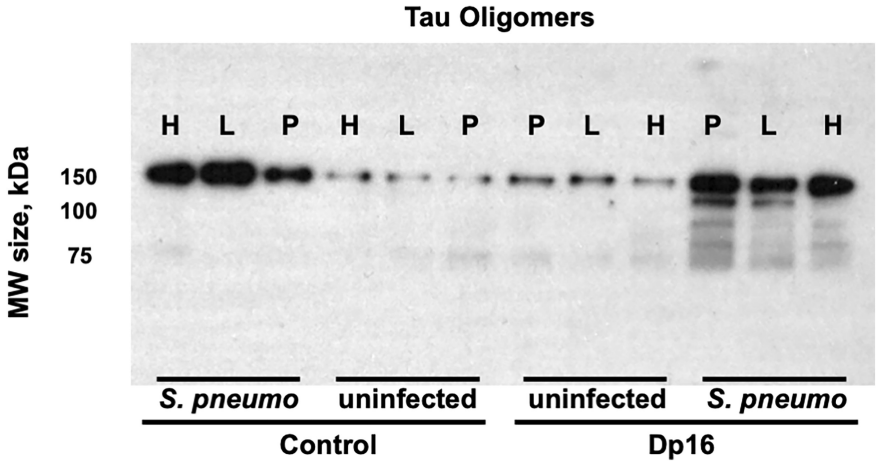

FIGURE 6 Infection with *S. pneumoniae* elicits tau oligomer distribution. Representative sarkosyl extractions of plasma, lung, and heart probed with T22 antioligomeric tau antibody. Compared to uninfected mice, the tissues of both control and Dp16 mice infected with *S. pneumoniae* contain tau oligomers. H, heart; L, lung $n = 3$ animals per group; P, plasma, pooled from n = 3 animals per group.

types of lung immune cell subsets are likely very different. Our finding of increased BALT in Dp16 mice at baseline support such an idea. A unique collection of lung immune cells would seem likely to give rise to a unique lung immune cell response when challenged with a microbial agent like *S. pneumoniae*. Second, given the global changes in gene expression in Dp16 mice, the heterotypic interactions between immune cells and lung epithelial cells, endothelial cells, and mesenchymal cells, etc., is likely to be very different. The characterization of such interactions will be an important step forward in our understanding of how the lung immunity in persons with DS is different. It should be noted that in these experiments we deliberately cultured mixed lung cells without cell selection or purification. It is likely that in these cultures, some phenotypic changes and intercellular crosstalk may be altered from lung tissue. Nevertheless, it is our experience that compared to mixed cultures, the use of purified cell lines or immortalized cell lines potentially represents a much larger distortion of the complex heterotypic lung cell interactions occurring in vivo.

We found no evidence of increased PAFR expression in Dp16 mice following infection. Our previous data showing no increase in PAFR expression prior to infection suggests that it does not play a major role in bacterial dissemination of the lung, at least for *S. pneumoniae*. We did find evidence of more extensive lymphocytic dissemination to distal lungs in Dp16 mice. *S. pneumoniae* is a Gram-positive, extracellular, commensal, opportunistic pathogen found in the mucosal surfaces of the upper respiratory tract. In people with DS, aspiration is a major clinical problem,[6,40] and may potentially contribute to local spreading or seeding to deeper regions of the lung.

The histopathology of the Dp16 mouse lung post-infection was remarkably worse compared to controls. Necrotizing vasculitis, hemorrhage, and hepatization were frequent and extensive in Dp16 mice. In addition, BALT were numerous and prominent. We previously observed that lungs of Dp16 mice are replete with BALT preinfection. This raises the possibility that the lung microbiome of Dp16 mice is unique compared to controls and it also suggests that BALT may play key roles in the susceptibility and response to lung infection in DS. Due to the scarcity of autopsy material from people with DS, we are unable to directly correlate our findings regarding BALT in Dp16 mice to DS. This will be an important endeavor moving forward, as there are many potential clinical avenues to explore to manipulate BALT.[41]

RTI is associated with development of cognitive dysfunction in typical individuals[25] as well as those with DS.[26] Pneumonia induces a tauopathy due to release of cytotoxic lung endothelial tau.[27,28] Furthermore, people with DS circulate elevated Tau in peripheral blood.[29,30] Our finding of increased oligomeric tau in the plasma, lung, and heart of both control and Dp16 mice groups that were infected with *S. pneumoniae,* but not in uninfected mice, is exciting. Although we did not find evidence of increased oligomeric tau in Dp16 mice compared to controls, our study was not empowered to do so. Our current work here confirms that RTI elicits significant increases in lung tau in mice and that tau oligomers are distributed in peripheral tissues. Indeed, lung infection with Pseudomonas aeruginosa in mice was found to be associated with blood–brain barrier injury and leakage, as well as behavioral changes.[42] This has important implications for people with DS, because it suggests that limiting the incidence and severity of RTI could potentially help to preserve cognition and perhaps lengthen the time to the development of symptoms of Alzheimer's disease related to tau pathobiology. It is important to point out that our finding of a tauopathy in Dp16 mice infected with *S. pneumoniae* may not be generalized to RTI caused by other pathogens. Future studies using viruses and bacterial strains should address this question.

There are several limitations to our study. Firstly, the lung is equipped with a formidable mechanical and immune host defense weaponry, much of which we do not directly account for here. For example, the production of mucus and the mucociliary elevator, and the arrangement of tight junctions are but two examples of mechanical barriers against microbial invasion. A previous study on 18 individuals with DS demonstrated the presence of normal ciliary architecture but reduced beat frequency.[43] These authors concluded that reduced mucociliary clearance in DS was thus the result of prolonged bacterial invasion with concomitant change in rheological properties of mucus. By contrast, overexpression of pericentrin, an Hsa21-encoded gene, causes a ciliopathy due to dysregulated centrosomal scaffolding.[44] Claudin 14, a tight junction protein, has been implicated in early onset hearing loss in DS.[45] Secondly, deficiencies in IgG subclasses have been previously noted in individuals with DS.[46] Any or all of these mechanical and immunological defenses (as yet, largely uninvestigated) could potentially account for, or contribute to, the increased severity of RTI that we observed in Dp16 mice. Thirdly, the Dp16 mouse model does not exactly duplicate human DS. The Dp16 mouse has segmental duplication of genes, some of which are syntenic to Hsa21, and some of which are mouse genes for which there is no human orthologue. Furthermore, the segmental duplications of Dp16 mice do not include additional elements of chromosomal architecture such as centromeres and telomeres, which are also duplicated in DS. These elements have unique biology and likely affect gene expression. Finally, we did not evaluate the potential contributions of hypotonia, upper airway abnormalities, and dysphagia in Dp16 mice to the intranasal delivery of *S. pneumoniae*. Any of these could have had a disparate impact on pathogen delivery and to bacteremia (ex: swallowing bacteria) in the Dp16 mice in our experiments.

In conclusion, we found that Dp16 mice mirror several key aspects of susceptibility to severe lung infection observed in people with Down syndrome. In addition, bronchus-associated lymphoid tissue may play key roles in controlling susceptibility and severity of infectious lung disease in DS. Interestingly, infection with *S. pneumoniae* is associated with the presence of oligomeric tau in multiple tissues. Unraveling the mechanisms controlling these responses may uncover new therapies to reduce the morbidity and mortality of infectious lung disease and perhaps cognition for people with and without Down syndrome.

AUTHOR CONTRIBUTIONS

K.L.C. and M.E.Y. were involved in conceptualization. K.L.C., R.J.E, J.H., E.G.B., and M.E.Y. were involved in methodology. K.L.C. and M.E.Y. were involved in validation. K.L.C., R.J.E., J.H., E.G.B., and M.E.Y were involved in writing—review and editing. K.L.C., D.M.G., R.J.E., J.H., E.G.B., and M.E.Y. were involved in investigation. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

Data are contained within the article and supplementary file.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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